



Toxicological profile for L-Menthol

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

(1R,2S,5R)-5-Methyl-2-propan-2-ylcyclohexan-1-ol (PubChem).

1.2. Synonyms

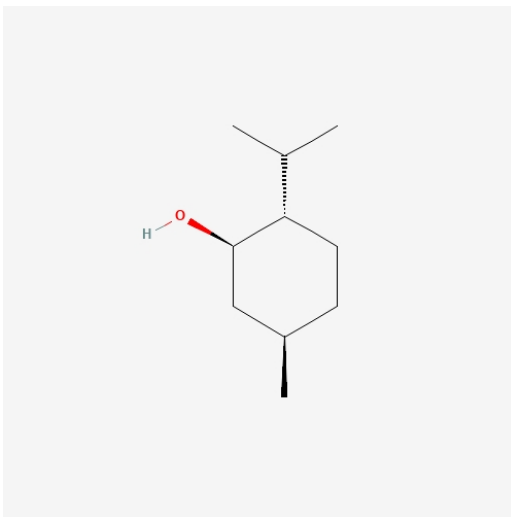
CAS 2216-51-5: l-Menthol; Levomenthol; Menthomenthol; Menthacamphor; Peppermint camphor; Racementhol; Hexahydrothymol; p-Menthan-3-ol; Racemic menthol; FEMA No. 2665; Thymomenthol; Menthol, cis-1,3,trans-1,4-; Racementol; EINECS 201-939-0; EINECS 218-690-9; EINECS 239-388-3; UNII-BZ1R15MTK7; . UNII-YS08XHA860; (PubChem)

CAS 89-78-1: MENTHOL; dl-Menthol; 2-Isopropyl-5-methylcyclohexanol; Cyclohexanol, 5-methyl-2-(1-methylethyl)-; p-Menthan-3-ol; Hexahydrothymol; 5-methyl-2-propan-2-ylcyclohexan-1-ol; rac-Menthol; Neomenthol; Menthyl alcohol; Menthol, cis-1,3,trans-1,4-; Mentholum; Mineral ice; 3-p-Menthol; EINECS 216-074-4; AKOS000119740; AKOS016843634; (PubChem)

1.3. Molecular formula

C₁₀H₂₀O (PubChem)

1.4. Structural Formula



1.5. Molecular weight (g/mol)

156.27 (EPISuite, 2017)

1.6. CAS registration number

2216-51-5, 89-78-1

1.7. Properties

1.7.1. Melting point

(°C): 41-45 (ChemSpider; EPISuite, 2017; PubChem); 79.5 (EPISuite, 2017); 39 or 100°F (38°C) (PubChem); 34-35 (ChemSpider) (CAS 2216-51-5)

1.7.2. Boiling point

(°C): 212-216 (ChemSpider; EPISuite, 2017; PubChem) (CAS 2216-51-5)

1.7.3. Solubility

490 mg/L at 25°C (PubChem); 456 mg/L at 25°C (PubChem); 420, 456 or 490 mg/L at 25°C (EPISuite, 2017)

456 mg/L (ChemIDplus), (CAS 89-78-1)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 93 or 101 (ChemSpider); 196°F (91°C) (PubChem) 93 or 101°C (ChemSpider) (CAS 89-78-1)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

Stable (ChemSpider)

1.7.10. Vapor pressure

0.0637 mmHg at 25°C (extrapolated) (EPISuite, 2017); 0.08 mmHg (PubChem)

1.7.11. log Kow

3.4 (PubChem); 3.19 or 3.4 (EPISuite, 2017); 3.3 (PubChem); 3.211 (ChemSpider) (CAS 2216-51-5)

2. General information

2.1. Exposure

The Maximised Survey-derived Daily Intake (MSDI) intake for menthol is 16 mg/person/day (EFSA, 2011).

Menthol (CAS RNs 1490-04-6/2216-51-5/89-78-1/15356-70-4) is used as a denaturing, fragrance, refreshing and soothing ingredient in cosmetics in the EU. As taken from CosIng, undated

L-Menthol (CAS RN 2216-51-5) is listed as a fragrance ingredient (IFRA; US EPA InertFinder Database, 2023).

Menthol (CAS RN 89-78-1) is listed in the US EPA InertFinder Database (2023) as approved for fragrance and non-food use pesticide products. (US EPA InertFinder Database, 2023)

Probable Routes of Human Exposure:

Occupational exposure to L-menthol may occur through inhalation and dermal contact with this compound at workplaces where L-menthol is produced or used(SRC). The general population may be exposed to L-menthol through dermal contact with cosmetic products and ingestion of products containing L-menthol (SRC).

As taken from HSDB, 2003.

Human Exposure:

“The major route of occupational exposure to menthol isomers is the inhalation route. Although dermal exposure may also occur, the volatility of menthol isomers is high enough to limit the extent of absorption through the skin (see Henry’s law constant). The main sources of occupational exposure are menthol manufacture, intertank transfers and cleaning processes. Further sources of occupational exposure in the processing industry are the following: manufacture of cosmetics, medical ointments, toothpastes, manufacture of sweets, liqueurs, and chewing gums (Haarmann and Reimer, 2002).”

“The Bayer menthol synthesis plant continuously produces an isomer mixture of D/L-menthol from m-cresol and propene via thymol in a closed system. The exhausts from menthol production are connected to a thermal exhaust purification. Some tanks are equipped with activated carbon filters to enable tank respiration. Although for menthol and thymol there are no occupational exposure limits (OELs) like MAK, to ensure protection of workers at the workplace, thymol was used as an indicator for menthol and other expositions in the Bayer menthol manufacturing plant in 1990/91. Thymol has chemical properties similar to menthol, e.g. a melting point of about 50 °C and a boiling point of 233 °C. The results of the thymol measurements were < 0,5 mg/m³ and < 0,8 mg/m³ in the Bayer menthol manufacturing factory (Bayer AG, 2002a).”

“Since menthol is a food ingredient (FAO/WHO) and generally recognized as safe (US FDA), it was decided not to monitor menthols at the workplace in the Bayer production plant (Bayer AG, 2002a). In the Haarmann and Reimer menthol processing plants e.g. from olfaction and measurements of solvents used in the manufacturing process it is assumed that the menthol concentrations at their workplaces are very low. The most significant routes of consumer exposure to menthol isomers from the use of mentholated products are likely to be dermal and oral. Consumers may also be exposed to menthol through the inhalational route, e.g. by smoking mentholated cigarettes, or by odour improving agents. Consumer exposure to menthol isomers is possible from a number of different sources including oral care products, pharmaceuticals, food and flavouring products, tobacco, and others. In a survey of flavoring usage levels, the Flavoring Extract Manufacturing Association has summarized "average maximum use levels" on which an expert panel based its judgements that the substances are generally recognized as safe (Hall and Oser 1965). For menthol these levels are (in ppm):

Beverages 35

Ice cream 68

Candy 400

Baked goods 130

Chewing gums 1100

Recommended levels for the addition of L-menthol to products are e.g. (Hopp 1993). Oral care products (concentrated mouthwashes) up to 2 % Pharmaceuticals (medicated oils) up to 4 % Menthol cigarettes up to 0.45 %.

Pipe tobacco up to 0.3 % Perfumed products (e.g. refreshing towels, cooling gels) up to 1 % Additional information of identified uses from the US are given under section 2, "General Information on Exposure". Information on consumer products from Product Registers revealed that the highest menthol concentrations are reported for cosmetics (2-20%), and odor agents (2-20%) (Danish Product Register, 2002). Concentrations in the range between 1 and 10% may be found in paints, animal care products, veterinary medicines, repellents, and cleaning products (Danish Product Register, 2002; Swedish Product Register, 2002; Swiss Product Register, 2002). Menthol concentrations of max. 1 % are reported for shoe- and leather care products and disinfectants (Swiss Product Register, 2002).

The Joint FAO/WHO Expert Committee on Food Additives derived in their 51st meeting in 1998 an acceptable daily intake (ADI) for L-menthol and D/L-menthol in the range of 0 - 4 mg/kg bodyweight (FAO/WHO 1999).

Exposure to menthol also occurs through the use of peppermint oil, since menthol is the primary component of peppermint oil (35 - 60 %) (Nair, 2001). Peppermint oil is used in cosmetic formulations, in the manufacture of chewing gum, confectionery, toothpastes and pharmaceutical products.

Potential exposure to menthol isomers from drinking water and ambient air is expected to be negligible (Haarmann and Reimer, 2002)."

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

Dermal systemic exposure to L-menthol via cosmetics products was reported to be 0.01 mg/kg bw/day (Belsito et al, 2008).

Menthol, L- (CAS RN 2216-51-5) is listed as an ingredient (at given concentrations, where specified) in personal care (0.1-10%), pet care (4%), inside the home and commercial/ institutional products by the CPID.

Occupational exposure limit: RUSSIA: STEL 1 mg/m³, JUN2003

As taken from RTECS, 2017

"Used as a flavor/fragrance in liqueurs, confectionary, perfumes, cigarettes, cough drops, and nasal inhalers. Also used as a topical antipruritic and veterinary local anesthetic, antiseptic, carminative, and gastric sedative. Used in Canada to control mites and in Europe in paints and lacquers, adhesives, metal care products, cleaning products, shoe and leather care products, disinfectants, solvents, repellents, cosmetics, odor agents, and animal care products.

WEEL: 1 ppm, STEL = 3 ppm (OARS WEEL)."

L-Menthol (CAS RN 2216-51-5) is used as an oral flavour enhancer, topical fragrance Ingredient and topical skin-conditioning agent in non-medicinal natural health products. It has an upper limit toxicity restriction of 4.0 mg/kg bw/day (Health Canada, 2021).

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
Menthone	376331495	7.65	455603753	9.66
Menthol	4218881056	85.76	4019682431	85.23
Menthol isomer	89681550	1.85	72408036	1.54
Total ion chromatogram	4927557692	100	4701820519	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker R and Bishop L. (2004). The pyrolysis of tobacco ingredients. J. Anal. Appl. Pyrolysis 71, 223–311.

Ingredients CAS Number Formulae or structure	Chemical Class	Mol. Wt. (M) Bp or Mp (°C)	Max cig Appln. Level (ppm)	Purity of sample Pyrolysed (%)	Composition of pyrolysate (Compound %)	Max level in smoke (µg)
I-Menthol CAS 89-78-1; 2216-51-5	Cyclic alcohol	M=156 mp 42- 43 bp 212	20,000	99	Methol 99.0 Menthone 0.9 Menthene 0.1	10,000 90 10

“When pyrolysed at 900 °C three pyrolysis compounds were identified – carbon dioxide, para-menth-3-ene and dl-menthol. Menthol was not extensively pyrolysed (Philip Morris USA Internal Report 2001)”.

Pyrolysis of d, l-menthol was carried out in a laboratory study at 860+5 °C, the assumed cigarette burn temperature. It was also performed at 600+5 °C to examine the effect of temperature. 15 products were produced including, benzo[a]pyrene and phenol (Schmeltz et al, 1968).

In a series of pyrolysis studies on tobacco ingredients, the intact transfer of 97.4% of menthol was reported. One extra compound at greater than 1% of total ion current was detected by gas chromatography-mass spectrometry. The authors comment that pyrolysis does not provide a robust prediction of the compounds that are formed from ingredients during cigarette smoking studies (Purkis et al. 2011).

2.3. Ingredient(s) from which it originates

A pale yellow oil extracted from the leaves of lantana orangerose contained 8.2% l-menthol. [BASLAS RK, KUMAR P; J SCI RES (BHOPAL, INDIA) 2 (3): 155 (1980)] **PEER REVIEWED**

L-Menthol is the main component of peppermint oils and cornmint oils obtained from *Mentha piperita* and *Mentha arvensis*(1). [(1) Bauer K et al; Ullmann's Encycl Indust Chem. Gerhartz W Ed. NY,NY: VCH Publ A11: 167-8 (1988)] **PEER REVIEWED**

Most l-menthol is obtained ... from *Mentha arvensis* oil [Kirk-Othmer Encyclopedia of Chemical Technology. 3rd ed., Volumes 1-26. New York, NY: John Wiley and Sons, 1978-1984., p. V22 742] **PEER REVIEWED**

As taken from HSDB, 2003.

Menthol is present in the volatile oils of doggrass (3.5%), Spanish lavender (*L. Stoechas*; 18%), peppermint (29–48%), spearmint and cornmint (70–95%), in geranium oils and in a cultivar of *M. arvensis* (21%).

As taken from Khan and Abourashed, 2010.

3. Status in legislation and other official guidance

Acceptable Daily Intakes:

FDA Requirements:

FDA 121.1164 [Fenaroli's Handbook of Flavor Ingredients. Volume 2. Edited, translated, and revised by T.E. Furia and N. Bellanca. 2nd ed. Cleveland: The Chemical Rubber Co., 1995., p. 330].

General Information

Chemical Names: l-3-p-MENTHANOL; dl-3-p-MENTHANOL

CAS number: 89-78-1 (dl); 2216-51-5 (l)

JECFA number: 427

COE number: 63

FEMA number: 2665

Functional Class: Flavouring Agent

FLAVOURING_AGENT

Evaluations

Evaluation year: 2018

ADI: 0-4 mg/kg bw

Comments: No safety concern at current levels of intake when used as a flavouring agent

Meeting: 86

Specs Code: M

Report: TRS 1014-JECFA 86/107

Specification: FAO JECFA Monographs 22/99

Evaluation year: 1998

Meeting: 20

Specs Code: S

Report: TRS 891-JECFA 51/25,111

Tox Monograph: FAS 42-JECFA 51/57,381

Specification: COMPENDIUM ADDENDUM 6/FNP 52 Add.6/186

Previous Years: 1976, FNS 1/TRS 599-JECFA 20/15, FAS 69.31/NMRS 44B-JECFA 11/35,36 (1967). 0-0.2. FU S 1974, NMRS 54/TRS 557-JECFA 18/14. 0-0.2. FU. S 1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/35,36; COMPENDIUM/923,925, FAS 68.33/NMRS 44A-JECFA 11

As taken from WHO, 2021. Available at <https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1519>

“No safety concern” was established and the ADI of 0–4 mg/kg bw (see above) was retained at the 86th JECFA meeting (WHO, 2021).

Menthol (CAS 89-78-1) is included on the US FDA's list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is generally recognised as safe (GRAS) under 21 CFR section 172.515 ([Synthetic flavoring substances and adjuvants](#)), and 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)). (FDA 2022, 2023)

There are REACH dossiers on L-menthol (CAS 2216-51-5) and menthol (CAS 89-78-1) (ECHA, undated).

L-menthol and menthol are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

L-menthol (CAS RN 2216-51-5) and menthol, racemic (CAS 89-78-1) are included on the US EPA's Safer Chemical Ingredients list (US EPA, 2023).

L-menthol (CAS RN 2216-51-5) is listed in the US EPA InertFinder Database (2023) as approved for fragrance use pesticide products.

Menthol (CAS RN 89-78-1) is listed in the US EPA InertFinder Database (2023) as approved for fragrance and non-food use pesticide products.

Levomenthol (CAS RN 2216-51-5) and (1R,2S,5R)-Menthol (CAS 89-78-1) are listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2020 CDR list (Chemical Data Reporting Rule).

The TSCA inventory and 2020 CDR list.

Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1.alpha.,2.beta.,5.alpha.)]- (CAS RN 2216-51-5) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” and data available on its function “indicate that it may be used in cosmetics, but only at low concentrations” (AICIS, 2017).

Levomenthol (CAS RN 2216-51-5) is included on the US FDA's list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potencies per unit dose and maximum daily exposure:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
LEVOMENTHOL	BUCCAL	GUM, CHEWING	2216515	BZ1R15MTK7	9.2mg	
LEVOMENTHOL	ORAL	SOLUTION	2216515	BZ1R15MTK7		15mg
LEVOMENTHOL	ORAL	TABLET	2216515	BZ1R15MTK7		5mg
LEVOMENTHOL	ORAL	TABLET, ORALLY DISINTEGRATING	2216515	BZ1R15MTK7		1mg
LEVOMENTHOL	SUBLINGUAL	SPRAY	2216515	BZ1R15MTK7	0.05mg/1ml	
LEVOMENTHOL	TOPICAL	LOTION	2216515	BZ1R15MTK7	0.08%w/w	
LEVOMENTHOL	TOPICAL	SPRAY	2216515	BZ1R15MTK7		2mg

As taken from FDA, 2023b

Menthol racemic (FEMA no. 2665; CAS RNs 1490-04-6, 2216-51-5, 89-78-1 and 15356-60-2) has been given GRAS (generally recognised as safe) status by FEMA (Hall RL and Oser BL, 21965)

I-Menthol (CAS RN 2216-51-5) is classified as a natural health product (NHP) under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations (Health Canada, 2021).

Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1.alpha.,2.beta.,5.alpha.)]- (CAS 2216-51-5) and Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1R,2S,5R)-rel- (CAS 89-78-1) are listed on Australian Inventory of Industrial Chemicals (AICIS, formerly NICNAS). As taken from AICIS, undated.

Menthol (CAS RN 89-78-1) is listed as authorised for use as a flavouring substance in all categories of flavoured food in the EU under Regulation (EU) No 872/2012.

As taken from European Commission, 2012.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

L-Menthol conjugates readily in rabbit forming l-menthyl-beta-d-glucuronide. about half of the l-menthol fed to rabbits is excreted combined with glucuronic acid; the fate of other half is not known, but it is possible that ring fission occurs with considerable degradation of the menthol molecule. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 519] **PEER REVIEWED**

In dogs, much oxidation of menthol takes place and only about 5% of the dose can be recovered in urine as the glucuronide. /menthol/ [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 519] **PEER REVIEWED**

L-Menthol was rapidly but incompletely glucuronidated. the output of l-menthol glucuronide was incr in all but 1 subject pretreated with cimetidine (1 g/day for 1 wk), an inhibitor of oxidative drug metabolism, & in all subjects pretreated with a drug-metabolizing enzyme inducer, phenobarbitone (60 MG NIGHTLY FOR 10 DAYS). [BELL GD ET AL; BR J CLIN PHARMACOL 12 (2): 274 (1981)]

****PEER REVIEWED****

Corynebacterium sp. strain RWM1 grew with (-)-menthol, (-)-menthone and other acyclic monoterpenes as sole carbon sources. Growth on menthol was very slow, with a doubling time of more than 24 h, and was not rapid with (-)-menthone (doubling time 12 h). Concentrations of either carbon source greater than 0.025% inhibited growth. (-)-Menthone-grown cultures transiently accumulated 3,7-dimethyl-6-hydroxyoctanoate during growth, and (-)-menthol-grown cells oxidized (-)-menthol, (-)-menthone, 3,7-dimethyl-6-octanolide and 3,7-dimethyl-6-hydroxyoctanoate. Although neither a menthol oxidase nor a menthol dehydrogenase could be detected in extracts of (-)-menthol- or (-)-menthone-grown cells, an induced NADPH-linked monooxygenase with activity towards (-)-menthone was readily detected. With crude cell extracts, only 3,7-dimethyl-6-hydroxyoctanoate was detected as the reaction product. When the (-)-menthone monooxygenase was separated from an induced 3,7-dimethyl-6-octanolide hydrolase by chromatography on hydroxyapatite, the lactone 3,7-dimethyl-6-octanolide was shown to be the product of oxygenation. [Williams DR, PW Trudgill; Microbiology (Reading) 140 (3): 611-6 (1994)] ****PEER REVIEWED****

As taken from HSDB, 2003.

“One of the main pathways for the candidate alcohols and the ketones (after reduction) is conjugation with glucuronic acid followed by excretion. Menthol, ... are also metabolised via this pathway. Menthol, ... are not anticipated to be oxidised to the corresponding ketone.”

“In vivo metabolism of l-menthol was studied in adult male rats by giving the rats 800 mg/kg bw l-menthol solved in 1 % methyl cellulose solution by gavage every day for 20 days. Control rats were given vehicle only. The following metabolites of l-menthol were found in the urine: p-menthane-3,8-diol, p-menthane-3,9-diol, 3,8-oxy-p-menthane-7-carboxylic acid and 3,8-dihydroxy-p-menthane-7-carboxylic acid. The main urinary metabolites were p-menthane-3,9-diol and 3,8-dihydroxy-pmenthane-7-carboxylic acid. Menthone was not detected (Madyastha and Srivatsan, 1988).”

“Oxidation of alkyl groups have been observed for menthol ...”

“The hydrolysis product menthol, as such or after the oxidation of the alkyl ring substituents, is mainly conjugated with glucuronic acid and excreted via the bile in rats. Low levels of oxidation products were found in the urine, but no unchanged menthol was detected in the urine, faeces or bile after oral administration of radioactive labelled menthol (Yamaguchi et al., 1994).”

As taken from EFSA, 2015.

“Metabolism of l-menthol in rats was investigated both in vitro and in vivo. Rat liver microsomes readily converted l-menthol to p-menthane-3,8-diol in the presence of NADPH and O₂ (Hawthorn et al., 1988). After oral administration of l-menthol (800 mg/kg bw per day) to rats, the following metabolites were isolated and characterised from the urine: p-menthane-3,8-diol, p-menthane-3,9-diol, 3,8-oxy-pmenthane-7-carboxylic acid and 3,8-dihydroxy-p-menthane-7-carboxylic acid. In vivo, the major urinary metabolites were p-menthane-3,8-diol and 3,8-dihydroxy-p-menthane-7-carboxylic acid. Repeated oral administration of l-menthol to rats for 3 days resulted in the increase of both liver microsomal CYP450 content and NADPH-cytochrome c-reductase activity by nearly 80%. Further treatment (for 7 days total) did not change these levels. [In] studies with tritiated l-menthol in rats ... the main metabolite identified was mentholglucuronide. Additional metabolites

are mono- or dihydroxylated menthol derivatives (EMEA, 2008). Similarly to rats, humans metabolise menthol primarily by conjugation with glucuronic acid and elimination in the urine. CYP450-mediated oxidation occurs in humans, yielding various alcohol and hydroxy acid derivatives, which would also be eliminated in the urine unchanged or conjugated with glucuronic acid (WHO, 1999)."

As taken from EFSA, 2016.

"To determine the disposition kinetics and to examine subjective and cardiovascular effects of menthol, a crossover placebo-controlled study that compared pure menthol versus placebo, along with an uncontrolled exposure to menthol in food or beverage was conducted. Twelve subjects were studied; each received a 100 mg l-menthol capsule, a placebo capsule, and 10 mg menthol in mint candy or mint tea on three different occasions. Plasma and urine levels of menthol and conjugated menthol (glucuronide), cardiovascular measurements, and subjective effects were measured at frequent intervals. Menthol was rapidly metabolized, and only menthol glucuronide could be measured in plasma or urine. The plasma half-life of menthol glucuronide averaged 56.2 minutes (95% confidence interval [CI], 51.0 to 61.5) and 42.6 minutes (95% CI, 32.5 to 52.7) in menthol capsule and mint candy/mint tea conditions, respectively (P < 0.05). The plasma area under the plasma concentration-time curve ratios for menthol capsule to mint candy/mint tea treatment averaged 9.2 (95% CI, 8.2 to 10.1) (Hadley & Gaarder, 2005)."

As taken from EMA, 2020

4.2. Absorption, distribution and excretion

...the percentage of a dose of l-menthol that is excreted combined with glucuronic acid in the rabbit depends on the magnitude of the dose; the larger the dose, the less is the conjugation. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 519]
PEER REVIEWED

As taken from HSDB, 2003.

'Absorbed menthol is largely eliminated as glucuronides. Thus, 79% of a 1-g (Quick, 1928) and 78% of a 10-20-mg (Atzl et al., 1972) oral dose of menthol was eliminated as the glucuronic acid conjugate within 6 h after administration to volunteers. When 750 mg (-)-menthol were given orally to three human volunteers, followed by oral or intravenous administration of 200 mg [6-¹³C]-glucuronolactone or [6-¹³C]-sodium glucuronate, menthyl glucuronide was excreted for two days, in average daily yields ranging from approximately 27 to 84% (Eisenberg et al., 1955).'

"From the studies on metabolism with L-, D/L- and the unspecified menthol isomer mixture it can be concluded that menthol is well absorbed by the oral route (Madhava Madyastha and Srivatsan, 1988; Yamaguchi, et al., 1994, Williams, 1938, Atzl et al., 1972). Dermal absorption is slower than oral absorption (Atzl et al., 1972). No quantitative data are available. From case reports it also can be concluded, that absorption by inhalation is very efficient (Atzl et al., 1972)."

Of a dose of 47 mg/kg bw [3-³H]-(-)-menthol, 82% was eliminated in the urine 17 h after administration. Smaller amounts were distributed in the faeces and ileum; only 1% of the activity remained in the liver (Clegg et al., 1982).

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

"The percentage of a dose of l-menthol that is excreted combined with glucuronic acid in the rabbit depends on the magnitude of the dose; the larger the dose, the less is the conjugation."

"In contrast to the results of studies with rats, L-menthyl glucuronide was detected in the urine of sheep fed with L-menthol in major amounts - within 24 hours after application, the excretion being almost complete (Wright, 1945). Similarly also rabbits excreted major amounts of L-menthol in the

urine. 48 % conjugated L-menthol was recovered after 2 days as glucuronide in the urine from rabbits fed with 1000 mg/kg bw L-menthol (Williams, 1938)."

"Also humans excrete considerable amounts of L-menthol as the glucuronide. In an investigation with two human volunteers between 17 and 38 % of menthol was excreted as menthyl glucuronide in urine within 24 hours after 8 daily doses of 750 mg L-menthol (Eisenberg et al., 1955)."

"Glucuronide excretion was investigated in the urine of several persons after oral ingestion of menthol (unspecified isomer) in a variety of studies. In older studies about 70% of the total dose (10 mg to 1560 mg) were found in the urine of humans as glucuronide 6 to 24 hours after ingestion (Quick, 1928; Atzl et al., 1972; Bolund et al., 1967). In a study of Somerville et al. (1984) 35 to 40% menthol was found in the urine of 6 volunteers 24 hours after ingestion of 180 to 190 mg menthol. In another study (Kaffenberger and Doyle, 1990) an average of 40 % menthol was recovered in the urine of 4 volunteers 14 hours after dosing with 72 mg menthol. In summary, the studies show that in humans the unspecified menthol isomer is rapidly glucuronidated and excreted mainly via urine."

"Investigations with male Fischer 344 rats showed that 48 h after administration of 500 mg/kg radiolabelled L-menthol > 70 % of the administered dose was found in urine and faeces (Yamaguchi, et al, 1994)."

"Rats were administered 470 mg/kg bw of [3-3H]-menthol (unspecified isomer) orally. After 17 hours 52 % of the administered radioactivity was found in the urine, 4.5 % and 3.5 % were found in the faeces and ileum (Clegg et al., 1982)."

"In humans urinary elimination of menthol after oral application was almost complete within about 12 to 24 hours (Atzl et al., 1972; Bolund et al., 1967; Kaffenberger and Doyle, 1990; Somerville et al., 1984)."

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

"When 750 mg (-)-menthol were given orally to three human volunteers, followed by oral or intravenous administration of 200 mg [6-¹³C]-glucuronolactone or [6-¹³C]-sodium glucuronate, menthyl glucuronide was excreted for two days, in average daily yields ranging from approximately 27 to 84% (Eisenberg et al., 1955)."

"The human capacity to eliminate menthol is indicated by research on patients with chronic liver disease. Doses of 2 g menthol were given to evaluate the glucuronization capacity of patients with alcohol-induced cirrhosis or steatosis when compared with normal subjects. While the mean excretion of menthol glucuronide was slightly lower in patients with liver disease, they retained a significant capacity to metabolize menthol (Horvath et al., 1984)."

"To determine the concentration of menthol in rat plasma by GC. Rats were administered with single dose of Zhike Chuanbei Pipa dropping pills (ZCPDP) and different doses of menthol herbs. DAS 3. 1.6 software was used to calculate pharmacokinetic parameters, and the accumulative absorption percentage of menthol was calculated by Loo-Riegelman method. The linear regression analysis was made in vitro/in vivo accumulative absorption percentages to detect the in vitro/in vivo correlation. The results of the study showed that the pharmacokinetics behavior of menthol in ZCPDP was in conformity with two-compartment model characteristics. The main parameters were: t_{max} was 10 min, t_{1/2β} was (183. 93 52. 75) min, CL/F was (0. 426 +/- 0. 194) L . min⁻¹ . kg⁻¹, all of which were no difference between ZCPDP and menthol herbs with the same dosage. There were significant differences in t_{max}, t_{1/2β}, CL/F between menthol herbs with different dosages (P < 0. 05) , with indirect proportion between AUC_{0-∞} and dosage. The regression equation of ZCPDP's accumulative absorption percentage and accumulative release percentage was Fa = 1. 160 3Q - 19. 968, r = 0. 981 3. These results suggested that the pharmacokinetics behavior was similar between ZCPDP and menthol herbs with the same dosage in rats, with good in vitro/in vivo correlation. There were significant differences in pharmacokinetics of menthol in the range of 19.2-

570 mg . kg⁻¹.” As taken from Yang B et al. 2013. Zhongguo Zhong Yao Za Zhi 38(9), 1421-5. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23944081>

“We investigated the absorption and metabolism pharmacokinetics of a single L-menthol oral versus skin administration and the effects on human thermogenesis and metabolic rate. Twenty healthy adults were randomly distributed into oral (capsule) and skin (gel) groups and treated with 10 mg kg⁻¹ L-menthol (ORALMENT; SKINMENT) or control (lactose capsule: ORALCON; water application: SKINCON) in a random order on two different days. Levels of serum L-menthol increased similarly in ORALMENT and SKINMENT ($p > 0.05$). L-menthol glucuronidation was greater in ORALMENT than SKINMENT ($p < 0.05$). Cutaneous vasoconstriction, rectal temperature and body heat storage showed greater increase following SKINMENT compared to ORALMENT and control conditions ($p < 0.05$). Metabolic rate increased from baseline by 18% in SKINMENT and 10% in ORALMENT and respiratory exchange ratio decreased more in ORALMENT (5.4%) than SKINMENT (4.8%) compared to control conditions ($p < 0.05$). Levels of plasma adiponectin and leptin as well as heart rate variability were similar to control following either treatment ($p > 0.05$). Participants reported no cold, shivering, discomfort, stress or skin irritation. We conclude that a single L-menthol skin administration increased thermogenesis and metabolic rate in humans. These effects are minor following L-menthol oral administration probably due to faster glucuronidation and greater blood menthol glucuronide levels.” As taken from Valente A et al. 2015. Food Chem. Toxicol. 86, 262-73. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26429629>

“Studies with tritiated l-menthol in rats indicated about equal excretion in faeces and urine... Similarly to rats, humans metabolise menthol primarily by conjugation with glucuronic acid and elimination in the urine.”

As taken from EFSA, 2016.

“1. l-Menthol, as a kind of monocyclic terpene, is widely used in inhalation formulations, food and tobacco. The purpose of this study was to investigate the pharmacokinetic behavior of l-menthol as well as its influence on the activities of cytochrome P450 enzymes. 2. The pharmacokinetic behaviors of l-menthol after inhalation (50 mg/kg) and intravenous injection (10 mg/kg) were investigated. A rat liver microsomal model was adopted to elucidate the inhibitory effect of l-menthol on CYP1A2, CYP2C11, CYP2D1/2, CYP2D4, CYP2E1 and CYP3A1 using phenacetin, tolbutamide, omeprazole, dextromethorphan, chlorzoxazone and testosterone as probe drugs, respectively. 3. The plasma concentration reached the C_{max} within 1.0 h (inhalation) and descended with the T_{1/2} of 8.53 and 6.69 h for inhalation and i.v. administration, respectively. IC₅₀ for inhibition of l-menthol on CYP 450 enzymes were 4.35 μ M for 2D4, 8.67 μ M for 1A2, 13.02 μ M for 3A1, 14.78 μ M for 2D1/2, 234.9 μ M for 2C11 and 525.4 μ M for 2E1, respectively. 4. The results illustrate the pharmacokinetic process of l-menthol in rats and provide information for further rational applications. l-Menthol had moderate inhibitions on CYP2D4 and 1A2, which might affect the disposition of medicines primarily dependent on these pathways.” As taken from Feng X et al. 2019. Xenobiotica 49(10), 1183-1191. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/30654691/>

“Eighteen lactating women were given 100 mg of l-menthol in a capsule on 3 test days. Milk samples were collected every 2 hours for 8 hours starting at the time of ingestion. Menthol was detected in milk at all collection times, with the average concentrations of 2.1 mcg/L at 0 hours, 4.9 mcg/L at 2 hours, 5.9 mcg/L at 4 hours, 5.8 mcg/L at 6 hours and 4.1 mcg/L at 8 hours after the dose. The average peak menthol concentration in milk was 8 mcg/L. Only small amounts of menthol glucuronide metabolites were present in the milk samples.”

As taken from LactMed, 2029

4.3. Interactions

The effects of l-menthol ((-)-menthol) combined with ethyl alcohol (ethanol) on the percutaneous absorption of model drugs were studied using 2 equations and a 2-layer in vitro skin model. A nonlinear least-squares method was employed to determine 6 coefficients using the 2 equations and experimentally obtained permeability coefficient through full-thickness skin and full-thickness skin/vehicle concentration ratio. Adding menthol to water and 40% ethyl alcohol increased the diffusion coefficient of drugs in lipid and pore pathways of stratum corneum. Adding ethyl alcohol to water and 5% menthol increased drug solubility in the vehicle, decreased skin polarity, and increased the role of the pore pathway to whole-skin permeation. It was concluded that the effects of l-menthol and ethyl alcohol on percutaneous drug absorption in vitro are synergistic. [Kobayashi D et al; Pharm Res 11 (Jan): 96-103 (1994)] **PEER REVIEWED**

Hydroxyurea (HU) is a swiftly acting cytotoxic agent and an inhibitor of DNA synthesis that is teratogenic in all mammals studied. By 2-4 hours after maternal injection, HU causes (1) a rapid episode of embryonic cell death that is observable by light microscopy and (2) profound inhibition of 3H-thymidine incorporation into DNA. Previous work has demonstrated that a variety of antioxidants administered before, after or with HU delay the onset of embryonic cell death and ameliorate the birth defects seen at term but do not block the early inhibition of DNA synthesis. This suggests that the early embryonic cell death is not caused by inhibited DNA synthesis. We have postulated that some HU molecules react within the embryo to produce H₂O₂ and intermediate free radicals (including the very reactive hydroxyl free radical) that in turn are responsible for the early cell death; antioxidants are believed to block this reaction. To test whether hydroxyl free radicals are the proximate active species, pregnant New Zealand white rabbits were injected sc with a teratogenic dose of HU (650 mg/kg) in the presence or absence of 550 mg/kg of D-mannitol, a specific scavenger of hydroxyl free radicals. The dosage of D-mannitol was equimolar to the doses of antioxidants used in previous work. When the rabbits were allowed to continue pregnancy to term, the teratogenic effects of HU were ameliorated as evidenced by decreased incidences of the expected malformations. When embryos were examined histologically at 4-16 hr after maternal injection, D-mannitol was found to delay the onset of cell death for 6-8 hr. These results are consistent with the hypothesis that hydroxyl free radicals may be the proximate reactive species in HU-induced early embryonic cell death. [DeSesso JM, GC Goeringer; Teratology 41 (5): 550 (1990)] **PEER REVIEWED**

As taken from HSDB, 2003.

"0.05% and 0.1% menthol significantly enhanced the penetration of dexamethasone in the cornea of rabbits, but did not change the dexamethasone penetration in sclera in vitro. When topical drops of dexamethasone containing 0.1% menthol were administered, a significantly increased concentration of dexamethasone in the cornea and aqueous humor tissues was reported, but dexamethasone concentrations remained unaffected in the retina-choroid tissues. No signs of irritation were observed when menthol was administered at concentrations ranging from 0.025%-0.1%; moreover, no substantial toxic reactions were observed in corneal hydration level, electrophysiological, or histological examinations after the addition of 0.1% menthol". Xu et al (2011) Available at <http://www.springerlink.com/content/2156121h7382477q/>

"To explore the structure-activity relationship for terpenes as transdermal penetration enhancers, unsaturated menthol analogues were synthesized in our study, including p-menth-1-en-3-ol (Compd 1), p-menth-4-en-3-ol (Compd 2), p-menth-4(8)-en-3-ol (Compd 3) and p-menth-8-en-3-ol (Compd 4). Their enhancing activity on the penetration of 5-fluorouracil through rat skin was evaluated by in vitro experiments. Attenuated total reflection-Fourier transform infrared spectroscopy, molecular modeling and transepidermal water loss (TEWL) were introduced to

investigate the enhancer induced alteration in different skin lipid domains. The results indicated that Compd 3 achieved the highest enhancement ability with an enhancement ratio of 3.08. Other analogues were less effective than Compd 3, and no significant difference was found between them and menthol. Treatment of rat skin with these enhancers did not produce any shift in the stretching vibration of the methylene in hydrophobic lipid chains, but significantly improved the polar pathway across the rat skin as suggested by the increased TEWL. Molecular modeling results suggested that polar head groups of the skin lipids provided the main binding site for enhancer action. These findings indicated that the studied compounds enhanced drug transport by interacting with the polar domain of the skin lipid, instead of by affecting the arrangement of the hydrophobic chains.” As taken from Chen Y et al. 2013. *Int. J. Pharm.* 443(1-2), 120-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23333756>

“INTRODUCTION: Despite the longstanding use and popularity of menthol as a flavorant in tobacco products, its sensory interactions with inhaled nicotine have never been measured independently of the other irritants in tobacco smoke. We therefore measured the perception of menthol in an E-cigarette with the primary goal of assessing its analgesic effect on the sensory irritation produced by inhaled nicotine. METHODS: Adult cigarette smokers sampled aerosolized E-liquids containing five different concentrations of nicotine with 0%, 0.5%, or 3.5% l-menthol, as well as two commercial menthol flavors with and without nicotine. For each of the E-liquids participants used a labeled magnitude scale to rate the Overall Sensation intensity, Coolness/Cold, and Irritation/Harshness they experienced, and a Labeled Hedonic Scale to indicate how much they liked/disliked the overall flavor. RESULTS: The main findings were that (1) perceived Irritation/Harshness was unaffected by a low (0.5%) menthol concentration, whereas a high menthol concentration (3.5%) led to higher perceived Irritation/Harshness at low nicotine concentrations but to lower Irritation/Harshness at the highest nicotine concentration (24mg/ml); (2) a commercial Menthol-Mint flavor produced similar results; (3) nicotine tended to enhance rather than suppress sensations of Coolness/Cold; and (4) menthol tended to slightly increase liking independently of nicotine concentration. CONCLUSION: In addition to adding a sensation of coolness, menthol can reduce perceived airway irritation and harshness produced by inhalation when nicotine concentration is high, and contributes to the sensory impact of E-liquids when nicotine concentration is low. IMPLICATIONS: The evidence presented here indicates that menthol can potentially improve the appeal of E-cigarettes not only via its coolness and minty flavor, but also by reducing the harshness from high concentrations of nicotine. As the first direct demonstration of an analgesic effect of menthol on inhaled nicotine in humans, these data also have implications for the role of menthol flavors in other inhaled tobacco products.” As taken from Rosbrook K and Green BG. 2016. *Nicotine Tob. Res.* 18(7), 1588-95. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26783293>

“OBJECTIVE: The aim of the study was to enhance the transdermal delivery of diclofenac potassium (DP) from hydrogels by constant voltage iontophoresis (CVI). The other objective was to establish the safety and efficacy of CVI in rats. MATERIALS AND METHODS: Hydrogels of DP were developed using hydroxyethyl cellulose as matrix material and geraniol, l-menthol and thymol as iontophoretic efficiency enhancers (IEE). In vitro permeation of hydrogels under CVI (1.5 V) was performed in Franz diffusion cells across porcine skin. The ability of CVI to deliver therapeutic amount of DP in vivo was assessed in rat paw edema model. RESULTS: CVI significantly ($p < 0.05$) increased the steady state flux of DP compared to the passive. The hydrogels containing geraniol and l-menthol enhanced the iontophoretic flux of DP by ~4.75 and ~4.49 fold, respectively compared to passive control. The in vivo studies indicated that CVI in combination with IEE, significantly reduced ($p < 0.05$) area under the curve (AUC) of % inflammation compared to passive treatment. An excellent correlation ($r = 0.996$) was noted between in vitro flux values and AUC of % inflammation. CONCLUSION: The preclinical studies conclusively demonstrated that CVI in combination with IEE's such as geraniol or l-menthol has the potential to safely deliver therapeutic amounts of DP.” As taken from Arunkumar S et al. 2018. *Pharm. Dev. Technol.* 23(8), 806-814. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/28814142>

"Menthol, which creates mint flavor and scent, is often added to tobacco in both menthol and nonmenthol cigarettes. A potent tobacco carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), is extensively metabolized to its equally carcinogenic metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) as (R)- or (S)-NNAL enantiomers. NNAL is detoxified by UDP-glucuronosyltransferase (UGT) enzymes, with glucuronidation occurring on either NNAL's pyridine ring nitrogen (NNAL-N-Gluc) or the chiral alcohol [(R)- or (S)-NNAL-O-Gluc]. To characterize a potential effect by menthol on NNAL glucuronidation, in vitro menthol glucuronidation assays and menthol inhibition of NNAL-Gluc formation assays were performed. Additionally, NNAL and menthol glucuronides (MG) were measured in the urine of smokers (n = 100) from the Southern Community Cohort Study. UGTs 1A9, 1A10, 2A1, 2A2, 2A3, 2B4, 2B7, and 2B17 all exhibited glucuronidating activity against both l- and d-menthol. In human liver microsomes, both l- and d-menthol inhibited the formation of each NNAL-Gluc, with a stereospecific difference observed between the formation of (R)-NNAL-O-Gluc and (S)-NNAL-O-Gluc in the presence of d-menthol but not l-menthol. With the exception of three nonmenthol cigarette smokers, urinary MG was detected in all menthol and nonmenthol smokers, with l-MG comprising >98% of total urinary MG. Levels of urinary NNAL-N-Gluc were significantly ($P < 0.05$) lower among subjects with high levels of total urinary MG; no significant changes in free NNAL were observed. These data suggest that the presence of menthol could lead to increases in alternative, activating metabolic pathways of NNAL in tobacco target tissues, increasing the opportunity for NNAL to damage DNA and lead to the development of tobacco-related cancers. SIGNIFICANCE STATEMENT: High levels of the major menthol metabolite, menthol-glucuronide, was observed in the urine of smokers of either menthol or nonmenthol cigarettes. The fact that a significant inverse correlation was observed between the levels of urinary menthol-glucuronide and NNAL-N-glucuronide, a major detoxification metabolite of the tobacco carcinogen, NNK, suggests that menthol may inhibit clearance of this important tobacco carcinogen." As taken from Kozlovich S et al. 2019. Drug Metab. Dispos. 47(12), 1388-1396. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31578206/>

"Objective: To determine whether 3% w/w levomenthol added to ibuprofen gel (5% w/w) improves its efficacy compared with ibuprofen gel alone or diclofenac gel (1.16%) for the treatment of soft-tissue injuries. Methods: A total of 182 patients with acute soft-tissue injuries participated in a randomised, single-blind, single-dose study to assess the efficacy and safety of three topical analgesic gels. Efficacy was assessed as the score change in a numeric rating scale for pain. Results: The median time to significant pain relief was 20 minutes for the ibuprofen/levomenthol and diclofenac gels but 25 minutes for ibuprofen gel. At 2 hours, significantly more patients treated with ibuprofen/levomenthol gel reported a cooling sensation (45.8%) compared with diclofenac (16.4%) or ibuprofen (14.7%) gels, and both ibuprofen/levomenthol and diclofenac gels provided significantly more effective global pain relief compared with ibuprofen gel. Few adverse events and no serious adverse events related to study medication were recorded. Conclusions: Although all gels effectively relieved pain, both ibuprofen/levomenthol and diclofenac gels provided superior global pain relief compared with ibuprofen gel, with a shorter median time to significant pain relief. Only ibuprofen/levomenthol gel provided cooling for up to 2 hours. None of the gels were associated with serious safety concerns." As taken from Wade AG et al. 2019. J. Int. Med. Res. 47(9), 4454-4468. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31353997/>

"This study designed the transdermal formulations containing indomethacin (IMC)-1% IMC was crushed with 0.5% methylcellulose and 5% 2-hydroxypropyl- β -cyclodextrin by the bead mill method, and the milled IMC was gelled with or without 2% l-menthol (a permeation enhancer) by Carbopol® 934 (without menthol, N-IMC gel; with menthol, N-IMC/MT gel). In addition, the drug release, skin penetration and percutaneous absorption of the N-IMC/MT gel were investigated. The particle sizes of N-IMC gel were approximately 50-200 nm, and the combination with l-menthol did not affect the particle characterization of the transdermal formulations. In an in vitro experiment using a Franz diffusion cell, the skin penetration in N-IMC/MT gel was enhanced than the N-IMC gel, and the percutaneous absorption (AUC) from the N-IMC/MT gel was 2-fold higher than the N-

IMC gel. On the other hand, the skin penetration from the N-IMC/MT gel was remarkably attenuated at a 4 °C condition, a temperature that inhibits all energy-dependent endocytosis. In conclusion, this study designed transdermal formulations containing IMC solid nanoparticles and l-menthol, and found that the combination with l-menthol enhanced the skin penetration of the IMC solid nanoparticles. In addition, the energy-dependency of the skin penetration of IMC solid nanoparticles was demonstrated. These findings suggest the utility of a transdermal drug delivery system to provide the easy application of solid nanoparticles (SNPs).” As taken from Nagai N et al. 2019. *Int. J. Mol. Sci.* 20(15), 3644. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31349657/>

“Estradiol (E2) modulates the central and peripheral thermoregulatory responses to cold. Menthol is an agonist of transient receptor potential melastatin type 8 (TRPM8), which is a peripheral cold receptor. E2 suppresses menthol-induced elevation of body temperature (T_b) in ovariectomized rats, but the mechanism is unknown. The aim of the present study is to investigate the effect of E2 on uncoupling protein 1 (UCP1), a thermogenic gene, and TRPM8 mRNA levels in ovariectomized rats applied menthol. A silastic tube was implanted in ovariectomized rats with and without E2 underneath the dorsal skin (E2(+) and E2(-) groups), and data loggers for T_b measurement into peritoneal cavity. After application of 10% L-menthol or vehicle to the skin of the whole trunk of rats, T_b was measured for 2 h. The interscapular brown adipose tissue (BAT) and spinal ganglia of cervical, thoracic, and lumbar parts were obtained for RT-qPCR assay. In the menthol application, T_b in the E2(+) group was lower than that in the E2(-) group. The UCP1 mRNA in the BAT, TRPM8 mRNA in the BAT and spinal ganglia in all areas did not differ between the E2(+) and E2(-) groups. In conclusion, the UCP1 and TRPM8 expression in the brown fat did not affect the restriction of the menthol-induced hyperthermia by estradiol in ovariectomized rats.” As taken from Uchida Y et al. 2021. *J. Nutr. Sci. Vitaminol. (Tokyo)* 67(2), 130-134. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33952734/>

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
Cat	LD50	intraperitoneal	800mg/kg (800mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.
Cat	LD50	oral	800mg/kg (800mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.
Cat	LDLo	intravenous	34mg/kg (34mg/kg)	LUNGS, THORAX, OR RESPIRATION: RESPIRATORY DEPRESSION	Archives Internationales de Pharmacodynamie et de Therapie. Vol. 63, Pg. 43, 1939.
guinea pig	LDL	intraperitoneal	4gm/kg (4000mg/kg)		Archives Internationales de

	o		)		Pharmacodynami e et de Therapie. Vol. 63, Pg. 43, 1939.
mouse	LD5 0	intraperitoneal	6600mg/kg (6600mg/kg)		Farmatsevtichnii Zhurnal Vol. 17(3), Pg. 53, 1962. Link to PubMed
mouse	LD5 0	oral	3400mg/kg (3400mg/kg)		Quarterly Journal of Pharmacy & Pharmacology. Vol. 5, Pg. 233, 1932.
mouse	LD5 0	subcutaneous	5gm/kg (5000mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.
rabbit	LD5 0	skin	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 14, Pg. 471, 1976.
rabbit	LDL o	intraperitoneal	2gm/kg (2000mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.
rat	LD5 0	intraperitoneal	700mg/kg (700mg/kg)	BEHAVIORAL: GENERAL ANESTHETIC BEHAVIORAL: ALTERED SLEEP TIME (INCLUDING CHANGE IN RIGHTING REFLEX) LUNGS, THORAX, OR RESPIRATION: RESPIRATORY DEPRESSION	Journal of Pharmacy and Pharmacology. Vol. 35, Pg. 110, 1983. Link to PubMed
Rat	LD5 0	oral	3300mg/kg (3300mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.
rat	LD5 0	subcutaneous	1gm/kg (1000mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.

As taken from ChemIDplus, (CAS 2216-51-5). Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Human	10 mg/kg	Lungs, Thorax, or Respiration - other changes Nutritional and Gross Metabolic - other changes	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 86,262,2015
TDLo - Lowest published toxic dose	Administration onto the skin	Human	10 mg/kg	Lungs, Thorax, or Respiration - other changes Nutritional and Gross Metabolic - other changes Vascular - measurement of regional blood flow	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 86,262,2015

As taken from RTECS, 2017.

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
cat	LDLo	intraperitoneal	1500mg/kg (1500mg/kg)	BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD LUNGS, THORAX, OR RESPIRATION: OTHER CHANGES	Muenchener Medicinische Wochenschrift. Vol. 73, Pg. 2011, 1926.

				PERIPHERAL NERVE AND SENSATION: SPASTIC PARALYSIS WITH OR WITHOUT SENSORY CHANGE	
cat	LDLo	intravenous	37mg/kg (37mg/kg)		Archives Internationales de Pharmacodynamie et de Therapie. Vol. 63, Pg. 43, 1939.
cat	LDLo	oral	1500mg/kg (1500mg/kg)		"Abderalden's Handbuch der Biologischen Arbeitsmethoden." Vol. 4, Pg. 1289, 1935.
cat	LDLo	oral	1500mg/kg (1500mg/kg)		Muenchener Medicinische Wochenschrift. Vol. 73, Pg. 2011, 1926.
guinea pig	LD50	intraperitoneal	865mg/kg (865mg/kg)	BEHAVIORAL: SLEEP BEHAVIORAL: "HALLUCINATIONS, DISTORTED PERCEPTIONS" BEHAVIORAL: MUSCLE WEAKNESS	Annales Pharmaceutiques Francaises. Vol. 10, Pg. 481, 1952.
mouse	LD50	intraperitoneal	14200mg/kg (14200mg/kg)		Farmatsevtichnii Zhurnal Vol. 17(3), Pg. 53, 1962.
mouse	LD50	oral	3100mg/kg (3100mg/kg)		Quarterly Journal of Pharmacy & Pharmacology. Vol. 5, Pg. 233, 1932.

mouse	LDLo	intraperitoneal	1800mg/kg (1800mg/kg)		Archives Internationales de Pharmacodynamie et de Therapie. Vol. 63, Pg. 43, 1939.
mouse	LDLo	subcutaneous	14gm/kg (14000mg/kg)	BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD PERIPHERAL NERVE AND SENSATION: SPASTIC PARALYSIS WITH OR WITHOUT SENSORY CHANGE LUNGS, THORAX, OR RESPIRATION: OTHER CHANGES	Muenchener Medizinische Wochenschrift. Vol. 73, Pg. 2011, 1926.
rabbit	LD50	intraperitoneal	2gm/kg (2000mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.
rabbit	LD50	skin	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 14, Pg. 473, 1976.
rat	LD50	intramuscular	10gm/kg (10000mg/kg)		Naunyn-Schmiedeberg's Archiv fuer Experimentelle Pathologie und Pharmakologie. Vol. 222, Pg. 244, 1954.
rat	LD50	intraperitoneal	670mg/kg (670mg/kg)	BEHAVIORAL: MUSCLE WEAKNESS BEHAVIORAL:	Annales Pharmaceutiques Francaises. Vol. 10, Pg. 481, 1952.

				SLEEP BEHAVIORAL: "HALLUCINATION S, DISTORTED PERCEPTIONS"	
rat	LD50	oral	2900mg/kg (2900mg/kg)		FAO Nutrition Meetings Report Series. Vol. 44A, Pg. 58, 1967.
rat	LD50	oral	3180mg/kg (3180mg/kg)	BEHAVIORAL: ATAXIA	Food and Cosmetics Toxicology. Vol. 2, Pg. 327, 1964.
rat	LDLo	intraperitoneal	1500mg/kg (1500mg/kg)	BEHAVIORAL: MUSCLE WEAKNESS BEHAVIORAL: SLEEP BEHAVIORAL: "HALLUCINATION S, DISTORTED PERCEPTIONS"	Annales Pharmaceutiques Francaises. Vol. 10, Pg. 481, 1952.
rat	LDLo	subcutaneous	1gm/kg (1000mg/kg)		Muenchener Medicinische Wochenschrift. Vol. 73, Pg. 2011, 1926.

As taken from ChemIDplus (CAS RN 2216-51-5) available via PubChem

"A considerably lower LD50 of 940 mg/kg bw was observed in a single study with L-menthol in rats (FDA, 1975). In this study a severe irritation of the mucosal lining of the stomach and intestine was reported. Such effects have not been reported by the other investigators mentioned above."

"The human lethal dose is reported to be in the range of 50-500 mg/kg bw (FAO/WHO, 1999)."

"Ingestion of high menthol doses may cause abdominal pain, convulsions, nausea, vomiting, vertigo, ataxia, drowsiness and coma (Dukes, 1980; Gleason et al., 1969) After drinking of about 200 – 250 mg menthol/kg bw a child became drowsy, somnolent, felt pain in the stomach and vomited. The symptoms were fully reversible within 4 days (Leiber, 1967). Oral intake of 8000 to 9000 mg of menthol (unspecified isomer) by three volunteers (corresponding approx. 120 mg menthol/kg bw) led to a cold burning sensation in mouth, throat and esophagus, a cold sensation on the mucous membranes of the nose, on the skin of the hand and feet, and fatigue (Schwenkenbecher, 1908). About 20 mg menthol/kg bw led only to a mild abdominal discomfort (Bolund, et al., 1967)."

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

Two young children exposed to menthol, either via a peppermint liqueur or the oil itself, fell into a deep sleep, and later vomited (BIBRA, 1990).

In a study that established the intraperitoneal LD50 at 700 mg/kg bw, reported toxic effects included general anaesthetic (Wei & Seid, 1983).

“The usual human oral dose is 60-120 mg menthol per person. It can be estimated from unreferenced citations in pharmaceutical texts, such as Gleason et al. (1969), that the lethal human dose is 50-500 mg/kg bw. In section 2.1.1, the maximum doses tested were 180 mg (Kaffenberger & Doyle, 1990) and 1 g (Quick, 1928).”

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

“Menthol is a cyclic monoterpene alcohol which possesses well-known cooling characteristics and a residual minty smell of the oil remnants from which it was obtained. Because of these attributes it is one of the most important flavouring additives besides vanilla and citrus. Due to this reason it is used in a variety of consumer products ranging from confections such as chocolate and chewing gum to oral-care products such as toothpaste as well as in over-the-counter medicinal products for its cooling and biological effects. Its cooling effects are not exclusive to medicinal use. Approximately one quarter of the cigarettes on the market contain menthol and small amounts of menthol are even included in non-mentholated cigarettes. Natural menthol is isolated exclusively from *Mentha canadensis*, but can also be synthesised on industrial scale through various processes. Although menthol exists in eight stereoisomeric forms, (-)-menthol from the natural source and synthesised menthol with the same structure is the most preferred isomer. The demand for menthol is high and it was previously estimated that the worldwide use of menthol was 30-32,000 metric tonnes per annum. Menthol is not a predominant compound of the essential oils as it can only be found as a constituent of a limited number of aromatic plants. These plants are known to exhibit biological activity in vitro and in vivo such as antibacterial, antifungal, antipruritic, anticancer and analgesic effects, and are also an effective fumigant. In addition, menthol is one of the most effective terpenes used to enhance the dermal penetration of pharmaceuticals. This review summarises the chemical and biological properties of menthol and highlights its cooling effects and toxicity.” As taken from Kamatou GP et al. 2013. *Phytochemistry* 96, 15-25. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24054028>

Record for (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexanol (CAS RN 2216-51-5):

Species Scientific Name	Species Common Name	Exposure Duration Mean (Days)	Observed Duration Mean (Days)	Test Location	Exposure Type	Endpoint	Effect	Effect Measurement	Response Site	Conc 1 (Author)	Conc 1 Units (Author)
<i>Rattus norvegicus</i>	Norway Rat	1	1	Lab	Gavage	LOEL	Enzyme(s)	Cytochrome P-450	Liver	800	mg/kg bw/d

As taken from the US EPA ECOTOX database, 2021.

“We investigated the absorption and metabolism pharmacokinetics of a single L-menthol oral versus skin administration and the effects on human thermogenesis and metabolic rate. Twenty healthy adults were randomly distributed into oral (capsule) and skin (gel) groups and treated with 10 mg kg(-1) L-menthol (ORALMENT; SKINMENT) or control (lactose capsule: ORALCON; water application: SKINCON) in a random order on two different days. Levels of serum L-menthol

increased similarly in ORALMENT and SKINMENT ($p > 0.05$). L-menthol glucuronidation was greater in ORALMENT than SKINMENT ($p < 0.05$). Cutaneous vasoconstriction, rectal temperature and body heat storage showed greater increase following SKINMENT compared to ORALMENT and control conditions ($p < 0.05$). Metabolic rate increased from baseline by 18% in SKINMENT and 10% in ORALMENT and respiratory exchange ratio decreased more in ORALMENT (5.4%) than SKINMENT (4.8%) compared to control conditions ($p < 0.05$). Levels of plasma adiponectin and leptin as well as heart rate variability were similar to control following either treatment ($p > 0.05$). Participants reported no cold, shivering, discomfort, stress or skin irritation. We conclude that a single L-menthol skin administration increased thermogenesis and metabolic rate in humans. These effects are minor following L-menthol oral administration probably due to faster glucuronidation and greater blood menthol glucuronide levels." As taken from Valente A et al. 2015. Food Chem. Toxicol. 86, 262-73. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26429629>

5.2. Repeated dose toxicity

Liver and kidney damage was seen in workers in a factory in which the atmospheric levels ranged from 12-380 mg/m³ (BIBRA, 1990).

No overt toxicity was observed when rats received diets providing up to 200 mg/kg bw/day of 5.5 weeks. However, the same dose administered via stomach tube, for 28 days, caused liver cell changes and increased liver weights (BIBRA, 1990).

"In the investigation of Madhava Madyastha it was further found, that repeated oral administration of L-menthol for 3 days induced cytochrome P450 and the NADPH-cytochrome c reductase activity in the liver of rats by nearly 80%. Further treatment (for 7 days) reduced their levels considerably, although the levels were still higher than the control values."

"Female and male Wistar rats (10/sex/dose) received 200, 400 and 800 mg/kg bw/d L-menthol in soybean oil daily by gavage for 28 days. This study was performed mainly according to OECD TG 407. It is reported that liver weights were significantly increased in male rats at doses of 200, 400 and 800 mg/kg bw/d and also in female rats at doses of 400 and 800 mg/kg bw/d. Additionally, rats of all menthol treatment groups showed vacuolization of hepatocytes (4/20; 5/17, 4/19; no distinction between sexes), which was not seen in the control animals. This effect was not dose related and may reflect an adaptation (Thorup, 1983). Since no information is available as to the magnitude and the incidence of increased liver weights in the various exposure groups, the relevance of this finding is questionable and a NOAEL or a LOAEL cannot be deduced from this study."

"There is one detailed study from 1954 available with exposure to L-menthol by whole body vapour inhalation for 71 to 79 days with male and female Sherman rats (groups of 12). Although the authors attempted to measure the menthol concentrations in the gas phase, there was no adequate analytic method available. Therefore the exposure concentrations are given as weight of menthol vaporized divided by the volume of air circulated. The exposure concentrations were determined to be 0.087, 0.148 and 0.259 ppm (according to 0.57, 0.96 and 1.68 mg/m³). Besides this shortcoming, the study is of good design with numerous parameters investigated. Histopathological organ examinations showed toxic effects in the lungs only, ranging from tracheitis to severe congestion of the lungs at the highest dose, changes indicative of irritation (Rakieten et al., 1954). As the measurement of the exposure concentration does not seem reliable, this study cannot be used to derive a NOAEL. However, it points to the respiratory system as possible target organ after exposure by inhalation."

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

"Groups of six male mice were given (-)-menthol at doses of 2000, 2500, 3200, 4000, or 5000 mg/kg bw by gavage for five days and examined for 14 days. Gross necropsy of animals that died or were killed at termination revealed no abnormal finding. The LD⁵⁰ was calculated to be 2600 mg/kg bw (Litton Bionetics, Inc., 1975)."

"In groups of three to four adult male Wistar rats given 1% menthol in the diet for two weeks, increased serum cholesterol and serum triglycerides were observed, but there was no effect on apo A-1 lipids, an indicator of high-density lipoprotein status. Body weight and liver weight were unaffected (Imaizumi et al., 1985)."

"14-day subacute oral LD₅₀ in male ICR mice is 2652 mg/kg with 95% confidence limits of 1951 to 3218 mg/kg. Signs of toxicity and abnormal behavior included depression, excitability, rapid respiration and unthrifty appearance."

"Menthol is a common pharmaceutical, food and tobacco flavouring ingredient used for its minty characteristics and cooling effects. A 13-wk comparative nose-only smoke inhalation toxicity study was conducted using an American-style, cellulose acetate-filtered, non-menthol reference cigarette and a similarly blended test cigarette containing 5000 ppm synthetic l-menthol tobacco. Male and female Fischer 344 rats were exposed for 1 hr/day, 5 days/wk for 13 wk at target mainstream smoke particulate concentrations of 200, 600 or 1200 mg/m³, while control rats were exposed to filtered air. Internal dose biomarkers (blood carboxyhaemoglobin, serum nicotine and serum cotinine) indicated equivalent exposures were obtained for the two cigarettes. Effects typically noted in rats exposed to high levels of mainstream tobacco smoke were similar for both cigarette types and included reduced body weights (males slightly more affected than females), increased heart-to-body weight ratios and lung weights, and histopathological changes in the respiratory tract. Rats exposed to reference cigarette smoke displayed a dose-related increase in nasal discharge that was not observed in menthol smoke-exposed rats. All smoke-related effects diminished significantly during a 6-wk non-exposure recovery period. The results of this 13-wk smoke inhalation study indicated that the addition of 5000 ppm menthol to tobacco had no substantial effect on the character or extent of the biological responses normally associated with inhalation of mainstream cigarette smoke in rats." As taken from Gaworski CL et al. Food Chem Toxicol. 1997 Jul; 35(7):683-92 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=9301652&query_hl=2&itool=pubmed_docsum

"The US Food and Drug Administration (FDA) consider menthol an effective substance, which help in common cold symptoms and labeled to have low toxicity profile. Direct exposure to high menthol amount has been reported in animal; but no studies have been done to show the effect of menthol on long-term use in humans. Up to our knowledge we are reporting a rare case of chronic exposure to significant amount of menthol associated with cutaneous, gastrointestinal and neurological manifestations". As taken from Baibars M et al. 2012. Case Rep. Med. 2012, 187039. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23251165>

"Menthol is a cyclic monoterpene alcohol which possesses well-known cooling characteristics and a residual minty smell of the oil remnants from which it was obtained. Because of these attributes it is one of the most important flavouring additives besides vanilla and citrus. Due to this reason it is used in a variety of consumer products ranging from confections such as chocolate and chewing gum to oral-care products such as toothpaste as well as in over-the-counter medicinal products for its cooling and biological effects. Its cooling effects are not exclusive to medicinal use. Approximately one quarter of the cigarettes on the market contain menthol and small amounts of menthol are even included in non-mentholated cigarettes. Natural menthol is isolated exclusively from *Mentha canadensis*, but can also be synthesised on industrial scale through various processes. Although menthol exists in eight stereoisomeric forms, (-)-menthol from the natural source and synthesised menthol with the same structure is the most preferred isomer. The demand for menthol is high and it was previously estimated that the worldwide use of menthol was 30-32,000 metric tonnes per annum. Menthol is not a predominant compound of the essential oils as it

can only be found as a constituent of a limited number of aromatic plants. These plants are known to exhibit biological activity in vitro and in vivo such as antibacterial, antifungal, antipruritic, anticancer and analgesic effects, and are also an effective fumigant. In addition, menthol is one of the most effective terpenes used to enhance the dermal penetration of pharmaceuticals. This review summarises the chemical and biological properties of menthol and highlights its cooling effects and toxicity.” As taken from Kamatou GP et al. 2013. Phytochemistry 96, 15-25. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24054028>

Record for (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexanol (CAS RN 2216-51-5):

Species Scientific Name	Species Common Name	Exposure Duration Mean (Days)	Observed Duration Mean (Days)	Test Location	Exposure Type	Endpoint	Effect	Effect Measurement	Response Site	Conc 1 (Author)	Conc 1 Units (Author)
Rattus norvegicus	Norway Rat	28	28	Lab	Gavage	LOEL	Morphology	Organ weight in relationship to body weight	Liver	200	mg/kg bwtd/d
Rattus norvegicus	Norway Rat	28	28	Lab	Gavage	LOEL	Morphology	Organ weight in relationship to body weight	Liver	400	mg/kg bwtd/d
Rattus norvegicus	Norway Rat	28	28	Lab	Gavage	LOEL	Feeding behavior	Water consumption	Not reported	800	mg/kg bwtd/d
Rattus norvegicus	Norway Rat	28	28	Lab	Gavage	NOEL	Morphology	Organ weight in relationship to body weight	Liver	200	mg/kg bwtd/d
Rattus norvegicus	Norway Rat	28	28	Lab	Gavage	NOEL	Feeding behavior	Water consumption	Not reported	400	mg/kg bwtd/d
Rattus norvegicus	Norway Rat	28	28	Lab	Gavage	NOEL	Growth	Weight	Whole organism	800	mg/kg bwtd/d
Rattus norvegicus	Norway Rat	28	28	Lab	Gavage	NOEL	Growth	Weight	Whole organism	800	mg/kg bwtd/d

As taken from the US EPA ECOTOX database, 2021.

"A single exposure to menthol can, depending on concentration, enhance both cool sensations and encourage body heat storage. This study tested whether there is an habituation in either response after repeated-daily exposures. Twenty-two participants were assigned to one of three spray groups: Control (CON; n=6), 0.05% L-menthol (M(0.05%); n=8), and 0.2% L-menthol (M(0.2%); n=8). On Monday (20°C, 50% rh) participants were sprayed with 100 mL of solution and undertook 40 min of cycling at 45% of their peak power (Ex1), from Tuesday to Thursday (30°C, 50% rh) they were sprayed twice daily whilst resting (R1 to R6), Friday was a repeat of Monday (Ex2). Thermal sensation (TS), thermal comfort, perceived exertion, irritation, rectal and skin temperature (Tsk), skin blood flow (SkBF) and sweat rate were monitored. A two-way ANOVA ($\alpha=0.05$) compared responses from the beginning (Ex1, R1) and end (Ex2, R5) of the testing week. M(0.2%) induced significantly ($P<0.05$) cooler TS at the beginning of the week (Ex1, R1) compared to the end (Ex2, R5), indicating habituation of TS; this was not observed in M(0.05%). No other perceptual or physiological responses habituated. 0.2% Menthol caused a heat storage response, mediated by vasoconstriction, at the beginning and end of the week, suggesting the habituation of TS occurred in a pathway specific to sensation. In summary, the cooling influence of 0.2% menthol habituates after repeated-daily exposures, but with no habituation in heat storage." As taken from Gillis DJ et al. 2015. *Physiol. Behav.* 139, 511-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25484357>

There are sufficient repeated dose toxicity data on menthol. In an OECD 407/GLP repeated dose toxicity study, groups of 10 rats/sex/dose were administered *l*-menthol (CAS # 2216- 51-5) via gavage at doses of 0 (soybean oil), 200, 400, and 800 mg/ kg/day. There was an increase in absolute and relative liver weight among all of the treated males and females at concentrations ≥ 400 mg/ kg/day as compared to the controls. Histopathological examination revealed vacuolation of the hepatocytes among the treated animals; however, there was no dose-response pattern. The report did not mention the magnitude of liver weight increases among treated animals; hence, the significance of liver weight alterations could not be determined. The OECD (2003) cites an unpublished report submitted to JECFA that states "no adverse effects on weight gain, excretion of glucuronides, water, or electrolytes, or interference with central nervous system reactions to stimulants were observed when groups of 40 rats of each sex were fed ()-or ($\pm$)-menthol in the diet for 5.5 weeks at doses of 0, 100, or 200 mg/kg per day." Based on these observations, the OECD SIDS dossier authors concluded that a NOAEL of 200 mg/kg/day could be determined since no effects on the liver were observed during a longer duration dietary study on *l*-menthol (Thorup et al., 1983).

ministered via diet to groups of 10 B6C3F1 mice/sex/dose at concentrations of 0, 930, 1870, 7500, and 15000 ppm. The study was conducted to determine the dietary concentrations for a following 2- year carcinogenicity study. Mortality was reported among the treated animals; however, this was not due to test material administration. There was a decrease in bodyweight gain among the high-dose females as compared to the controls. There were reports of increases in the incidences of perivascular lymphoid hyperplasia and interstitial nephritis among the female mice in the 2 high-dose groups. Thus, the 2 concentrations selected for the chronic 2-year study were 2000 and 4000 ppm.

A subsequent 2-year carcinogenicity study was conducted on *d,l*-menthol in 2% corn oil administered via diet to B6C3F1 mice (50/sex/ dose) at concentrations of 0, 2000, or 4000 ppm for 103 weeks followed by a 1-week treatment-free period. There was a significant decrease in survival among the high-dose females; however, there were no reports of test material-related tumors observed among the treated animals. Thus, under the conditions of this study, *d,l*-menthol was concluded to be non-carcinogenic for B6C3F1 mice. The NOAEL in mice was considered to be 2000 ppm (equivalent to 300 mg/kg/day, as per the conversion factors for mice, available in the JECFA guidelines for the preparation of toxicological working papers on food additives), based on decreased survival among the high-dose females (National Cancer Institute, 1979).

administered test material, *d,l*-menthol (CAS # 1490-04-6), via diet in 2% corn oil for 13 weeks at concentrations of 0, 930, 1870, 7500, and 15000 ppm. The study was conducted to determine the dietary concentrations for a subsequent 2-year carcinogenicity study. There were incidences of

interstitial nephritis reported among the high-dose males. There were no other treatment-related alterations reported during the 13-week treatment. Based on these results, the concentrations for the chronic 2-year study were determined to be 3700 and 7500 ppm. *d,l*-Menthol in 2% corn oil was administered via diet to Fischer 344 (50/ sex/dose) at concentrations of 3700 and 7500 ppm. There were no significant differences in survival rates among the treated animals. Based on the histopathologic examination, *d,l*-menthol was neither toxic nor carcinogenic to Fischer 344 rats under the conditions of this study. Thus, the NOAEL was considered to be 7500 ppm or 750 mg/kg/day (using conversion factors for rats, available in the JECFA guidelines for the preparation of toxicological working papers on food additives), the highest dose tested (National Cancer Institute, 1979).

The most conservative NOAEL of 300 mg/kg/day from the long-term 2-year carcinogenicity study in mice was considered for the repeated dose toxicity endpoint. **Therefore, the menthol MOE for the repeated dose toxicity endpoint can be calculated by dividing the *d,l*-menthol NOAEL in mg/kg/day by the total systemic exposure to menthol, 300/0.035 or 8571.**

As taken from Api AM et al. (2022)

5.3. Reproduction toxicity

"2.18, 10.15, 47.05 and 218.0 mg/kg bw/d were administered by gavage to Wistar rats from gestation day 6 to 15. There was no effect on maternal and fetal survival and the number of abnormalities in soft or skeletal tissues observed did not differ from sham treated control. No clinical signs of maternal toxicity were observed. Therefore the NOEL derived for maternal and fetal toxicity and teratogenicity in rats can be determined as 218.0 mg/kg bw/d."

"Doses of 1.85, 8.59, 39.9 and 185.0 mg/kg bw/d were administered to CD-1 mice from gestation day 6 to 15. No effect on maternal and fetal survival and no dose-related increase in the number of abnormalities in soft or skeletal tissues was observed. No clinical signs of maternal toxicity were observed. The NOEL derived for maternal and fetal toxicity and teratogenicity was 185.0 mg/kg bw/d."

"Rabbits were administered menthol from gestation day 6 to 18 in following doses: 4.25, 19.75, 91.7 and 425.0 mg/kg bw/d. Few of the rabbits died or aborted before day 29 (4.25/2 out of 13 animals, 19.75/3 out of 12 animals, 91.7/1 out of 11 animals, 425.0/4 out of 14 animals), however, these effects were not dose related and are not considered to be a consequence of test substance administration. Also in rabbits no effect on maternal and fetal survival and no dose-related increases in the number of abnormalities in soft or skeletal tissues were observed. No clinical signs of maternal toxicity were observed. The NOEL derived for maternal and fetal toxicity and teratogenicity was therefore 425.0 mg/kg bw/d."

"4.05, 21.15, 98.2 and 405.0 mg/kg bw/d were administered to Syrian hamsters during gestation days 6-10. No effect on maternal and fetal survival and no dose-related number of abnormalities in soft or skeletal tissues were observed. The NOEL for maternal and fetal toxicity and teratogenicity was therefore 405.0 mg/kg bw/d."

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

"(-)-Menthol was administered in corn oil by gavage to 22 or 23 pregnant CD-1 mice at 0, 1.9, 8.6, 40, or 190 mg/kg bw per day on days 6-15 of gestation; to 22 or 23 pregnant Wistar rats at 0, 2.2, 10, 47, or 220 mg/kg bw per day on days 6-15 of gestation; to 21-23 golden hamsters at 0, 4.1, 21, 98, or 400 mg/kg bw per day on days 6-10 of gestation; and to 11 to 14 Dutch-belt rabbits at 0, 4.3, 20, 92, or 430 mg/kg bw per day on days 6-18 of gestation. Control groups for each species were sham treated; positive control groups for each species were given 150 or 250 mg/kg bw per day aspirin. Body weights were recorded on three or four days during the gestation period. All animals were observed daily for appearance, behaviour, and food consumption. On the scheduled day, the fetuses were removed from all dams and dams and fetuses examined. One-third of the fetuses

from each group underwent detailed visceral examination; the other two-thirds were examined for skeletal defects. There were no effects on nidation, maternal survival, fetal survival, or fetal abnormalities. The numbers of abnormalities seen in soft or skeletal tissues of treated animals did not differ from those occurring spontaneously in the sham-treated controls (Food and Drug Research Labs, Inc., 1973)."

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

There are sufficient developmental toxicity data on menthol. Menthol (CAS # 89-78-1) has gavage developmental toxicity studies conducted in mice, rats, hamsters, and rabbits. Groups of 22–23 pregnant albino CD-1 mice/dose group were administered menthol in corn oil via gavage at doses of 0, 1.85, 8.59, 39.9, and 185 mg/kg/day from day 6 through day 15 of gestation. There were no effects on implantation, maternal or fetal survival among treated animals as compared to the control group up to the highest dose tested (FDA, 1973). The NOEL for maternal and developmental toxicity was considered to be 185 mg/kg/day. In another study, groups of 22–25 pregnant Wistar rats/dose group were administered menthol in corn oil via gavage at doses of 0, 2.18, 10.15, 47.05, and 218 mg/kg/day from day 6 through day 15 of gestation. Menthol produced no effects among the treated animals when compared to the control group up to the highest dose tested. The NOEL for maternal and developmental toxicity was considered to be 218 mg/kg/day (FDA, 1973). In another study, groups of 21–23 pregnant Syrian hamsters/dose group were administered menthol in corn oil via gavage at doses of 0, 4.05, 21.15, 98.2, and 405 mg/kg/day from day 6 through day 10 of gestation. Menthol produced no effects among the treated animals when compared to the control group up to the highest dose tested. The NOEL for maternal and developmental toxicity was considered to be 405 mg/kg/day (FDA, 1973). In another study, groups of 11–14 pregnant rabbits/dose group were administered menthol in corn oil via gavage at doses of 0, 4.25, 19.75, 91.7, and 425 mg/kg/day from day 6 through day 18 of gestation. Mortality was reported among the treated and control animals; however, there were no dose responses and no alterations in clinical signs reported; hence, this finding was not considered to be treatment-related. In addition, no effect on maternal and fetal survival and no dose-related increases in the number of abnormalities in soft or skeletal tissues were observed up to the highest dose tested. Thus, the NOAEL for maternal and developmental toxicity was considered to be 425 mg/kg/day, the highest dosage tested (FDA, 1973k). The NOAEL for developmental toxicity was determined to be 425 mg/kg/day, the highest dosage tested among the treated rabbits (FDA, 1973).

Further, an OECD 443/GLP Extended One-Generation Reproductive Toxicity (EOGRT) study with F2 generation extension was conducted in Sprague Dawley rats, groups of 25 rats/sex/dose were administered *d,l*-menthol (CAS # 1490-04-6), in diet at concentrations 0, 4000, 8000 or 16000 ppm (equivalent to 203–247, 419–499, and 837–1016 mg/kg/day in males and 229–300, 455–594 and 892–1205 mg/kg/day). Male rats received *d,l*-menthol orally via the diet for 10 weeks before pairing and until termination. Female rats received *d,l*-menthol orally via the diet for 10 weeks before pairing, throughout pairing and gestation, and during lactation. In the F1 generation, 40 males and 40 females were treated from weaning to their scheduled termination (relevant to each cohort). There was no treatment-related mortality observed in F0 and F1 generations. In F1 and F2 generations, offspring growth was reduced from day 4 of age to weaning in the high-dose litters (absolute body weight on day 21 of age being 17–18% lower than control). The extent of the reduction in offspring growth in the high-dose group in both generations was considered to be adverse. There was no adverse effect on F1 or F2 offspring birth weight, ano-genital distance, sex ratio, survival from day 1 of age to weaning. Thus, the NOAEL for developmental toxicity was considered to be 8000 ppm (equivalent to 419 mg/kg/day) based on reduced offspring weight in F1 and F2 generation at the highest dose (ECHA, 2011b).

The NOAEL for OECD 443 (419 mg/kg/day) was considered for the safety assessment. Therefore, the menthol MOE for the developmental toxicity endpoint can be calculated by dividing the menthol NOAEL in mg/kg/day by the total systemic exposure to menthol, 419/0.035, or 11971.

There are sufficient fertility data on menthol. A dietary 13-week study was conducted where test material, *d,l*-menthol (CAS # 1490- 04-6; isomer unspecified), was administered to groups of 10 B6C3F1 mice/sex/dose at dietary concentrations of 0, 930, 1870, 7500, and 15000 ppm. There were no changes observed in the histopathological examination of testes, prostate, uterus, ovaries, mammary glands, and adrenals in the treated mice at any of the doses administered. In a following 2-year carcinogenicity study, no changes in reproductive organs (testes, prostate, uterus, ovaries, mammary gland, and adrenals) were observed in histopathological examinations at concentrations of 2000 or 4000 ppm (National Cancer Institute, 1979). Another dietary 13-week study was conducted, where the test material *d,l*-menthol (CAS # 1490-04-6; isomer unspecified) was administered to groups of 10 Fischer 344 rats/sex/dose at dietary concentrations of 0, 930, 1870, 7500, and 15000 ppm. There were no changes observed in the histopathological examination of testes, prostate, uterus, ovaries, mammary glands, and adrenals in the treated mice at any of the doses administered. In a following 2-year carcinogenicity study, no changes in reproductive organs (testes, prostate, uterus, ovaries, mammary gland, and adrenals) were observed in histopathological examinations at concentrations of 3700 and 7500 ppm (National Cancer Institute, 1979). However, since there were no sperm analysis or estrous cycling parameters reported in any of the studies conducted, a NOAEL for the reproductive toxicity endpoint could not be determined.

Further, an OECD 443/GLP EOGRT study with F2 generation extension was conducted in Sprague Dawley rats, groups of 25 rats/sex/ dose were administered *d,l*-menthol (CAS # 1490-04-6), in diet at concentrations 0, 4000, 8000, or 16000 ppm (equivalent to 203–247, 419–499, and 837–1016 mg/kg/day in males and 229–300, 455–594 and 892–1205 mg/kg/day). No test material-related changes in general clinical condition, and estrous cycle regularity pre-coital interval, mating performance, conception rate, and fertility index were observed. Similarly, there was no treatment-related effect on the hematological parameters in F0 and F1 animals or sperm motility/counts/morphology of the F0 and F1 males. The macroscopic examination did not reveal any treatment-related abnormalities. There was no adverse effect on F1 or F2 offspring birth weight, ano-genital distance, sex ratio, survival from day 1 of age to weaning. When compared to controls, for F1 litters in the high-dose group, the mean number of uterine implantation sites was statistically significantly low, resulting in a lower total number of offspring born and subsequently live offspring on Day 1 and Day 4 of age. In addition, mean values of litter size in the high-dose F1 and F2 litters were lower than all other study groups in both generations. The lower number of uterine implantation sites and subsequently small litter size in the high-dose group in both F0 and F1 generations were considered treatment-related and adverse (ECHA, 2011b).

The NOAEL for fertility was considered to be 8000 ppm (419 mg/kg/ day), based on the lower number of uterine implantation sites and subsequently small litter size in the high-dose group in both F0 and F1 generations.

Therefore, the menthol MOE for the fertility endpoint can be calculated by dividing the menthol NOAEL in mg/kg/day by the total systemic exposure to menthol, 419/0.035, or 11971.

As taken from API AM et al. (2022)

5.4. Mutagenicity

Type of Test	Route of Exposure	Species Observed	Dose Data	Reference
DNA repair		Bacteria - Bacillus subtilis	10 mg/disc	OIGZDE Osaka-shi Igakkai Zasshi. Journal of Osaka City Medical Association. (Osaka-shi Igakkai, c/o Osaka-shiritsu Daigaku Igakubu, 1-4-54 Asahi-cho, Abeno-ku, Osaka, 545, Japan) V.24- 1975- Volume(issue)/page/year: 34,267,1985

As taken from RTECS, 2017.

Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM

Strain Indicator:	TA100
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537

Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254

Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	6.4-800 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[ANDERSEN,PH AND JENSEN,NJ; MUTAGENIC INVESTIGATION OF PEPPERMINT OIL IN THE SALMONELLA/MAMMALIAN-MICROSOME TEST; MUTAT. RES. 138(1):17-20, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.001-0.1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)

Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.001-0.1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.001-0.1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU

	EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.001-0.1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	100-600 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO.MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]
Test System:	AMES SALMONELLA TYPHIMURIUM

Strain Indicator:	TA98
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	100-600 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO,MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	RAT LIVER S-9, AROCLOR 1254
Method:	STANDARD PLATE
Dose:	200-700 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO,MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98

Metabolic Activation:	RAT LIVER S-9, AROCLOR 1254
Method:	STANDARD PLATE
Dose:	200-800 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO,MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97A
Metabolic Activation:	RAT LIVER S-9, AROCLOR 1254
Method:	STANDARD PLATE
Dose:	200-800 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO,MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97A
Metabolic Activation:	NONE

Method:	STANDARD PLATE
Dose:	100-700 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO,MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	5-400 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO,MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	RAT LIVER S-9, AROCLOR 1254
Method:	STANDARD PLATE

Dose:	50-500 UG/PLATE (TEST MATERIAL SOLVENT:ETHANOL)
Results:	NEGATIVE
Reference:	[GOMES-CARNEIRO,MR, FELZENSZWALB,I AND PAUMGARTTEN,FJR; MUTAGENICITY TESTING OF (+/-)-CAMPHOR, 1,8-CINEOLE, CITRAL, CITRONELLAL, (-)-MENTHOL AND TERPINEOL WITH THE SALMONELLA/MICROSOME ASSAY; MUTAT. RES. 416(1,2):129-136, 1998]

As taken from CCRIS, 2001.

"L-Menthol was not mutagenic in Ames tests using the tester strains *S. typhimurium* TA 97a, TA 98, TA 100, TA 102, TA 1535, TA 1537, and TA 2637 with and without metabolic activation (Nohmi, et al., 1985, Andersen and Jensen, 1984, Gomes-Carneiro, et al., 1998). The tests were performed also at cytotoxic concentrations (800 µg/plate, Gomes-Carneiro et al., 1998). A reverse mutation assay with *E. coli* WP2 *uvrA* (*trp*-) was negative in concentrations up to 0.8 mg/plate; a recombination assay with *Bacillus subtilis* M 45 and H 17 gave positive results in doses up to 10 mg/disk (Yoo, 1986)"

"In a detailed study of Murthy et al. (1991) peripheral lymphocytes of 24 human donors were treated in vitro with L-menthol in the absence and in the presence of rat S9-mix. The concentration range tested was 0.1 to 10 mM L-menthol. The authors investigated chromosomal aberrations (in at least 100 cells per donor) and sister chromatid exchanges (in at least 25 second division metaphases per donor). In both test systems L-menthol did not induce chromosomal damage. In a further cytogenetic assay the anaphase chromosome aberrations of human fibroblasts were investigated. The concentration range tested was 0.1 to 10 µg/ml. There was no indication for chromosomal aberrations induced by L-menthol (FDA, 1975)."

"Chromosomal aberration tests with Chinese hamster cells (CHL) tested in concentrations of 0.1 to 0.3 mg/ml L-menthol (Sofuni et al., 1985; Matsuoka et al., 1998) were negative with and without metabolic activation."

"L-menthol did not lead to an increased rate of chromosomal aberrations in the bone marrow of rats at oral doses up to 3000 mg/kg bw (single dose; sacrifice 6, 24 and 48 h after treatment) and 1150 mg/kg bw/d (5 applications; sacrifice 6 h after last dose). Besides the restriction that only 50 metaphases have been investigated per animal, the experiments were performed in accordance with current standards. L-menthol was not mutagenic in a dominant lethal test in rats (FDA, 1975) with doses up to 3000 mg/kg bw (single dose; providing 14 to 20 pregnant females per mating group) and 1150 mg/kg bw/d (5 applications; providing 13 to 19 pregnant females per mating group)."

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

In vivo Genotoxicity

When administered by stomach tube, l-menthol at doses up to 145 mg/kg bw/ day for up to five days, did not affect bone marrow chromosomes (BIBRA, 1990).

"Menthol was administered at 725 mg/kg bw or the maximum tolerated dose of 1450 mg/kg bw to male Fischer 344 rats and male B6C3F1 mice. Hepatocytes were removed at 24, 39, and 49 h, and replicative DNA synthesis was measured. Synthesis was increased in 6% of the rats and 1.7% of the mice. This assay indicates cell replication (i.e. mitogenesis), however, and not genotoxicity (Uno et al., 1994; Yoshikawa, 1996)."

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

GENETIC TOXICITY 'IN VIVO'

Type:	Cytogenetic assay
Species:	rat
Sex:	male
Strain:	other: albino rats
Route of admin.:	gavage
Exposure period:	acute
Doses:	1.45, 14.5, 145.0 mg/kg and 500, 3000 mg/kg (second test)
Result:	negative
Method:	other
Year:	1975
GLP:	no
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	Treated animals showed no increased number of aberrations (max. 0.8 %) compared to control animals (max. 0.66 %). Positive control animals showed 22.8 - 37 % aberrant metaphases.
Test condition:	Analysis of chromosome aberrations in bone marrow Age: 10-12 weeks Groups: Animals were killed 6 hours, 24 hours and 48 hours after treatment respectively Number of animals/group: 5 number of negative control animals/group: 3 Number of positive control animals: 5 animals killed after 48 hours No. of metaphases investigated/animal: 50 Negative control: Vehicle (saline) Positive control: 0.30 mg/kg Triethylene melamine injected intraperitoneally Colcemid injection: 4 mg/kg administered 2 hrs prior killing.

Reliability:	(2) valid with restrictions. Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: only 50 metaphases per animal investigated.
Flag:	Critical study for SIDS endpoint 10.07.2002 (39)
Type:	Cytogenetic assay
Species:	Rat
Sex:	Male
Strain:	other: albino rats
Route of admin.:	Gavage
Exposure period:	5 days
Doses:	1.45, 14.5, 145.0 mg/kg and 1150 mg/kg (second test)
Result:	Negative
Method:	other
Year:	1975
GLP:	No
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	Treated animals showed a comparable number of aberrations (max. 0.8 %) to that of control animals (0 %).
Test condition:	Analysis of chromosome aberrations in bone marrow Age: 10-12 weeks Number of animals/dose group: 5 Number of negative control animals: 3 No. of metaphases investigated/animal: 50

Negative control:	vehicle (saline) Exposure: Five doses 24 hours apart, animals killed 6 hours after last dose.
Colcemid injection:	4 mg/kg administered 2 hrs prior killing No positive control animals.
Reliability:	(2) valid with restrictions. Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: only 50 metaphases per animal investigated.
Flag:	Critical study for SIDS endpoint 10.07.2002 (39)
Type:	Dominant lethal assay
Species:	rat
Sex:	male
Strain:	no data
Route of admin.:	gavage
Exposure period:	acute
Doses:	1.45, 14.5, 145.0 mg/kg and 500, 3000 mg/kg (second test)
Result:	negative
Method:	other
Year:	1975
GLP:	no
Test substance:	other TS: Menthol natural brazilian, FDA 71-57

Result:	Positive control showed strong effects on implantation, fertility, number of dead implants etc. The values calculated for menthol did not significantly vary from those of the negative control.
Test condition:	Following treatment 10 males/dose were mated with 2 females/week for 8 weeks - resulting in 14 to 20 pregnant females per dose, negative and positive control per mating interval. females were killed 14 days after separation from males Positive control: triethylene melamine (TEM 0.3 mg/kg, i.p.) Negative control: saline Fertility index, preimplantation loss and lethal effects on the embryos were determined and compared to those calculated from negative (saline dosed) and positive (TEM-dosed) control animals.
Statistics: fertility index:	chi-square test number of implants: t-test number of corpora lutea: t-test preimplantation lossess: Freeman-Tukey transformation and t-test
Reliability:	(2) valid with restrictions Limited documentation
Flag:	Critical study for SIDS endpoint 25.02.2003 (39)
Type:	Dominant lethal assay
Species:	rat
Sex:	male
Strain:	no data
Route of admin.:	gavage
Exposure period:	5 days
Doses:	1.45, 14.5, 145.0 mg/kg and 1150 mg/kg (second test)
Result:	negative
Method:	other
Year:	1975

GLP:	no
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	The values calculated for menthol did not significantly vary from those of the negative control.
Test condition:	Subacute study. Following treatment 10 males/dose were mated with 2 females/week for 7 weeks - resulting in 13 to 19 pregnant females per dose, negative and positive control per mating interval. females were killed 14 days after separation from males Positive control: triethylene melamine (TEM 0.3 mg/kg, i.p.) Negative control: saline Fertility index, preimplantation loss and lethal effects on the embryos were determined and compared to those calculated from negative (saline dosed) and positive (TEM-dosed) control animals.
Statistics:	fertility index: chi-square test, number of implants: t-test, number of corpora lutea: t-test, preimplantation lossess: Freeman-Tukey transformation and t-test.
Reliability:	(2) valid with restrictions. Limited documentation.
Flag:	Critical study for SIDS endpoint. 25.02.2003 (39)
Type:	other: Host mediated Assay
Species:	other: mouse (indicator organism: Salmonella typhimurium G-46)
Sex:	male
Strain:	ICR
Route of admin.:	gavage
Exposure period:	acute
Doses:	1.45, 14.5, 145.0 (first test) and 500, 5000 mg/kg bw (second test)
Result:	negative

Method:	other
Year:	1975
GLP:	no
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	The corresponding in vitro tests gave negative results.
Test condition:	Acute Ex-vivo study: Number of animals/dose level: 10 Indicator Organisms (reverse mutation): Salmonella typhimurium (his G-46) Negative control: solvent Positive control: dimethyl nitrosamine (only at 100 mg/kg) Injection of indicator organisms in mice: 2.0 ml (each ml contained 3.0×10^8 cells) intraperitoneally Three hours later mice were killed, Salmonella species were collected and the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample
Reliability:	(2) valid with restrictions Limited documentation 25.02.2003 (39)
Type:	other: Host mediated assay
Species:	other: mouse (indicator organism: Salmonella typhimurium TA 1530)
Sex:	Male
Strain:	ICR
Route of admin.:	Gavage
Exposure	Acute

period:	
Doses:	1.45, 14.5, 145.0 (first test) and 500, 5000 mg/kg bw (second test)
Result:	Ambiguous
Method:	other
Year:	1975
GLP:	No
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	Positive effects occurred only at intermediate dose levels with no dose response (significant increased mutant frequency at 145.0 mg/kg bw; 500 and 5000 mg/kg were negative). Effects can therefore be considered as non-relevant. The corresponding in vitro test gave negative results.
Test condition:	Acute Ex-vivo study: Number of animals/dose level: 10 Indicator Organisms (reverse mutation): Salmonella typhimurium (TA-1530) Negative control: solvent Positive control: dimethyl nitrosamine (only at 100 mg/kg) Injection of indicator organisms in mice: 2.0 ml (each ml contained 3.0×10^8 cells) intraperitoneally Three hours later mice were killed, Salmonella species were collected and the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample
Reliability:	(2) valid with restrictions Limited documentation 25.02.2003 (39)
Type:	other: Host mediated assay

Species:	other: mouse (indicator organism: Salmonella typhimurium G-46)
Sex:	Male
Strain:	ICR
Route of admin.:	Gavage
Exposure period:	5 days
Doses:	1.45, 14.5, 145.0, mg/kg (first test) and 1150 mg/kg (second test)
Result:	Negative
Method:	other
Year:	1975
GLP:	No
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	The in vitro test results were negative as well.
Test condition:	Subacute Ex-vivo study: Number of animals/dose level: 10 Dosages: Aute dosage was given once a day. Indicator Organisms (reverse mutation): Salmonella typhimurium (his G-46) Negative control: solvent Positive control: dimethyl nitrosamine (only at 100 mg/kg) Injection of indicator organisms in mice: Within 30 minutes after the last dosage each animal was given 2.0 ml (each ml contained 3.0×10^8 cells) intraperitoneally Three hours later mice were killed, Salmonella species were collected and the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample

Reliability:	(2) valid with restrictions Limited documentation 25.02.2003 (39)
Type:	other: Host mediated assay
Species:	other: mouse (indicator organism: <i>Saccharomyces cerevisiae</i> (D-3))
Sex:	Male
Strain:	ICR
Route of admin.:	Gavage
Exposure period:	Acute
Doses:	1.45, 14.5, 145.0 mg/kg (first test) 500, 5000 mg/kg (second test)
Result:	Negative
Method:	other
Year:	1975
GLP:	No
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	The recombinant frequency in the in vitro test with D3 was slightly elevated.
Test condition:	Acute Ex-vivo study: Number of animals/dose level: 10 Indicator Organisms (mitotic recombination): <i>Saccharomyces cerevisiae</i> (D-3)

	Negative control: solvent Positive control: ethyl methane sulfonate (intramuscularly injected at a dose of 350 mg/kg) Injection of indicator organisms in mice: 2.0 ml (each ml contained 5.0×10^8 cells) intraperitoneally. Three hours later mice were killed, Yeast species were collected and the number of recombinants was counted. Recombinant frequency (RF): total recombinants counted/total number colonies screened
Reliability:	(2) valid with restrictions Limited documentation 25.02.2003 (39)
Type:	other: Host mediated assay
Species:	other: mouse (indicator organism: <i>Saccharomyces cerevisiae</i> (D-3))
Sex:	Male
Strain:	ICR
Route of admin.:	Gavage
Exposure period:	5 days
Doses:	1.45, 14.5, 145 (first test), 1150 mg/kg (second test)
Result:	Ambiguous
Method:	other
Year:	1975
GLP:	No
Test substance:	other TS: Menthol natural brazilian, FDA 71-57

Result:	Slightly enhanced recombinant frequencies in all subacute dose levels. In a second test with a single subacute dose level of 1150 mg/kg the test result was negative. The recombinant frequency in the in vitro test with D3 was slightly elevated too.
Test condition:	Subacute Ex-vivo study: Number of animals/dose level: 10 Indicator Organisms (mitotic recombination): <i>Saccharomyces cerevisiae</i> (D-3) Negative control: solvent Positive control: ethyl methane sulfonate (intramuscularly injected at a dose of 350 mg/kg) Injection of indicator organisms in mice: Within 30 minutes after last dosage mice were given 2.0 ml (each ml contained 5.0×10^8 cells) intraperitoneally. Three hours later mice were killed, Yeast species were collected and the number of recombinants was counted. Recombinant frequency (RF): total recombinants counted/total number colonies screened
Reliability:	(2) valid with restrictions Limited documentation 25.02.2003 (39)
Type:	other: Host mediated assay
Species:	other: mouse (indicator organism: <i>Salmonella typhimurium</i> TA 1530)
Sex:	Male
Strain:	ICR
Route of admin.:	Gavage
Exposure period:	5 days
Doses:	1.45, 14.5, 145.0, mg/kg (first test) and 1150 mg/kg (second test)
Result:	Negative
Method:	other
Year:	1975

GLP:	No
Test substance:	other TS: Menthol natural brazilian, FDA 71-57
Result:	The in vitro test results were negative as well.
Test condition:	Subacute Ex-vivo study: Number of animals/dose level: 10 Dosages: Aute dosage was given once a day. Indicator Organisms (reverse mutation): Salmonella typhimurium (TA 1530) Negative control: solvent Positive control: dimethyl nitrosamine (only at 100 mg/kg). Injection of indicator organisms in mice: Within 30 minutes after the last dosage each animal was given 2.0 ml (each ml contained 3.0×10^8 cells) intraperitoneally. Three hours later mice were killed, Salmonella species were collected and the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample
Reliability:	(2) valid with restrictions Limited documentation 25.02.2003 (39)

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

"Menthol was lethal to E. coli at a concentration of 0.5% (Wokes, 1932) or 0.05% (Morris et al., 1979; Megalla et al., 1980; Jay & Rivers, 1984); to Salmonella typhimurium at a concentration of 0.05% (Karapinar & Aktug, 1987); to Clostridium at 0.05% (Ueda et al., 1982); to Staphylococcus at 0.05% (Wokes, 1932; Morris et al., 1979; Karapinar & Aktug, 1987; Moleyar & Narasimham 1992) or 0.003% (Jay & Rivers, 1984); to Vibrio spp. at 0.05% (Karapinar & Aktug;1987); and to Bacillus spp. at 0.05% (Morris et al., 1979; Megalla et al., 1980; Ueda et al., 1982; Moleyar & Narasimham, 1992). It was cytotoxic to spoilage fungi at 0.08-0.3% (Yousef et al., 1978; Kurita et al., 1981), 0.05% (Morris et al., 1979; Megalla et al., 1980; Mahmoud, 1994; Muller-Reibau et al., 1995), or 0.025% (Jay & Rivers, 1984)."

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

Test system	Test object	Concentration	Result	Reference	Comments

Rec assay	B. subtilis H17, M45	20 µg/disk	Negative	Oda et al. 1979	I-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (rec-assay) has poor predictive value.
Rec assay	B. subtilis H17, M45	Up to 10,000 µg/disk	Positive	Yoo, 1986	I-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation or a control group was used. The validity of this study cannot be assessed. The method (rec-assay) has poor predictive value
Ames test	S. typhimurium TA98, TA100, TA2637	0, 20–500 µg/plate (+/- S9)	Negative	Nohmi et al. 1985	I-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid
Ames test	E. coli WP2 uvrA (Trp-)	100–800 µg/plate	Negative	Yoo, 1986	I-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used
Ames test	S. typhimurium TA97A, TA98, TA100, TA102	0, 5–800 µg/plate (+/- S9)	Negative	Gomes-Carneiro et al. 1998	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid

As taken from EFSA, 2015.

System Test	Route/Organism/Tissue	Dose	Reference
DNA Damage	human	2000 µmol/L/2H	Mutagenesis. (Oxford Univ. Press, Pinkhill House, Southfield Road, Eynsham, Oxford OX8 1JJ, UK) V.1- 1986-29,267,2014

DNA Damage	human	1000 umol/L/4H	Mutagenesis. (Oxford Univ. Press, Pinkhill House, Southfield Road, Eynsham, Oxford OX8 1JJ, UK) V.1- 1986-29,267,2014

As taken from RTECS, 2019

The mutagenic activity of menthol has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with menthol in dimethyl sulfoxide (DMSO) at concentrations up to 5000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2018e). Under the conditions of the study, menthol was not mutagenic in the Ames test.

The clastogenicity of menthol was assessed in an *in vitro* chromosome aberration study. Chinese hamster ovary cells were treated with menthol at concentrations up to 5 mg/mL (5000 µg/mL) in the presence and absence of metabolic activation (solvent not specified). No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (ECHA, 2011b). Under the conditions of the study, menthol was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

Based on the data available, menthol does not present a concern for genotoxic potential.

As taken from Api AM et al. (2022)

5.5. Cytotoxicity

“Menthol has been tested for potential bactericidal and fungicidal effects on microorganisms that are foodborne or found in the oral cavity. Menthol at concentrations of 0.1-5 mmol/L was cytotoxic and affected tissue processes in trachea from chick embryos, ascites sarcoma BP8 cells, isolated hamster brown adipocytes, and rat liver mitochondria (Bernson & Pettersson, 1983). Menthol had no cytotoxic effect on human HeLa cells *in vitro* at concentrations of 1, 10, and 100 µg/ml (Nachev et al., 1967).

“Menthol was lethal to *E. coli* at a concentration of 0.5% (Wokes, 1932) or 0.05% (Morris et al., 1979; Megalla et al., 1980; Jay & Rivers, 1984); to *Salmonella typhimurium* at a concentration of 0.05% (Karapinar & Aktug, 1987); to *Clostridium* at 0.05% (Ueda et al., 1982); to *Staphylococcus* at 0.05% (Wokes, 1932; Morris et al., 1979; Karapinar & Aktug, 1987; Moleyar & Narasimham 1992) or 0.003% (Jay & Rivers, 1984); to *Vibrio* spp. at 0.05% (Karapinar & Aktug;1987); and to *Bacillus* spp. at 0.05% (Morris et al., 1979; Megalla et al., 1980; Ueda et al., 1982; Moleyar & Narasimham, 1992). It was cytotoxic to spoilage fungi at 0.08-0.3% (Yousef et al., 1978; Kurita et al., 1981), 0.05% (Morris et al., 1979; Megalla et al., 1980; Mahmoud, 1994; Muller-Reibau et al., 1995), or 0.025% (Jay & Rivers, 1984).”

“The results of these studies are difficult to extrapolate to the situation in experimental animal *in vivo*, and cannot be used directly in the safety assessment of menthol. Furthermore, the concentration at which cytotoxicity was observed is considerably higher than those present in the diets tested.”

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

“Menthol, a natural product of the peppermint plant *Mentha x piperita* (Lamiaceae), is a monoterpene which is widely used as a natural product in cosmetics, a flavoring agent, and as an intermediate in the production of other compounds. Various extracts from peppermint contain menthol as a major active constituent and have been used for centuries as traditional medicines for a number of ailments including infections, insomnia, and irritable bowel syndrome as well as an

insect repellent. Menthol's characteristic cooling sensation is due, in part, to the activation of sensory neurons generally termed transient receptor potential (TRP) channels, in particular transient receptor potential melastatin family member 8 (TRPM8) and transient receptor potential subfamily A, member 1 (TRPA1). Menthol acts upon TRPM8 receptors by rapidly increasing intracellular calcium and mobilizing calcium flux through the channels to induce cold response signals at the application site. Aside from its cold-inducing sensation capabilities, menthol exhibits cytotoxic effects in cancer cells, induces reduction in malignant cell growth, and engages in synergistic excitation of GABA receptors and sodium ion channels resulting in analgesia. Notwithstanding its plethora of benefits, menthol's cold-sensitivity response mechanism has been shown to inhibit mucosal recognition of nicotine and cigarette toxins common in mentholated cigarette brands thus potentially leading to toxic effects. Menthol may prove a valuable lead structure for the synthesis of drugs that target multiple receptors involved with a number of pharmacological effects." As taken from Farco JA and Grundmann O, 2013. Mini Rev Med Chem. 13(1), 124-31. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23061635>

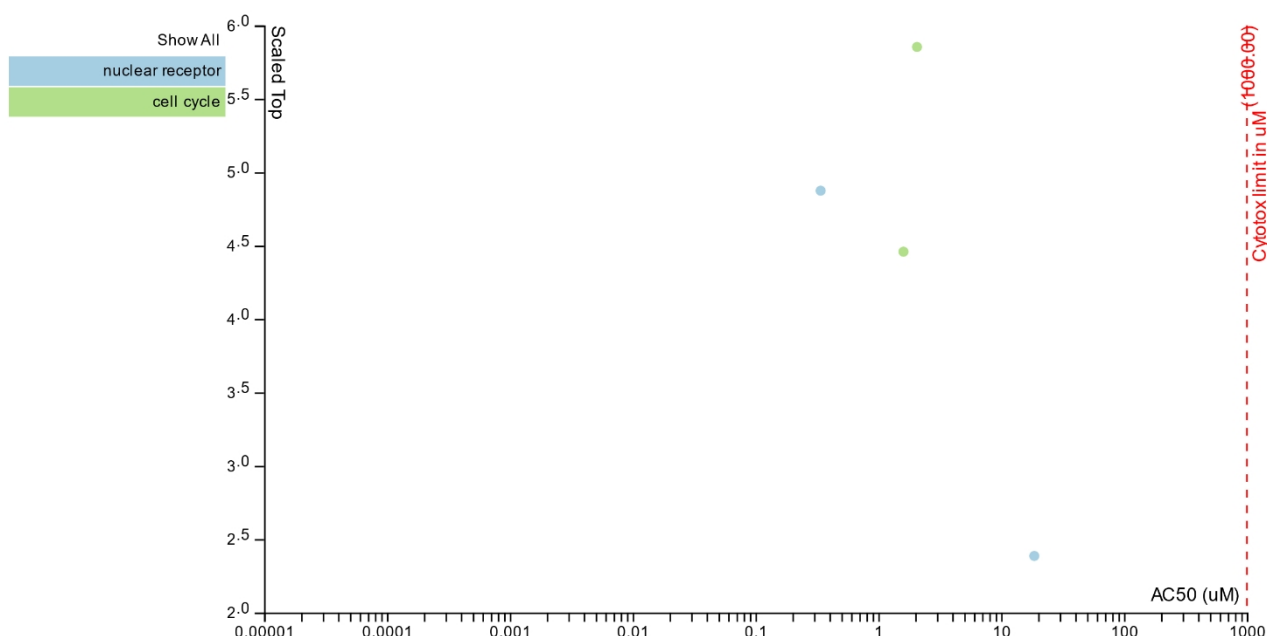
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated L-menthol (CAS RN 2216-51-5) in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: "The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve."

A summary of the ToxCast assay data on L-menthol is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



The US Environmental Protection Agency (EPA) evaluated menthol (CAS RN 89-78-1) in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.”

A summary of the ToxCast assay data on menthol is provided below in Figure 2. Figure 2 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 2



“E-cigarette-related hospitalizations and deaths across the U.S. continue to increase. A high percentage of patients have elevated liver function tests indicative of systemic toxicity. This study was designed to determine the effect of e-cigarette chemicals on liver cell toxicity. HepG2 cells were exposed to flavoring chemicals (isoamyl acetate, vanillin, ethyl vanillin, ethyl maltol, l-menthol, and trans-cinnamaldehyde), propylene glycol, and vegetable glycerin mixtures, and cell viability was measured. Data revealed that vanillin, ethyl vanillin, and ethyl maltol decreased HepG2 cell viability; repeated exposure caused increased cytotoxicity relative to single exposure, consistent with the hypothesis that frequent vaping can cause hepatotoxicity.” As taken from Rickard BP et al. 2021. ACS Omega 6(10), 6708-6713. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33748584/>

E-cigarettes utilize a wide range of flavoring chemicals with respiratory health effects that are not well understood. In this study, we used pulmonary-associated cell lines to assess the in vitro cytotoxic effects of 30 flavoring chemicals. Human bronchial epithelial cells (BEAS-2B) and both naïve and activated macrophages (THP-1) were treated with 10, 100, and 1000 μM of flavoring chemicals and analyzed for changes in viability, cell membrane damage, reactive oxygen species

(ROS) production, and inflammatory cytokine release. Viability was unaffected for all chemicals at the 10 and 100 μM concentrations. At 1000 μM , the greatest reductions in viability were seen with decanal, hexanal, nonanal, cinnamaldehyde, eugenol, vanillin, alpha-pinene, and limonene. High amounts of ROS were elicited by vanillin, ethyl maltol, and the diketones (2,3-pentanedione, 2,3-heptanedione, and 2,3-hexanedione) from both cell lines. Naïve THP-1 cells produced significantly elevated levels of IL-1 β , IL-8, and TNF- α when exposed to ethyl maltol and hexanal. Activated THP-1 cells released increased IL-1 β and TNF- α when exposed to ethyl maltol, but many flavoring chemicals had an apparent suppressive effect on inflammatory cytokines released by activated macrophages, some with varying degrees of accompanying cytotoxicity. The diketones, L-carvone, and linalool suppressed cytokine release in the absence of cytotoxicity. These findings provide insight into lung cell cytotoxicity and inflammatory cytokine release in response to flavorings commonly used in e-cigarettes. As taken from Morris AM et al. (2021). Available at <https://pubmed.ncbi.nlm.nih.gov/34769627/>

“To explore the role of transient receptor potential melastatin 8 (TRPM8) in the expression of airway epithelial-derived cytokines interleukin 25 (IL-25), IL-33 and thymic stromal lymphopoietin (TSLP) in human bronchial epithelial BEAS-2B cells induced by menthol and its related signal transduction mechanism. Methods BEAS-2B cells were treated with 2 mmol/L menthol for 1, 2, 3 and 4 hours. The groups with the higher expression of IL-25, IL-33, TSLP or Ca^{2+} were chosen to carry out the following experiments. The cells were transfected with siRNA of TRPM8 (si-TRPM8) or negative control siRNA (si-NC) and pretreated with intracellular calcium chelator BAPTA-AM, nuclear factor κB (NF- κB) inhibitor pyrrolidine dithiocarbamate (PDTC), and then intervened with menthol. Cell survival rate was measured by CCK-8 assay. The mRNA levels of IL-25, IL-33 and TSLP were detected by real-time quantitative PCR. The protein levels of IL-25, IL-33 and TSLP were detected by ELISA. The intracellular Ca^{2+} fluorescence intensity was detected by flow cytometry with Fluo-4 AM loading. Western blotting was used to detect the interference efficiency of si-TRPM8 on BEAS-2B cells and its effect on the protein expression of NF- κB p65. Results Compared with the blank control group, the mRNA and protein expression of IL-25, IL-33 and TSLP, the level of Ca^{2+} , and the protein expression of NF- κB p65 were significantly up-regulated in the menthol group. After knock-down of TRPM8, the menthol-induced increases of Ca^{2+} , IL-25, IL-33, TSLP and NF- κB p65 expression were inhibited. Both BAPTA-AM and PDTC could inhibit the high expression of IL-25, IL-33 and TSLP induced by menthol. Conclusion Menthol can induce the secretion of IL-25, IL-33 and TSLP in human BEAS-2B cells by activating TRPM8 which leads to increased Ca^{2+} concentration and activation of the NF- κB pathway.”

As taken from Hu X. and Li M. (2021). Available at <https://pubmed.ncbi.nlm.nih.gov/34140067/>

Cell type	Main findings	Reference
Immortalized human bronchial epithelial cells (16-HBE, BEAS-2B)	Classic Menthol induced the most significant mitochondrial ROS production in 16-HBE.	(Muthumalage et al. 2019)
BEAS-2B and human pulmonary fibroblast (HPF)	Both flavor chemicals were highly cytotoxic following a MTT assay at concentrations 30 (menthol) and 100 times (ethyl maltol) lower than the highest concentrations in the refill fluids. BEAS-2B cells ($\text{IC}_{50} = 0.15 \text{ mg/ml}$) were more sensitive to ethyl maltol than hPF ($\text{IC}_{50} = 0.28 \text{ mg/ml}$).	(Omaiye et al. 2019)
BEAS-2B	Menthol- mitochondrial effects: proton leakage, a decrease in coupling efficiency, and a reduction of complex I, II, and IV 24-h post-menthol exposure reduction of basal respiration, maximal respiration, spare capacity, and complex I. Tobacco had no significant effects on mitochondrial respiration, but immediately post final exposure increased complex I, IV, and V. Whether this effect is synergistic with nicotine was unclear.	(Lamb, Muthumalage, and Rahman 2020)
Human lung fibroblasts (HPF)	Most significant cytotoxicity following MTT assay was observed with cinnamon and menthol flavors which did not contain	(Bahl et al. 2012)

	nicotine.	
Normal human bronchial epithelial (NHBE) cells	Only menthol elicited dose-dependent cytotoxic effects on activating G0/G1 cycle arrest, decreasing glutathione concentration, activation of caspase 3/7 activity, increasing nuclear factor-kB (NF-kB), & increased mitochondrial mass. Tobacco, vanilla, and menthol decreased mitochondrial membrane potential dose-dependently.	Czekala et al. 2019
Human pulmonary fibroblasts (HPF), alveolar epithelial cells (A549)	Mint/Menthol was cytotoxic following MTT assay only as e-liquid.	(Behar et al., 2018)

The mint/menthol (10 articles) was the most frequently reported to demonstrate harmful effects, followed by cinnamon flavor (9 articles) and strawberry (5 articles) in cytotoxicity assays, metabolic activity assays, and pro-inflammatory biomarkers assays. As taken from Effah F. et. al. (2022). J Toxicol Environ Health B Crit Rev. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9590402/>

5.6. Carcinogenicity

“Menthol has been studied for its chemopreventive effect on ras-mediated tumour growth in four types of rat liver cell in vitro, with lovastatin as the positive control. Concentrations of 0.1-2.5 mmol/L of menthol inhibited tumour growth, but the authors concluded that the mechanism of the chemopreventive effect of menthol is different from that of lovastatin (Ruch & Sigler, 1994).”

“In a study of the preventive activity of menthol against 7,12-dimethylbenz [a]anthracene (DMBA)-induced mammary tumours in rats, a diet containing 1% menthol was fed from two weeks before DMBA treatment to up to 22 weeks after treatment. A reduction in mammary tumour incidence was seen. A diet containing 0.5% menthol fed from two weeks before DMBA treatment until one week after treatment also resulted in a reduction in tumour incidence (Russin et al., 1989; Gould et al., 1990).”

“In studies of mentholated cigarettes in animals and humans, the presence of menthol in cigarettes did not enhance the incidence of lung cancer over that due to smoking unmentholated cigarettes (Kabat & Hebert, 1991; Gaworski et al., 1997).”

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

Tumor Inhibition Studies

Species:	RAT
Number of Animals Tested:	(?,46)/(?,52)
Strain/Sex:	SPRAGUE-DAWLEY/FEMALE
Dose (Inhibitor):	0; 1% IN DIET BEGINNING 2 WK PRIOR TO CARCINOGEN DOSE AND CONTINUING FOR DURATION OF STUDY (STUDY DURATION: 20 WK)

Route (Inhibitor):	ORAL
Carcinogen:	7,12-DIMETHYLBENZ[A]ANTHRACENE ; 57-97-6
Route (Carcinogen):	GAVAGE
Dose (Carcinogen):	65 MG/KG IN 0.5 ML SESAME OIL ONCE
Promoter:	NONE USED
Target Tissue: Type of Lesion:	MAMMARY GLAND: TUMOR
Endpoint (Multiplicity):	3.3, 2.0, 39%, P<0.001
Endpoint (Latency):	63 D, 80 D, P<0.001
Comments:	MORE THAN 95% OF THE TUMORS WERE CARCINOMAS
Reference:	[RUSSIN,WA, HOESLY,JD, ELSON,CE, TANNER,MA AND GOULD,MN; INHIBITION OF RAT MAMMARY CARCINOGENESIS BY MONOTERPENOID; CARCINOGENESIS (LONDON) 10(11):2161-4, 1989]
Species:	RAT
Number of Animals Tested:	(?,52)/(?,52)
Strain/Sex:	SPRAGUE-DAWLEY/FEMALE
Dose (Inhibitor):	0; 0.5% IN DIET BEGINNING 2 WK PRIOR TO CARCINOGEN DOSE AND CONTINUING FOR 1 WK POST CARCINOGEN DOSE (STUDY DURATION: 20 WK)
Route (Inhibitor):	ORAL

Carcinogen:	7,12-DIMETHYLBENZ[A]ANTHRACENE ; 57-97-6
Route (Carcinogen):	GAVAGE
Dose (Carcinogen):	65 MG/KG IN 0.5 ML SESAME OIL ONCE
Promoter:	NONE USED
Target Tissue: Type of Lesion:	MAMMARY GLAND: TUMOR
Endpoint (Multiplicity):	2.5, 1.9, 24%, P<0.003
Endpoint (Latency):	58 D, 82 D, P<0.01
Comments:	MORE THAN 95% OF THE TUMORS WERE CARCINOMAS
Reference:	[RUSSIN,WA, HOESLY,JD, ELSON,CE, TANNER,MA AND GOULD,MN; INHIBITION OF RAT MAMMARY CARCINOGENESIS BY MONOTERPENOID; CARCINOGENESIS (LONDON) 10(11):2161-4, 1989]
Species:	RAT
Number of Animals Tested:	(19,18)/(19,19)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 5 MG/G IN DIET FOR 22 WK BEGINNING 3 D POST LAST CARCINOGEN DOSE (STUDY DURATION: 25 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2

Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	15 MG/KG BW 2/WK FOR 3 WK
Promoter:	NONE USED
Target Tissue: Type of Lesion:	LARGE BOWEL: ADENOMATOUS POLYP
Endpoint (Incidence):	82%, 88%, -7%, NOT SIGNIFICANT
Endpoint (Multiplicity):	1.5, 1.2, 20%, NOT SIGNIFICANT
Reference:	[WATTENBERG,LW; INHIBITION OF AZOXYMETHANE-INDUCED NEOPLASIA OF THE LARGE BOWEL BY 3-HYDROXY-3,7,11-TRIMETHYL-1,6,10-DODECATRIENE (NEROLIDOL); CARCINOGENESIS 12(1):151-152, 1991]
Species:	RAT
Number of Animals Tested:	(19,18)/(19,19)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 5 MG/G IN DIET FOR 22 WK BEGINNING 3 D POST LAST CARCINOGEN DOSE (STUDY DURATION: 25 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose	15 MG/KG BW 2/WK FOR 3 WK

(Carcinogen):	
Promoter:	NONE USED
Target Tissue: Type of Lesion:	DUODENUM: ADENOCARCINOMA
Endpoint (Incidence):	50%, 42%, 16%, NOT SIGNIFICANT
Endpoint (Multiplicity):	0.6, 0.4, 33%, NOT SIGNIFICANT
Reference:	[WATTENBERG,LW; INHIBITION OF AZOXYMETHANE-INDUCED NEOPLASIA OF THE LARGE BOWEL BY 3-HYDROXY-3,7,11-TRIMETHYL-1,6,10-DODECATRIENE (NEROLIDOL); CARCINOGENESIS 12(1):151-152, 1991]

As taken from CCRIS, 2001.

“Menthol is a cyclic monoterpene alcohol which possesses well-known cooling characteristics and a residual minty smell of the oil remnants from which it was obtained. Because of these attributes it is one of the most important flavouring additives besides vanilla and citrus. Due to this reason it is used in a variety of consumer products ranging from confections such as chocolate and chewing gum to oral-care products such as toothpaste as well as in over-the-counter medicinal products for its cooling and biological effects. Its cooling effects are not exclusive to medicinal use. Approximately one quarter of the cigarettes on the market contain menthol and small amounts of menthol are even included in non-mentholated cigarettes. Natural menthol is isolated exclusively from *Mentha canadensis*, but can also be synthesised on industrial scale through various processes. Although menthol exists in eight stereoisomeric forms, (-)-menthol from the natural source and synthesised menthol with the same structure is the most preferred isomer. The demand for menthol is high and it was previously estimated that the worldwide use of menthol was 30-32,000 metric tonnes per annum. Menthol is not a predominant compound of the essential oils as it can only be found as a constituent of a limited number of aromatic plants. These plants are known to exhibit biological activity in vitro and in vivo such as antibacterial, antifungal, antipruritic, anticancer and analgesic effects, and are also an effective fumigant. In addition, menthol is one of the most effective terpenes used to enhance the dermal penetration of pharmaceuticals. This review summarises the chemical and biological properties of menthol and highlights its cooling effects and toxicity.” As taken from Kamatou GP et al. 2013. *Phytochemistry*. 96, 15-25. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24054028>

“The objective of the present study was to repurpose L-menthol, which is frequently used in oral health and topical formulations, for cancer therapeutics. In this article, we argue that monoterpenes such as L-menthol might offer veritable potentials in systems medicine, for example, as cheaper anti-cancer compounds. Other monoterpenes such as limonene, perillyl alcohol, and geraniol have been shown to induce apoptosis in various cancer cell lines, but their mechanisms of action are yet to be completely elucidated. Earlier, we showed that L-menthol modulates tubulin polymerization and apoptosis to inhibit cancer cell proliferation. In the present report, we used an apoptosis-related gene microarray in conjunction with proteomics analyses, as well as in silico interpretations, to study gene expression modulation in human adenocarcinoma Caco-2 cell line in response to L-menthol treatment. The microarray analysis identified caspase 10 as the important initiator

caspase, instead of caspase 8. The proteomics analyses showed downregulation of HSP90 protein (also corroborated by its low transcript abundance), which in turn indicated inhibition of AKT-mediated survival pathway, release of pro-apoptotic factor BAD from BAD and BCLxL complex, besides regulation of other factors related to apoptosis. Based on the combined microarray, proteomics, and in silico data, a signaling pathway for L-menthol-induced apoptosis is being presented for the first time here. These data and literature analysis have significant implications for "repurposing" L-menthol beyond oral medicine, and in understanding the mode of action of plant-derived monoterpenes towards development of cheaper anticancer drugs in future." As taken from Faridi U et al. 2016. OMICS 20(1), 53-64. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26760959>

"No evidence of ... carcinogenicity".

"Cervical cancer is a major cause of gynecological related mortalities in developing countries. Cisplatin, a potent chemotherapeutic agent used for treating advanced cervical cancer exhibits side effects and resistance development. The current study was aimed to investigate the repurposing of l-menthol as a potential therapeutic drug against cervical cancer. L-menthol was predicted to be non-toxic with good pharmacokinetic properties based on SwissADME and pkCSM analysis. Subsequently, 543 and 1664 targets of l-menthol and cervical cancer were identified using STITCH, BATMAN-TCM, PharmMapper and CTD databases. STRING and Cytoscape analysis of the merged protein-protein interaction network revealed 107 core targets of l- menthol against cervical cancer. M-CODE identified highly connected clusters between the core targets which through KEGG analysis were found to be enriched in pathways related to apoptosis and adherence junctions. Molecular docking showed that l- menthol targeted E6, E6AP and E7 onco-proteins of HPV that interact and inactivate TP53 and Rb1 in cervical cancer, respectively. Molecular docking also showed good binding affinity of l-menthol toward proteins associated with apoptosis and migration. Molecular dynamics simulation confirmed stability of the docked complexes. In vitro analysis confirmed that l-menthol was cytotoxic towards cervical cancer CaSki cells and altered expression of TP53, Rb1, CDKN1A, E2F1, NFKB1, Akt-1, caspase-3, CDH1 and MMP-2 genes identified through network pharmacology approach. Schematic representation of the work flow depicting the potential of l-menthol to target cervical cancer." As taken from Sidhu S. et al (2022) Available at <https://pubmed.ncbi.nlm.nih.gov/35467269/>

5.7. Irritation/immunotoxicity

Type of Test	Route of Exposure	Species Observed	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration into the eye	Rodent - rabbit	1%		FRZKAP Farmatsevtichnii Zhurnal (Kiev). (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.3-1930- Volume(issue)/page/year: 17(3),53,1962
Standard Draize test	Administration into the eye	Mammal species unspecified	0.7%	Mild	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 46,S1,2008

TDLo - Lowest published toxic dose	Administration onto the skin	Human	10 mg/kg	Lungs, Thorax, or Respiration - other changes Nutritional and Gross Metabolic - other changes Vascular - measurement of regional blood flow	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 86,262,2015

As taken from RTECS, 2017.

Route/Organism	Dose	Effect	Reference
administration into the eye (irritant), rabbit	250 ug	severe irritation effect	American Journal of Ophthalmology. (Ophthalmic Pub. Co., 435 N. Michigan Ave., Suite 1415, Chicago, IL 60611) Series 3: V.1-1918-29,1363,1946
administration into the eye (irritant), mammal (species unspecified)	100%	severe irritation effect	Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982-46,S1,2008

As taken from RTECS, 2019

"INTRODUCTION: Among other effects, menthol added to cigarettes may modulate sensory response to cigarette smoke either by masking "harshness" or contributing to a desirable "impact." However, harshness and impact have been imprecisely defined and assessed using subjective measures. Thus, the current experiments used an objective measure of sensitivity to chemical irritation in the nose to test the hypothesis that menthol vapor modulates sensitivity to chemical irritation in the airways. METHODS: Nasal irritation thresholds were measured for 2 model compounds (acetic acid and allyl isothiocyanate) using nasal lateralization. In this technique, participants simultaneously sniff clean air in one nostril and chemical vapor in the other and attempt to identify the stimulated nostril. People cannot lateralize based on smell alone but can do so when chemicals are strong enough to feel. In one condition, participants were pretreated by sniffing menthol vapor. In a control condition, participants were pretreated by sniffing an odorless blank (within-subjects design). RESULTS: Pretreatment with menthol vapor decreased sensitivity to nasal irritation from acetic acid (participants required higher concentrations to lateralize) but increased sensitivity to allyl isothiocyanate (lower concentrations were required). CONCLUSIONS: The current experiments provide objective evidence that menthol vapor can modulate sensitivity to chemical irritation in the upper airways in humans. Cigarette smoke is a mixture of chemicals and particulates, and further work will be needed to determine exactly how menthol modulates smoking sensation. A better understanding could lead to treatments tailored to help menthol smokers quit by replacing the sensation of mentholated cigarettes." As taken from Wise PM et al. 2011. Nicotine Tob. Res. 13(10), 989-97. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/21652736>

Tested at 8% in petrolatum, /l-menthol/...produced no irritation after a 48-hr closed-patch test in human subjects, although it has been reported that menthol may act as a mild irritant in man. a maximization test was carried out on 24 volunteers. ...concn of 8% in petrolatum...produced no sensitization reactions. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 518] **PEER REVIEWED**

Moderate irritant to mucous membranes on inhalation. [Hawley, G.G. The Condensed Chemical Dictionary. 9th ed. New York: Van Nostrand Reinhold Co., 1977., p. 544] **PEER REVIEWED**

The psychotropic action...is always transient & it is unlikely that it would have any effects of this kind in man at the concentrations that occur in food or drinks. /menthol/ [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 518] **PEER REVIEWED**

"Cooling" effect of l-menthol was found...to be superior to that produced by other isomers; odor and taste, too of l-menthol were superior, with some of the isomers producing sharp, irritating and disagreeable perceptions. [Osol, A., and R. Pratt. (eds.). The United States Dispensatory. 27th ed. Philadelphia: J.B. Lippincott, 1973., p. 697] **PEER REVIEWED**

Sensitivity reactions associated with the use of mentholated products (including cigarettes) have been reported. Use of mentholated nasal drops in infants has evidently caused isolated cases of spasm of the larynx, and a few cases of nervous or digestive system disturbance have been associated with excessive inhalation or oral exposure to menthol. Menthol was reported to be a mild skin irritant in man, but caused more severe damage to the mucous membranes. [BIBRA working group; Toxicity profile. The British Industrial Biological Research Association 7 (1986)] **PEER REVIEWED**

An eye irritant. [Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 2108] **PEER REVIEWED**

L-Menthol applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 519] **PEER REVIEWED**

In rabbit eyes, application of menthol has been reported to cause severe damage, graded 9 on scale of 1-10 @ 24 hr. /most severe injuries have been rated 10/ ...application of 10 mg of l-menthol crystals for 5 min to rabbit's cornea. slight corneal haze...intense hyperemia of iris, & miosis... [Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 576] **PEER REVIEWED**

In experimental animals it was of low acute toxicity by oral, injection and dermal routes. Liver and kidney changes have been seen in a number of studies in laboratory animals, mainly involving oral administration. Inhalation of menthol may produce respiratory tract injury. In oral studies, there was no convincing evidence of carcinogenicity in rats and mice or of reproductive effects in mice, rats, rabbits and hamsters. A limited range of assays (including a bacterial Ames test) have not identified any genotoxic potential. It was severely irritating to the eye of the rabbit. [BIBRA working group; Toxicity profile. The British Industrial Biological Research Association 7 (1986)] **PEER REVIEWED**

As taken from HSDB, 2003.

"The presence of menthol and menthol-containing flavour and fragrance oils at high concentrations in consumer products such as cigarettes, toothpaste, and topical medications has led to sensitivity reactions in the oral and nasal cavity (e.g. Millard, 1973; Dooms-Goossens et al., 1977; Lewis et al., 1995; Morton et al., 1995; Shah et al., 1996)." "The preferred method for testing sensitivity or food intolerance is the double-blind placebo-controlled food challenge. In a study on the prevalence of food allergy and food intolerance in 1483 Dutch adults by this method, it was estimated that the prevalence of sensitivity or intolerance was 0.8-2.4% of the adult population. Within the sampled population, 73 subjects reported such effects, and 12 of the responses were confirmed. Only one of these responders had a reaction to menthol challenge; the subject reported 'aggravation of aphthae' (whitish spots in the mouth that characterize aphthous stomatitis) 1 h after administration (Niestijl Janson et al., 1994)."

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htmA>

A number of papers have been identified in TRACE that report investigations into the skin sensitizing potential of various isomers of menthol [not always specified]. The SIDS dossier summarizes 9 reports on patch tests on a total of 6227 patients, where 82 [1.3%] had positive reactions to menthol (SIAR, 2003).

Dermal sensitivity to menthol has been reported at a frequency of up to 6% in various groups of dermatitis patients (BIBRA, 1990).

Oral and nasal skin sensitization has also been reported in people exposed to menthol via flavourings in cigarettes, toothpaste and medications (Camarasa & Alomar, 1978; Morton et al., 1995; Shah et al., 1996).

No skin sensitization was reported in 25 volunteers in a maximization test with 8% dl-menthol in petrolatum (Kligman, 1975).

No skin sensitization was reported in a Buehler test in guinea pigs [or rabbits? – species unclear in secondary source] with 0.5 ml of 25% l-menthol nor in a local lymph node assay in mice (two unpublished studies cited in SIAR, 2003), whilst a modified Draize test [apparently in guinea pigs] was equivocal (Sharp, 1978).

Erythema multiforme (EM)-like eruptions were seen in a woman exposed to poultices containing L-menthol. The eruption sites were remote from the sites of application of the poultices. The woman also tested positive to 1.5% L-menthol in a patch test (Nakagawa et al. 2009).

A review of patch-test data for allergic contact dermatitis among 1147 patient tested with menthol [CAS no.1490-04-6 – isomer not specified] showed the material to be a rare allergen (Uter et al. 2010).

Menthol is a fragrance substance known to cause dose-related contact urticaria (SCCS, 2011).

"AIM: This systematic review was aimed at critically evaluating the evidence regarding the adverse effects associated with aromatherapy. METHOD: Five electronic databases were searched to identify all relevant case reports and case series. RESULTS: Forty two primary reports met our inclusion criteria. In total, 71 patients experienced adverse effects of aromatherapy. Adverse effects ranged from mild to severe and included one fatality. The most common adverse effect was dermatitis. Lavender, peppermint, tea tree oil and ylang-ylang were the most common essential oils responsible for adverse effects. CONCLUSION: Aromatherapy has the potential to cause adverse effects some of which are serious. Their frequency remains unknown. Lack of sufficiently convincing evidence regarding the effectiveness of aromatherapy combined with its potential to cause adverse effects questions the usefulness of this modality in any condition." As taken from Posadzki P et al. 2012. Int. J. Risk Saf. Med. 24(3), 147-61. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22936057>

"0.05% and 0.1% menthol significantly enhanced the penetration of dexamethasone in the cornea of rabbits, but did not change the dexamethasone penetration in sclera in vitro. When topical drops of dexamethasone containing 0.1% menthol were administered, a significantly increased concentration of dexamethasone in the cornea and aqueous humor tissues was reported, but dexamethasone concentrations remained unaffected in the retina–choroid tissues. No signs of irritation were observed when menthol was administered at concentrations ranging from 0.025%-0.1%; moreover, no substantial toxic reactions were observed in corneal hydration level, electrophysiological, or histological examinations after the addition of 0.1% menthol". Xu et al (2011) Available at <http://www.springerlink.com/content/2156121h7382477q/>

"INTRODUCTION: Despite the longstanding use and popularity of menthol as a flavorant in tobacco products, its sensory interactions with inhaled nicotine have never been measured independently of the other irritants in tobacco smoke. We therefore measured the perception of menthol in an E-cigarette with the primary goal of assessing its analgesic effect on the sensory irritation produced by inhaled nicotine. METHODS: Adult cigarette smokers sampled aerosolized E-liquids containing five different concentrations of nicotine with 0%, 0.5%, or 3.5% l-menthol, as well as two commercial menthol flavors with and without nicotine. For each of the E-liquids participants used a labeled magnitude scale to rate the Overall Sensation intensity, Coolness/Cold, and Irritation/Harshness they experienced, and a Labeled Hedonic Scale to indicate how much they liked/disliked the overall flavor. RESULTS: The main findings were that (1) perceived Irritation/Harshness was unaffected by a low (0.5%) menthol concentration, whereas a high menthol concentration (3.5%) led to higher perceived Irritation/Harshness at low nicotine concentrations but to lower Irritation/Harshness at the highest nicotine concentration (24mg/ml); (2) a commercial Menthol-Mint flavor produced similar results; (3) nicotine tended to enhance rather than suppress sensations of Coolness/Cold; and (4) menthol tended to slightly increase liking independently of nicotine concentration. CONCLUSION: In addition to adding a sensation of coolness, menthol can reduce perceived airway irritation and harshness produced by inhalation when nicotine concentration is high, and contributes to the sensory impact of E-liquids when nicotine concentration is low. IMPLICATIONS: The evidence presented here indicates that menthol can potentially improve the appeal of E-cigarettes not only via its coolness and minty flavor, but also by reducing the harshness from high concentrations of nicotine. As the first direct demonstration of an analgesic effect of menthol on inhaled nicotine in humans, these data also have implications for the role of menthol flavors in other inhaled tobacco products." As taken from Rosbrook K and Green BG. 2016. Nicotine Tob. Res. 18(7), 1588-95. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26783293>

"Immediate skin reactions are common in dermatological practice, but may often be overlooked. The main objective of this article is to provide an update of the literature concerning immediate-type reactions or contact urticaria/contact urticaria syndrome caused by cosmetic ingredients in terms of immediate clinical symptoms, positive reactions following open, scratch or, most often, prick testing, and sometimes the detection of specific IgE antibodies. To this end, a selective search in different medical literature databases was performed. This yielded a list of cosmetic ingredients causing immediate reactions, including hair dyes and bleaches, preservatives, fragrance and aroma chemicals, sunscreens, hair glues, plant-derived and animal-derived components, permanent makeup and tattoos, glycolic acid peel, lip plumper, and alcohols. Many of the reported cases, however, lack appropriate controls and detailed investigation. Contact urticaria may occur with or without systemic symptoms, which are sometimes life-threatening." As taken from Verhulst L and Goossens A. 2016. Contact Dermatitis 75(6), 333-344. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27593503>

"Levomenthol..... has a counterirritant effect on skin and mucous membranes, thereby producing a local analgesic or anesthetic effect."

As taken from PubChem.

According to the US EPA's Safer Chemical Ingredients list, L-menthol (CAS RN 2216-51-5) is a known sensitizer (US EPA, 2023).

Menthol (CAS RN 2216-51-5) is an established contact allergen in humans (Danish EPA, 2016).

"Menthol has a considerable cooling effect, but the use range of menthol is limited because of its extremely low solubility in water and inherent flavor. (-)-Menthol β -glucoside was determined to be more soluble in water (>27 times) than (-)-menthol α -glucoside; hence, β -anomer-selective glucosylation of menthol is necessary. The in vitro glycosylation of (-)-menthol by uridine diphosphate glycosyltransferase (BLC) from *Bacillus licheniformis* generated (-)-menthol β -glucoside and new (-)-menthol β -galactoside and (-)-menthol N-acetylglucosamine. The maximum conversion rate of menthol to (-)-menthol β -D-glucoside by BLC was found to be 58.9%. Importantly, (-)-menthol β -D-glucoside had a higher cooling effect and no flavor compared with menthol. In addition, (-)-menthol β -D-glucoside was determined to be a non-sensitizer in a skin allergy test in the human cell line activation test, whereas menthol was a sensitizer." As taken from Choi HY et al. 2017. *AMB Express* 7(1), 167. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28853018>

"L-Menthol is the main ingredient of peppermint which affects various pharmacological effects such as anti-inflammation and anti-oxidative activity. In this study, we aimed to evaluate the potential effects of L-menthol on cigarette smoke extract (CSE) induced lung injury in rats. Morphology assessment results revealed that administration with L-menthol (5, 10 or 20 mg kg⁻¹ d⁻¹) significantly alleviated CSE-induced lung injury. Besides, L-menthol significantly reduced the inflammatory response by suppressing the production of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) via downregulating nuclear factor kappa B (NF- κ B) and p38 MAPK pathways. Meanwhile, L-menthol decreased the levels of oxidative stress markers including malondialdehyde (MDA) and myeloperoxidase (MPO) whereas it increased the amount of glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) through activation of the Nrf2 pathway. Furthermore, the expression of MMP-9 and TIMP-1 in lungs was reduced after treatment with L-menthol, and this indicated that L-menthol might have a potential effect on airway remodeling. Moreover, immunohistochemistry analyses indicated that L-menthol could suppress the infiltration of CD4⁺ and CD8⁺ T cells in lung tissues and this was probably due to the immune regulation activity of L-menthol. Taken together, our findings support that L-menthol might be a potential candidate for the treatment of CSE-induced lung injury in rats." As taken from Liu Y et al. 2018. *RSC Adv.* 8, 9353-9363. Available at <http://pubs.rsc.org/en/content/articlehtml/2018/ra/c8ra00160j>

"BACKGROUND: Natural ingredients have variable compositions, so their allergenic potencies may differ. OBJECTIVES: To retrospectively analyse subjects reacting to herbal remedies over the past 27 years, with the aim of (i) evaluating demographic characteristics and lesion locations, (ii) describing the frequencies of positive patch test reactions, (iii) identifying sensitization sources, and (iv) studying concomitant sensitivity. PATIENTS AND METHODS: In total, 15980 patients were patch tested between 1990 and 2016 with the European baseline series and/or other series, product(s) used, and, whenever possible, the respective ingredients. RESULTS: Altogether, 8942 (56%) of 15 980 patients presented with at least one positive reaction. Reactions to topical herbal medicines, most often applied to treat an eczematous condition, leg ulcers, or other wounds, were seen in 125 (0.8%), that is, 1.4% of the contact-allergic subjects. Hands, legs and feet were the most frequently affected body sites. Twenty-one botanical allergens were identified, the commonest being *Myroxylon pereirae* (balsam of Peru), *Compositae* plants, and tincture of benzoin. Many patients presented with multiple positive test reactions, and some did not react to the commercial allergens but only to the products used. CONCLUSIONS: Topical herbal remedies should not be applied on damaged skin, as multiple sensitization may develop. Moreover, patch testing with the culprit products is important for the diagnosis." As taken from Gilissen L et al. 2018. *Contact Dermatitis* 78(3), 177-184. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29214642>

“Background: An essential step in ensuring the toxicological safety of cosmetic or personal care products is the evaluation of the skin sensitizing potential of product ingredients. Objective: We used a standardized protocol from cosmetic trade industry and consumer safety groups to evaluate the sensitization potential of ingredients in 3 commercially available cleansing conditioners. Methods: A total of 33 ingredients were evaluated. Each ingredient underwent (1) dermatological evaluation, (2) *in silico* analysis for irritation and sensitization potential, and (3) a literature evaluation to determine risk of sensitization. Consumer exposure level was compared with the weight-of-evidence no-expected sensitization induction level for the constituent. If a no-expected sensitization induction level for a specific ingredient was not available, the dermal sensitization threshold approach was used. A margin of safety was calculated for each constituent. Results: The margins of safety for all evaluated ingredients in the cleansing conditioners were greater than 1. Conclusions: This analysis indicates that exposure to the individual ingredients present in these cleansing conditioners would not be expected to induce dermal sensitization in a consumer under the examined exposure scenario.” As taken from Monnot AD et al. 2019. *Dermatitis* 30(2), 116-128. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/30829810/>

“Background: Naturally derived cosmetic product ingredients of both plant and animal origin are being included increasingly in product formulations in order to cater to consumer preferences. They may be an overlooked cause of reactions to cosmetic products in some patients with dermatitis. Objectives: To identify naturally derived cosmetic product ingredients with allergenic potential (type I and type IV) and propose a cosmetic screening test series. Methods: The study was conducted in two steps. The first step was a market survey using a nonprofit application helping consumers avoid problematic substances in cosmetic products. The application contained 10 067 cosmetic products that were label checked for naturally derived cosmetic product ingredients. The second step was a literature search to examine how frequently the naturally derived ingredients were described and related to allergic reactions in cosmetics or other topically administered products. Results: We identified 121 different naturally derived cosmetic product ingredients that were included in at least 30 cosmetic products. In total, 22 ingredients were selected for a screening test series. Conclusions: We propose a supplemental patch test and a prick test screening series with naturally derived cosmetic product ingredients for patients with skin reactions to cosmetic products, aiming to identify a cause in more patients than is currently possible.” As taken from Bruusgaard-Mouritsen MA et al. 2020. *Contact Dermatitis* 83(4), :251-270. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32248558/>

Based on the existing data on menthol and menthol isomers (*l*-menthol (CAS # 2216-51-5); *d*-menthol (CAS # 15356-60-2); menthol racemic (CAS # 15356-70-4); and *dl*-isomenthol (CAS # 3623-52-7)), menthol does not present a concern for skin sensitization under the current, declared levels of use. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; OECD Toolbox v4.2; Toxtree v3.1.0). *dl*-Isomenthol was found to be negative in an *in vitro* KeratinoSens and human cell line activation test (h-CLAT) (RIFM, 2018g; RIFM, 2018c). In a murine local lymph node assay (LLNA), *l*-menthol was not sensitizing up to the highest tested concentration of 30% (7500 µg/cm²) (RIFM, 1995). In guinea pig sensitization tests, including a Buehler test and an open epicutaneous test, no reactions indicative of sensitization were observed with *l*-menthol and menthol isomers (Brazilian, racemic, *l*-menthol, *d*-menthol) (RIFM, 1990a; RIFM, 1974b). In another guinea pig study conducted according to the modified Draize procedure, a positive response was reported for *l*-menthol only after the same animals were re-tested utilizing the full induction and challenge procedure (Sharp, 1978). This result is not considered to be of significance as the test was not conducted on naïve animals (Sharp, 1978; ECHA, 2011a). Furthermore, in 2 separate human maximization tests, no skin sensitization reactions were observed when conducted using 8% (5520 µg/cm²) concentration of *l*-menthol and menthol racemic, respectively (RIFM, 1974a; RIFM, 1973).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies, menthol does not present a safety concern.

As taken from API AM et al. (2022)

5.8. All other relevant types of toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to oral product (snus/nicotine pouch). Non-clinical in vitro studies were conducted to investigate the characteristics of extracts from tobacco free nicotine pouches alongside a reference snus product containing L-Menthol and/or 1R6F reference cigarette. Within the sensitivity and specificity of the bioassay(s) all smokeless products extracts were non-mutagenic, non-genotoxic, non-cytotoxic and non-irritant in contrast to results obtained for the 1R6F reference cigarette (Miller-Holt et al., 2022). Table below provides highest tested level(s) and specific endpoint(s).

Product type	Endpoint	Tested level (ppm)	Reference
Nicotine Pouch	In vitro genotoxicity	1600 1500	Labstat International Inc. (2020) Ökolab Test Report (2021)
	In vitro cytotoxicity	1600 1500	Labstat International Inc. (2020) Ökolab Test Report (2021)
	In vitro irritation (EpiGingival™ 3D)	1600	Charles River Laboratories (2022)

Hives, flushing, and headaches in a 31-year-old woman were attributed to her use of mentholated products, such as toothpaste, peppermint cany, cigarettes, mint jelly, and facial cream (Papa, 1964). [...] Black and blue patches on the skin were attributed to the use of mentholated cigarettes. The condition was reproduced by a small oral dose of menthol (Highstein, 1951).

“Menthol is a cyclic monoterpene alcohol which possesses well-known cooling characteristics and a residual minty smell of the oil remnants from which it was obtained. Because of these attributes it is one of the most important flavouring additives besides vanilla and citrus. Due to this reason it is used in a variety of consumer products ranging from confections such as chocolate and chewing gum to oral-care products such as toothpaste as well as in over-the-counter medicinal products for its cooling and biological effects. Its cooling effects are not exclusive to medicinal use. Approximately one quarter of the cigarettes on the market contain menthol and small amounts of menthol are even included in non-mentholated cigarettes. Natural menthol is isolated exclusively from *Mentha canadensis*, but can also be synthesised on industrial scale through various processes. Although menthol exists in eight stereoisomeric forms, (-)-menthol from the natural source and synthesised menthol with the same structure is the most preferred isomer. The demand for menthol is high and it was previously estimated that the worldwide use of menthol was 30-32,000 metric tonnes per annum. Menthol is not a predominant compound of the essential oils as it can only be found as a constituent of a limited number of aromatic plants. These plants are known to exhibit biological activity in vitro and in vivo such as antibacterial, antifungal, antipruritic, anticancer and analgesic effects, and are also an effective fumigant. In addition, menthol is one of the most effective terpenes used to enhance the dermal penetration of pharmaceuticals. This review summarises the chemical and biological properties of menthol and highlights its cooling effects and toxicity.” As taken from Kamatou GP et al. 2013. *Phytochemistry* 96, 15-25. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24054028>

“Menthol, a natural product of the peppermint plant *Mentha x piperita* (Lamiaceae), is a monoterpene which is widely used as a natural product in cosmetics, a flavoring agent, and as an intermediate in the production of other compounds. Various extracts from peppermint contain menthol as a major active constituent and have been used for centuries as traditional medicines for a number of ailments including infections, insomnia, and irritable bowel syndrome as well as an insect repellent. Menthol's characteristic cooling sensation is due, in part, to the activation of sensory neurons generally termed transient receptor potential (TRP) channels, in particular transient receptor potential melastatin family member 8 (TRPM8) and transient receptor potential subfamily A, member 1 (TRPA1). Menthol acts upon TRPM8 receptors by rapidly increasing

intracellular calcium and mobilizing calcium flux through the channels to induce cold response signals at the application site. Aside from its cold-inducing sensation capabilities, menthol exhibits cytotoxic effects in cancer cells, induces reduction in malignant cell growth, and engages in synergistic excitation of GABA receptors and sodium ion channels resulting in analgesia. Notwithstanding its plethora of benefits, menthol's cold-sensitivity response mechanism has been shown to inhibit mucosal recognition of nicotine and cigarette toxins common in mentholated cigarette brands thus potentially leading to toxic effects. Menthol may prove a valuable lead structure for the synthesis of drugs that target multiple receptors involved with a number of pharmacological effects.” As taken from Farco JA and Grundmann O, 2013. *Mini Rev Med Chem.* 13(1), 124-31. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23061635>

“Objective: To identify natural bioactive molecules with potential to inhibit DNA methyltransferase 1 (DNMT1) and cause reactivation of genes silenced due to promoter hypermethylation. Methods and Results: -(-) [sic] Menthol and epigallocatechin-3-gallate (EGCG) (reference molecule) were investigated using an in vitro methylation assay, which indicated potential of -(-)menthol as an epigenetic modulator with the ability to directly inhibit M.SssI (an analogue of DNMT1) activity at 100µM. Methylation specific PCR and bisulphite sequencing revealed complete hypomethylation of 15 CpG sites in the Fanconi anemia, complementation group F (FANCF) gene between +280 and +432 nucleotides relative to the transcription start site, which resulted in significant ($P<0.001$) up-regulation of FANCF gene expression by 2.1 and 2.5 fold respectively after treatment with menthol (80µM) and EGCG (80µM) for 4 days in the SiHa cell line as analyzed by qRT PCR. Conclusion: The present work highlighted the potential of -(-) menthol, a naturally occurring cyclic monoterpene, as an epigenetic modulator causing promoter hypomethylation induced reactivation of the FANCF gene mediated by possible inhibition of DNMT1 activity in the SiHa cell line.” As taken from Parashar G et al. 2017. *Asian Pac. J. Cancer Prev.* 18(5), 1365-1370. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28612587>

“Chirality plays a vital role in biological membranes and has a significant effect depending on the type and arrangement of the isomer. Menthol has two typical chiral forms, d- and l-, which exhibit different behaviours. l-Menthol is known for its physiological effect on sensitivity (i.e. a cooling effect), whereas d-menthol causes skin irritation. Menthol molecules may affect not only the thermoreceptors on biomembranes, but also the membrane itself. Membrane heterogeneity (lipid rafts, phase separation) depends on lipid packing and acyl chain ordering. Our interest is to elaborate the chirality dependence of d- and l-menthol on membrane heterogeneity. We revealed physical differences between the two optical isomers of menthol on membrane heterogeneity by studying model membranes using nuclear magnetic resonance and microscopic observation.” As taken from Gusain P et al. 2017. *Membranes (Basel)* 7(4), E69. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29244740>

“Menthol induces a cooling sensation on the skin upon inhalation, oral ingestion, or topical application by stimulating the cold-sensitive receptors expressed on the skin, without actually causing a drop in the skin temperature.”

As taken from PubChem.

High-throughput Assay Data

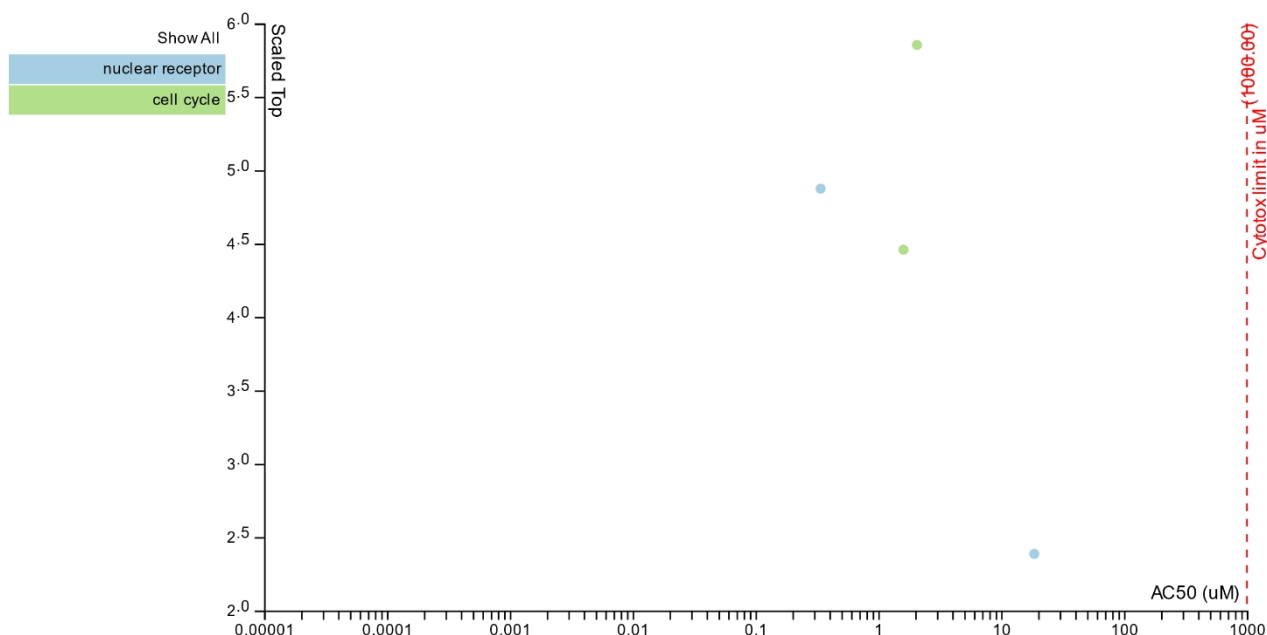
The US Environmental Protection Agency (EPA) evaluated L-menthol (CAS RN 2216-51-5) in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision

contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.”

A summary of the ToxCast assay data on L-menthol is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

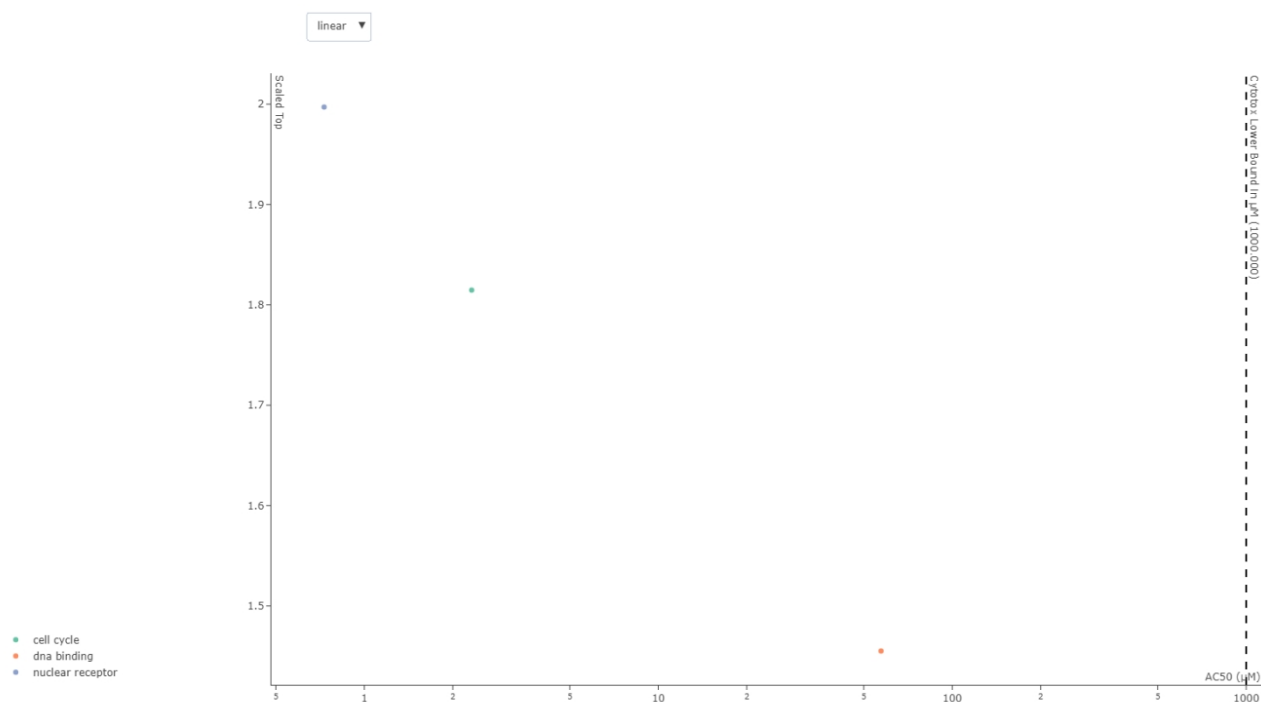
Figure 1



The US Environmental Protection Agency (EPA) evaluated menthol (CAS RN 89-78-1) in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

A summary of the ToxCast assay data on menthol is provided below in Figure 2. Figure 2 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 2



This ingredient was considered as part of an overall safety assessment of ingredients added to oral product (snus/nicotine pouch). Non-clinical in vitro studies were conducted to investigate the characteristics of extracts from tobacco free nicotine pouches alongside a reference snus product containing I-Menthol and/or 1R6F reference cigarette. Within the sensitivity and specificity of the bioassay(s) all smokeless products extracts were non-mutagenic, non-genotoxic, non-cytotoxic and non-irritant in contrast to results obtained for the 1R6F reference cigarette (Miller-Holt et al., 2022). Table below provides highest tested level(s) and specific endpoint(s).

Product type	Endpoint	Tested level (ppm)	Reference
Nicotine Pouch	In vitro genotoxicity	1600 1500	Labstat International Inc. (2020) Ökolab Test Report (2021)
	In vitro cytotoxicity	1600 1500	Labstat International Inc. (2020) Ökolab Test Report (2021)
	In vitro irritation (EpiGingival™ 3D)	1600	Charles River Laboratories (2022)

6. Functional effects on

6.1. Broncho/pulmonary system

“GeloMyrtol® forte (Myrtol®) is a phytomedicine obtained by distillation from essential oils. The trial was conducted to confirm the efficacy of Myrtol® in the treatment of acute bronchitis. METHODS: Patients with acute bronchitis and without confounding co-morbidity or co-medication were randomly assigned to treatment with either Myrtol® 300 mg 4 times daily or matched placebo in double-blind, parallel-group fashion. Signs and symptoms were evaluated by the investigator at baseline and after 7, 10 and 14 days of treatment; intake of medication, wellbeing and symptoms were recorded daily by the patient in the patients' diaries. FINDINGS: 413 patients were enrolled and randomised (Myrtol®: 202; Placebo: 211); 398 had at least one on-treatment efficacy evaluation (Myrtol®: 196; Placebo: 202). The mean change in coughing fits from D01 (baseline) to D07-D09 (after about one week treatment) was 62.1% (95% CI: 57.6-66.6%) and 49.8% (95% CI: 44.6-55.0%) for treatment with Myrtol® and placebo, respectively (p<0.0001). With Myrtol®, the

median time to 50% reduction in coughing fits was statistically significantly shorter and there were more patients without day-time coughing fits; there also were statistically significantly less day-time coughing fits, less difficulty coughing up, less sleep disturbance due to night-time coughing; with Myrtol® there was less symptomatic impairment (composite bronchitis severity score and subscores) and significant more patients had a clinically satisfying response to the investigational treatment. Both treatments were generally well tolerated. CONCLUSIONS: Myrtol® is statistically significantly superior to placebo in treating acute bronchitis.” As taken from Gillissen A et al. 2013. Drug Res. (Stuttg.) 63(1), 19-27. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23447044>

“The cold-sensitive cation channel TRPM8 is a target for menthol, which is used routinely as a cough suppressant and as an additive to tobacco and food products. Given that cold temperatures and menthol activate neurons through gating of TRPM8, it is unclear how menthol actively suppresses cough. In this study we describe the antitussive effects of (-)-menthol in conscious and anesthetized guinea pigs. In anesthetized guinea pigs, cough evoked by citric acid applied topically to the tracheal mucosa was suppressed by menthol only when it was selectively administered as vapors to the upper airways. Menthol applied topically to the tracheal mucosa prior to and during citric acid application or administered continuously as vapors or as an aerosol to the lower airways was without effect on cough. These actions of upper airway menthol treatment were mimicked by cold air delivered to the upper airways but not by (+)-menthol, the inactive isomer of menthol, or by the TRPM8/TRPA1 agonist icilin administered directly to the trachea. Subsequent molecular analyses confirmed the expression of TRPM8 in a subset of nasal trigeminal afferent neurons that do not coincidentally express TRPA1 or TRPV1. We conclude that menthol suppresses cough evoked in the lower airways primarily through a reflex initiated from the nose.” As taken from Plevkova J et al. 1985. J. Appl. Physiol. 115(2), 268-74. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23640596>

Irritation of the respiratory tract was seen in workers in a factory in which the atmospheric levels ranged from 12-380 mg/m³ (BIBRA, 1990).

Damage to the nasal passages and sinuses was reported in rabbits which inhaled an aerosol of a 1 % solution of menthol daily for nine months. Similarly, lung changes indicative of irritation occurred in rats inhaling about 1.6 mg l-menthol/m³ for 6¾ hours/day, 5 days/week for 26 to 58 exposures, although no clear effects were observed at 0.9 mg/m³ (BIBRA, 1990).

No overt toxicity or damage to the respiratory tract occurred when groups of 4 rats inhaled up to approximately 0.9 mg l-menthol/m³, 6.75 hours/day, 5 days/week for a maximum of 52 exposures. At a dose of 1.6 mg/m³ the blood picture was normal, and detailed examination of a range of tissues revealed only effects on the lungs, with nearly all animals showing congestion and inflammation (BIBRA, 1990).

No overt toxicity was reported when four monkeys inhaled, for 8 hour/day, an aerosol providing about 40 mg menthol/kg bw/day for 14 days (BIBRA, 1990).

“Moderate irritant to mucous membranes on inhalation.” As taken from HSDB, 2003.

“In very few cases, all in children younger than 1 year, menthol applied to the nostrils or near the nose caused reflex apnea. Clinical signs were laryngospasm, spasm of the glottis or instant collapse, dyspnea, apnea, unconsciousness, cyanosis and hyperextensive extremities (Champeau, 1935, Kleinschmidt, 1935, Klinke, 1967, Lesoine, 1965, Leiber 1967, Martindale, 1982, Melis, 1989). This effect is assumed not to be a poisoning effect but a reflectory reaction of the nervus trigeminus (Kratschmer reflex) (Leiber, 1967).”

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

Human

Airway hyperresponsiveness was improved in 23 patients with mild asthma in which 10 mg menthol [not further specified] was administered twice daily by inhalation for 4 weeks; acute bronchodilatory effects were not found in a preliminary study (Tamaoki et al. 1995).

Guinea pig

Menthol is reported to be an antitussive agent [it suppresses coughing] in guinea pigs [no further details in secondary source] (Laude et al. 1994).

“BACKGROUND: Menthol (l-menthol) is a naturally-occurring cold receptor agonist commonly used to provide symptomatic relief for upper airway congestion. Menthol can also reduce the sensation of dyspnea. It is unclear whether the physiological action of menthol in dyspnea reduction is through its cold receptor agonist effect or whether associated mechanical changes occur in the upper airway. OBJECTIVE: To determine whether menthol inhalation alters upper airway resistance in humans. METHODS: A randomized, sham-controlled, single-blinded crossover study of inhaled menthol on upper airway resistance during semirecumbent quiet breathing in healthy subjects was conducted. Ten healthy participants (eight female) with a mean ($\pm$ SD) age of 21 ± 1.6 years completed the study. RESULTS: Nasal resistance before testing was similar on both occasions. No differences were found in respiratory frequency (mean $\pm$ SEM) (menthol 17.0 ± 1.1 cmH₂O/L/s; sham 16.9 ± 0.9 cmH₂O/L/s), minute ventilation (menthol 7.7 ± 0.5 cmH₂O/L/s; sham 7.9 ± 0.5 cmH₂O/L/s) or total inspiratory time/total breath time (menthol 0.4 ± 0.1 cmH₂O/L/s; sham 0.4 ± 0.1 cmH₂O/L/s). The upper airway resistance was similar during menthol (3.47 ± 0.32 cmH₂O/L/s) and sham (3.27 ± 0.28 cmH₂O/L/s) ($P = 0.33$) inhalation. CONCLUSION: Inhalation of menthol does not alter upper airway resistance in awake human subjects”. As taken from Pereira EJ et al. 2013. Can. Resp. J. 20, e1-e4. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23457678>

“INTRODUCTION: Chronic cough is a common clinical problem and there is a shortage of effective treatments for it. Within the group of transient receptor potential ion channels a receptor for the cooling substance menthol has been identified. This study aimed to assess whether pre-inhalation of dissolved, nebulised menthol could increase capsaicin cough thresholds and influence spirometric values. METHODS: Fourteen patients with chronic cough and airway sensitivity to environmental irritants and 15 control subjects were tested on three occasions. Each one inhaled a 1 mL of nebulised menthol solution of 0.5% or 1% or placebo (saline with 0.05% menthol) at each visit in a randomized and double-blind order. They were then provoked by capsaicin inhalation. RESULTS: Patients' cough thresholds differed significantly from the controls' on all three provocations ($P < 0.0001$). After inhalation of 1% menthol, the patients' cough thresholds were significantly higher ($P < 0.02$) compared to after placebo inhalation and to after 0.5% menthol inhalation ($P < 0.05$). The patients' peak inspiratory flows were significantly reduced after inhalation of the placebo (saline) ($P < 0.05$) but not after inhalation of 0.5% or 1% menthol. Forced inspiratory flows 50% were lowered after inhalation of placebo and of 0.5% menthol ($P < 0.05$) but not after 1% menthol. Among the controls, forced inspiratory flows 50% were lowered after only placebo inhalation ($P < 0.05$). CONCLUSIONS: In patients with chronic cough, pre-inhalation of menthol reduces cough sensitivity to inhaled capsaicin and influences inspiratory flows. The findings may provide scientific support for the common practice of using menthol as a reliever for variant airway discomfort. The use of menthol in different cigarette brands could be questioned since it could conceal the natural irritation following smoking”. As taken from Millqvist E et al. 2013. Resp. Med. 107, 433-438. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23266255>

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference

TDLo - Lowest published toxic dose	Oral	Human	10 mg/kg	Lungs, Thorax, or Respiration - other changes Nutritional and Gross Metabolic - other changes	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 86,262,2015
TDLo - Lowest published toxic dose	Administration onto the skin	Human	10 mg/kg	Lungs, Thorax, or Respiration - other changes Nutritional and Gross Metabolic - other changes Vascular - measurement of regional blood flow	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 86,262,2015

As taken from RTECS, 2017.

“Tobacco products containing flavorings, such as electronic nicotine delivery devices (ENDS) or e-cigarettes, cigars/cigarillos, waterpipes, and heat-not-burn devices (iQOS) are continuously evolving. In addition to increasing the exposure of teenagers and adults to nicotine containing flavoring products and flavoring enhancers, chances of nicotine addiction through chronic use and abuse also increase. These flavorings are believed to be safe for ingestion, but little information is available about their effects on the lungs. In this review, we have discussed the in vitro and in vivo data on toxicity of flavoring chemicals in lung cells. We have further discussed the common flavoring agents, such as diacetyl and menthol, currently available detection methods, and the toxicological mechanisms associated with oxidative stress, inflammation, mucociliary clearance, and DNA damage in cells, mice, and humans. Finally, we present potential biomarkers that could be utilized for future risk assessment. This review provides crucial parameters important for evaluation of risk associated with flavouring agents and flavoring enhancers used in tobacco products and ENDS. Future studies can be designed to address the potential toxicity of inhaled flavorings and their biomarkers in users as well as in chronic exposure studies.” As taken from Kaur G et al. 2018. Toxicology Letters 288, 143-155. PubMed, 2018, available at <https://www.ncbi.nlm.nih.gov/pubmed/29481849>

“Background: Inhalation of L-menthol as a transient receptor potential melastatin 8 agonist induces a cooling sensation in the airway. This cooling sensation induced through olfactory stimulation by L-menthol (OSM) alleviates dyspnea in healthy humans. Therefore, we investigated effects of OSM on the neural respiratory drive (NRD) and multidimensional aspects of dyspnea induced by inspiratory resistive loaded breathing in COPD. Methods: In total, 28 patients with COPD and 14 control participants were included in this randomized, single-blinded, placebo-controlled, crossover study. OSM was administered using an L-menthol-scented patch, whereas placebo was administered using olfactory stimulation by strawberry-scented patch: placebo (OSP). Sham condition was measured by breathing through a face mask without a OSP. Dyspnea was evoked by breathing through the inspiratory resistance of 20 or 30 cm H₂O/L/s and measured using the Multidimensional Dyspnea Profile. NRD was estimated using the electromyogram activities of the parasternal intercostal muscles. Results: Compared with sham and OSP, OSM did not alter the breathing pattern/timing and NRD during inspiratory resistive loading; however, it significantly alleviated the physical and mental breathing effort, air hunger, breathing discomfort, anxiety, and fear during inspiratory resistive loaded breathing in patients with COPD. However, although OSM reduced the air hunger, mental breathing effort, and unpleasantness, no significant improvement on affective dimension of dyspnea by OSM was observed in control participants. Conclusions: Our findings suggest that OSM activated the sense of inspiratory flow that achieved dyspnea relief in

patients with COPD.” As taken from Kanezaki M et al. 2020. Chest 157(6), 1455-1465. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/31981567/>

“Purpose of review: Chronic breathlessness is a troublesome symptom experienced by people with advanced malignant and nonmalignant disease. Disease-directed therapies are often insufficient in the management of chronic breathlessness. Therefore, pharmacological and nonpharmacological breathlessness-specific interventions should be considered for select patients. Recent findings: There is some evidence to support the use of low-dose opioids (≤ 30 mg morphine equivalents per day) for the relief of breathlessness in the short term. However, additional studies are needed to understand the efficacy of opioids for chronic breathlessness in the long term. Nonopioid therapies, including inspiratory muscle training, fan-to-face therapy, L-menthol and inhaled nebulized furosemide show some promise for the relief of breathlessness in advanced disease. There is insufficient evidence to support the use of anxiolytics and benzodiazepines and cannabis for chronic breathlessness. Summary: More research is needed to identify therapies for the management of chronic breathlessness.” As taken from Abdallah SJ et al. 2019. Curr. Opin. Support. Palliat. Care 13(3), 167-173. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31335450/>

6.2. Cardiovascular system

Type:	Biochemical or cellular interactions
Result:	Menthol depressed the isolated heart of both the frog and the rabbit directly and dilated the coronary vessels. The frequency of beat was diminished, the power was increased. Ultimately the heart stopped in diastole.
Test substance:	unspecified isomer
Reliability:	(4) not assignable Documentation insufficient 25.02.2003 (36) (134) (135)

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

Heartbeat irregularities were observed in two individuals with excessive intakes of sweets containing peppermint oil (BIBRA, 1990).

“Menthol, a naturally occurring compound in mint, is known to give cold sensation. However, previous findings about its pharmacological activity in blood vessels are full of paradox. The present study investigated the action of menthol on vascular reactivity in different arteries isolated from male Sprague-Dawley rats, e.g. aortae, main mesenteric arteries and coronary arteries. The arterial segments were suspended in organ bath or in wire myograph for measurement of isometric force. Menthol concentration-dependently relaxed all three arteries with similar relaxing sensitivity in arteries contracted by different contractors, KCl, U46619 (9,11-dideoxy-9 α ,11 α -methanoepoxy Prostaglandin F₂ α) and phenylephrine. Menthol-induced relaxations were unaffected by nitric oxide synthase inhibitor L-NAME, soluble guanylyl cyclase inhibitor ODQ, or mechanical removal of endothelium. Menthol also concentration-dependently suppressed 60mM KCl-induced constriction

and CaCl₂-induced contraction in Ca²⁺-free K⁺-containing solution. Calcium fluorescent imaging using fluo-4 showed that 10min-incubation with 1mM menthol inhibited 60mM KCl-induced Ca²⁺ influx in rat aortic smooth muscle cell line A7r5 and vascular smooth muscle of coronary arteries. To conclude, menthol induces relaxation and inhibits contraction in rat aortae, mesenteric and coronary arteries primarily through inhibiting Ca²⁺ influx via nifedipine-sensitive Ca²⁺ channels in vascular smooth muscle". As taken from Cheang WS et al. 2013. Eur. J. Pharmacol. 702, 79-84. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23380688>

"CONTEXT: Injury management commonly includes decreasing arterial blood flow to the affected site in an attempt to reduce microvascular blood flow and edema and limit the induction of inflammation. Applied separately, ice and menthol gel decrease arterial blood flow, but the combined effects of ice and menthol gel on arterial blood flow are unknown. OBJECTIVES: To compare radial artery blood flow, arterial diameter, and perceived discomfort before and after the application of 1 of 4 treatment conditions. DESIGN: Experimental crossover design. SETTING: Clinical laboratory. PARTICIPANTS OR OTHER PARTICIPANTS: Ten healthy men, 9 healthy women (mean age = 25.68 years, mean height = 1.73 m, mean weight = 76.73 kg). INTERVENTION(S): Four treatment conditions were randomly applied for 20 minutes to the right forearm of participants on 4 different days separated by at least 24 hours: (1) 3.5 mL menthol gel, (2) 0.5 kg of crushed ice, (3) 3.5 mL of menthol gel and 0.5 kg of crushed ice, or (4) no treatment (control). MAIN OUTCOME MEASURE(S): Using high-resolution ultrasound, we measured right radial artery diameter (cm) and blood flow (mL/min) every 5 minutes for 20 minutes after the treatment was applied. Discomfort with the treatment was documented using a 1-to-10 intensity scale. RESULTS: Radial artery blood flow decreased ($P < .05$) from baseline in the ice (-20% to -24%), menthol (-17% to -24%), and ice and menthol (-36% to -39%) treatments but not in the control (3% to 9%) at 5, 10, and 15 minutes. At 20 minutes after baseline, only the ice (-27%) and combined ice and menthol (-38%) treatments exhibited reductions in blood flow ($P < .05$). Discomfort was less with menthol than with the ice treatment at 5, 10, and 20 minutes after application ($P < .05$). Arterial diameter and heart rate did not change. CONCLUSIONS: The application of 3.5 mL of menthol was similar to the application of 0.5 kg of crushed ice in reducing peripheral blood flow. Combining crushed ice with menthol appeared to have an additive effect on reducing blood flow." As taken from Topp R et al. 2013a. J. Athl. Train. 48(2), 220-5. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23672386>

"To investigate whether capsaicin and menthol stimuli elicit characteristic responses in facial skin blood flow (SkBF), we observed the facial SkBF response to low and high concentrations of capsaicin and menthol stimuli of 1-ml solution applied to the oral cavity for 20s in 17 healthy subjects. High concentration of capsaicin significantly increased the SkBF in all of the facial areas monitored. High concentration of menthol stimulus significantly decreased SkBF in the nose and increased that in the eyelid, and upper and lower lips. These results demonstrated that capsaicin and menthol stimuli in the oral cavity elicit characteristic responses in facial SkBF." As taken from Kashima H and Hayashi N. 2013. Auton. Neurosci. 174(1-2), 61-5. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23265982>

"BACKGROUND: Menthol chemically triggers cold-sensitive receptors in the skin without conductive skin cooling. We investigated the effects of menthol-induced activation of cutaneous cold receptors on the cold-induced vasodilation (CIVD) of the finger. We hypothesized that the menthol application would attenuate typical CIVD responses. METHODS: 1.5% L-menthol was fully applied over the left hand and forearm, and then, the middle finger of the left hand was immersed into 4 °C water for 30 min. A trial consisted of 10-min rest followed by 30-min immersion and 20-min recovery in 28 °C air temperature with 20% relative humidity. Another trial without the menthol application was carried out as a control. Seventeen females (24.2 ± 2.6 years in age, 160.5 ± 5.1 cm in height, and 51.2 ± 5.7 kg in body weight) participated in the two trials. RESULTS: The results showed that the maximum and average temperatures of the finger during the water immersion were lower in the menthol application when compared to control ($P < 0.05$), whereas no significant differences appeared in the onset time of CIVD, the frequency of CIVD, and minimum finger

temperature. These results imply that stronger stimulation of cold receptors without additional conductive skin cooling did not attenuate the triggering of CVD responses but intensified vasoconstriction after the first occurrence of CVD. CONCLUSION: It is suggested that substantial and conductive heat loss through the skin along with activation of cold receptors may be required to retain rewarming at a certain level.” As taken from Kim S and Lee JY. 2018. J. Physiol. Anthropol. 37(1), 14. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29739454>

6.3. Nervous system

Human

“Excessive” smoking of mentholated cigarettes (to deliver approximately 20 mg/kg bw, 3 times per day, for 7 days) caused an individual to report difficulty in mental concentration (Luke, 1962).

According to an early report, ingestion of menthol [unspecified quantity] by a young child induced dizziness, muscular rigidity and effects on reflexes (Wetzel, 1943).

“Recent research has shown that chewing gum increases subjective alertness. Upper respiratory tract illnesses (URTIs) lead to reduced alertness, and it has been shown that stimulants such as caffeine can reverse this effect. It is now important to determine whether menthol chewing gum can produce a similar effect. Young adults with an URTI (N = 16) and a healthy control group (N = 12) rated their mood and symptoms on two occasions (the first when those with a URTI were ill and the second when both groups were healthy). During each session, volunteers provided a rating while chewing gum and when they were not chewing. Those with a URTI reported reduced alertness, which persisted into convalescence. Chewing gum was associated with greater alertness in both those with URTIs and the healthy group. Chewing gum reduced the severity of nasal symptoms in those with a cold. The effects of the URTI and menthol gum may reflect changes in trigeminal stimulation.” As taken from Smith AP and Boden C. 2013. Stress Health 29(2), 138-42. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22674677>

“This study examined the separate effects of caffeine and menthol on cognition and mood during simulated firefighting in the heat. Participants (N = 10) performed three trials in a counterbalanced order, either with 400 mg caffeine, menthol lozenges, or placebo. The simulated firefighting consisted of 2 bouts of 20-min treadmill exercise and one bout of 20-min stepping exercise in the heat with two brief 15-min rest periods between each exercise phase. Exercise induced significant dehydration (>3%) and elevated rectal temperature (>38.9 °C), for all three conditions. Neither caffeine nor menthol reduced perceived exertion compared to placebo (p > 0.05). Mood ratings (i.e., alertness, hedonic tone, tension) significantly deteriorated over time (p < 0.05), but there was no difference among the three conditions. Simple reaction time, short-term memory, and retrieval memory did not alter with treatments or repeated evaluations. Reaction accuracy from a math test remained unchanged throughout the experimental period; reaction time from the math test was significantly faster after exposure to the heat (p < 0.05). It is concluded that, exhaustive exercise in the heat severely impacted mood, but minimally impacted cognition. These treatments failed to show ergogenic benefits in a simulated firefighting paradigm in a hot environment.” As taken from Zhang Y et al. 2014. Appl. Ergon. 45(3), 510-4. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23891504>

Rat

The reaction of the central nervous system to stimulants was unaffected in animals (40 males and 40 females per group) fed diets providing either l-menthol or dl-menthol at up to approximately 200 mg/kg bw/day for 5.5 weeks (Herken, 1961, unpublished study cited in IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>).

Signs in rats given a lethal oral dose of menthol (LD50 2.9-4.0 g/kg bw) included “unsteadiness” (Jenner et al., 1964).

Mouse

Psycholeptic [tranquilizing] effects were reported in mice treated with menthol [no further details in English abstract] (Le Bourhis & Soenen, 1973).

Excitation was induced in mice exposed dermally to l-menthol at approximately 1.25 g/kg bw in ethanol (Macht, 1939).

“Menthol is one of the most commonly used chemicals in our daily life, not only because of its fresh flavor and cooling feeling but also because of its medical benefit. Previous studies have suggested that menthol produces analgesic action in acute and neuropathic pain through peripheral mechanisms. However, the central actions and mechanisms of menthol remain unclear. Here, we report that menthol has direct effects on the spinal cord. Menthol decreased both ipsilateral and contralateral pain hypersensitivity induced by complete Freund's adjuvant in a dose-dependent manner. Menthol also reduced both first and second phases of formalin-induced spontaneous nocifensive behavior. We then identified the potential central mechanisms underlying the analgesic effect of menthol. In cultured dorsal horn neurons, menthol induced inward and outward currents in a dose-dependent manner. The menthol-activated current was mediated by Cl^- and blocked by bicuculline, suggesting that menthol activates γ -aminobutyric acid type A receptors. In addition, menthol blocked voltage-gated sodium channels and voltage-gated calcium channels in a voltage-, state-, and use-dependent manner. Furthermore, menthol reduced repetitive firing and action potential amplitude, decreased neuronal excitability, and blocked spontaneous synaptic transmission of cultured superficial dorsal horn neurons. Liquid chromatography/tandem mass spectrometry analysis of brain menthol levels indicated that menthol was rapidly concentrated in the brain when administered systemically. Our results indicate that menthol produces its central analgesic action on inflammatory pain probably via the blockage of voltage-gated Na^+ and Ca^{2+} channels. These data provide molecular and cellular mechanisms by which menthol decreases neuronal excitability, therefore contributing to menthol-induced central analgesia”. As taken from Pan R et al. 2012. J. Pharmacol. Exp. Ther. 343(3), 661-72. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22951274>

“BACKGROUND AND PURPOSE: Menthol, a naturally occurring compound in the essential oil of mint leaves, is used for its medicinal, sensory and fragrant properties. Menthol acts via transient receptor potential (TRPM8 and TRPA1) channels and as a positive allosteric modulator of recombinant GABAA receptors. Here, we examined the actions of menthol on GABAA receptor-mediated currents in intact midbrain slices. EXPERIMENTAL APPROACH: Whole cell voltage-clamp recordings were made from periaqueductal grey (PAG) neurons in midbrain slices to determine the effects of menthol on GABAA receptor-mediated phasic inhibitory postsynaptic currents (IPSCs) and tonic currents. KEY RESULTS: Menthol (150 - 750 μM) produced a concentration-dependent prolongation of spontaneous GABAA receptor-mediated IPSCs, but not non-NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) throughout the PAG. Menthol actions were unaffected by TRPM8 and TRPA1 antagonists, tetrodotoxin and the benzodiazepine antagonist, flumazenil. Menthol also enhanced a tonic current, which was sensitive to the GABAA receptor antagonists, picrotoxin (100 μM), bicuculline (30 μM) and Zn^{2+} (100 μM), but unaffected by gabazine (10 μM) and the GABAC receptor antagonist TPMPA (50 μM). In addition, menthol potentiated currents induced by the extrasynaptic GABAA receptor agonist THIP/gaboxadol (10 μM). CONCLUSIONS AND IMPLICATIONS: These results suggest that menthol positively modulates both synaptic and extrasynaptic populations of GABAA receptors in native PAG neurons. The development of agents which potentiate GABAA-mediated tonic currents and phasic IPSCs in a manner similar to menthol could provide a basis for novel GABAA-related pharmacotherapies.” As taken from Lau BK et al. 2014. Br. J. Pharmacol. 171(11), 2803-13. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/24460753>

“Both spinal and trigeminal somatosensory systems use the TRPM8 channel as a principal transducer for detecting cold stimuli. It is currently unclear whether this cold transducer may play a

role in trigeminal neuropathic pain manifesting cold allodynia and hyperalgesia. In the present study, trigeminal neuropathy was induced by chronic constrictive nerve injury of the infraorbital nerve (ION-CCI). Behavioral responses to cold stimuli in orofacial regions were assessed by the newly developed orofacial operant test in the ION-CCI rats. We tested menthol and capsazepine, two compounds that can activate and inhibit TRPM8 respectively, on orofacial operant responses to cold stimuli in ION-CCI rats. Testing animals performed operant tasks by voluntarily contacting their orofacial regions to a cold stimulation module in order to access sweetened milk as a reward, and contact time and number of the operant behaviors were automatically recorded. Total contact time was significantly reduced at the cooling temperatures of 17°C and 12°C in ION-CCI group in comparison with sham group, indicating the presence of cold allodynia and hyperalgesia in ION-CCI rats. When menthol was administered to ION-CCI rats, total contact time was further reduced and total contact number increased at the cooling temperatures. In contrast, after administration of capsazepine to ION-CCI rats, total contact time was significantly increased at the cooling temperatures. The behavioral outcomes support the idea that TRPM8 plays a role in cold allodynia and hyperalgesia following chronic trigeminal nerve injury.” As taken from Zuo X et al. 2013. Mol. Pain 9, 28. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23767981>

“AIMS: Transient receptor potential (TRP) vanilloid-1 (TRPV1) and melastatin-8 (TRPM8) channels play a role in transmitting sensory information in primary-afferent neurons. TRPV1 agonists at high concentrations inhibit action potential conduction in the neurons and thus have a local anesthetic effect. The purpose of the present study was to know whether TRPM8 agonist menthol at high concentrations has a similar action and if so whether there is a structure-activity relationship among menthol-related chemicals. MAIN METHODS: Compound action potentials (CAPs) were recorded from the frog sciatic nerve by using the air-gap method. KEY FINDINGS: (-)-Menthol and (+)-menthol concentration-dependently reduced CAP peak amplitude with the IC(50) values of 1.1 and 0.93 mM, respectively. This (-)-menthol activity was resistant to non-selective TRP antagonist ruthenium red; TRPM8 agonist icilin did not affect CAPs, indicating no involvements of TRPM8 channels. p-Menthane, (+)-limonene and menthyl chloride at 7-10 mM minimally affected CAPs. On the other hand, (-)-menthone, (+)-menthone, (-)-carvone, (+)-carvone and (-)-carveol (in each of which chemicals OH or O group was added to p-menthane and limonene) and (+)-pulegone inhibited CAPs with extents similar to that of menthol. 1,8-Cineole and 1,4-cineole were less effective while thymol and carvacrol were more effective than menthol in inhibiting CAPs. SIGNIFICANCE: Menthol-related chemicals inhibited CAPs and were thus suggested to exhibit local anesthetic effects comparable to those of lidocaine and cocaine as reported previously for frog CAPs. This result may provide information to develop local anesthetics on the basis of the chemical structure of menthol.” As taken from Kawasaki H et al. 2013. Life Sci. 92(6-7), 359-67. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23352972>

“Oral analgesics are commonly prescribed for the treatment of acute and chronic pain, but these agents often produce adverse systemic effects, which sometimes are severe. Topical analgesics offer the potential to provide the same analgesic relief provided by oral analgesics but with minimal adverse systemic effects. This article describes the results of a systematic review of the efficacy of topical analgesics in the management of acute and chronic pain conditions. A literature search of MEDLINE/PubMed was conducted using the keywords topical analgesic AND chronic pain OR acute pain OR neuropathic pain and focused only on individual clinical trials published in English-language journals. The search identified 92 articles, of which 65 were eligible for inclusion in the review. The most commonly studied topical analgesics were nonsteroidal anti-inflammatory drugs (n=27), followed by lidocaine (n=9), capsaicin (n=6), amitriptyline (n=5), glyceryl trinitrate (n=3), opioids (n=2), menthol (n=2), pimecrolimus (n=2), and phenytoin (n=2). The most common indications were acute soft tissue injuries (n=18), followed by neuropathic pain (n=17), experimental pain (n=6), osteoarthritis and other chronic joint-related conditions (n=5), skin or leg ulcers (n=5), and chronic knee pain (n=2). Strong evidence was identified for the use of topical diclofenac and topical ibuprofen in the treatment of acute soft tissue injuries or chronic joint-related conditions, such as osteoarthritis. Evidence also supports the use of topical lidocaine in the treatment of

postherpetic neuralgia and diabetic neuropathy. Currently, limited evidence is available to support the use of other topical analgesics in acute and chronic pain.” As taken from Argoff CE. 2013. Mayo Clin. Proc. 88(2), 195-205. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23374622>

“This study was designed to investigate the effect of surrogate orofacial pain models on the quantitative sensory testing (QST) profile in healthy participants. Capsaicin, menthol, or saline (control) were applied topically onto the gingiva of 15 healthy subjects for 15 min. During application, the subjects rated pain intensity on a score of 0-10, on an electronic visual analog scale (VAS). A standardized intra-oral QST protocol was performed before and immediately after application. Data obtained before and after application were compared using rank-sum tests, and QST profiles were made after Z-transformation. Application of capsaicin caused moderate levels of pain (VAS(peak) = 6.0 ± 0.7), and application of menthol produced mild levels of pain (VAS(peak) = 1.8 ± 0.6). Capsaicin induced hypersensitivity to warmth, heat pain and cold pain and hyposensitivity to mechanical stimuli. Menthol induced hypersensitivity to cold and warmth. Saline caused hypersensitivity to heat pain and hyposensitivity to mechanical stimuli. However, somatosensory profiles from Z-scores demonstrated sensory gains regarding warmth detection and heat pain only after application of capsaicin. In conclusion, a standardized battery of QST showed somatosensory changes after application of capsaicin, menthol and saline to the gingiva. However, the Z-score-based profiles may only reflect the most prominent somatosensory changes and thus represent a conservative approach for evaluation of data.” As taken from Lu S et al. 2013. Eur. J. Oral Sci. 121(1), 29-35. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23331421>

“Osteoarthritis (OA) is a common health problem with symptoms including reduced functioning and joint pain. Protracted pharmacological management of knee OA is associated with side effects including gastrointestinal, renal, and neurological dysfunction. Menthol gels have been used with limited empirical support to relieve pain and improve functioning among individual with OA. The purpose of this study was to compare the ability to complete functional tasks and knee pain while completing functional tasks among patients with knee OA after topical application of either 3.5% menthol gel or an inert placebo gel. Twenty individuals with knee OA volunteered to complete 2 data collection visits 1 week apart. Subjects underwent the same data collection at each visit including the performance of functional tasks and self-reporting knee pain while performing each task. The functional tasks included a 6-Minute Walk (6-MW), the Timed Get Up and Go (TUG), 30-second timed chair stand (TCS), and time to ascend (Up stairs) and descend (Down stairs) a flight of stairs. Subjects reported their knee pain immediately following each functional task using a 100-mm visual analog scale. These assessments of pain and functioning were measured twice at each subject visit: upon arrival at the facility without any intervention and again during the same visit after random application to the OA knee of 5 mL of 3.5% menthol gel or 5 mL of an inert gel. There were no significant between-group differences or time by treatment interaction in performance of any of the functional tasks, or measures of pain, at any of the data collection time points. However, there were significant within-group differences. Scores on the 6-MW, TCS, and Down stairs functional tasks improved significantly following the application of menthol gel. Scores on the Down stairs functional task improved significantly following application of the placebo gel. The menthol intervention resulted in significant reductions in pain during the TUG, TCS, Up stairs, and Down stairs tasks. The placebo condition did not result in any significant changes in pain during the functional tasks. There were no differences detected in functional tasks or pain following the placebo and menthol conditions. These findings provide partial support regarding the efficacy of menthol gel to improve functioning and reduce pain among knee OA patients.” As taken from Topp R et al. 2013b. J Geriatr Phys Ther. 36(2), 92-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22976810>

“Menthol, a natural product of the peppermint plant *Mentha x piperita* (Lamiaceae), is a monoterpene which is widely used as a natural product in cosmetics, a flavoring agent, and as an intermediate in the production of other compounds. Various extracts from peppermint contain

menthol as a major active constituent and have been used for centuries as traditional medicines for a number of ailments including infections, insomnia, and irritable bowel syndrome as well as an insect repellent. Menthol's characteristic cooling sensation is due, in part, to the activation of sensory neurons generally termed transient receptor potential (TRP) channels, in particular transient receptor potential melastatin family member 8 (TRPM8) and transient receptor potential subfamily A, member 1 (TRPA1). Menthol acts upon TRPM8 receptors by rapidly increasing intracellular calcium and mobilizing calcium flux through the channels to induce cold response signals at the application site. Aside from its cold-inducing sensation capabilities, menthol exhibits cytotoxic effects in cancer cells, induces reduction in malignant cell growth, and engages in synergistic excitation of GABA receptors and sodium ion channels resulting in analgesia. Notwithstanding its plethora of benefits, menthol's cold-sensitivity response mechanism has been shown to inhibit mucosal recognition of nicotine and cigarette toxins common in mentholated cigarette brands thus potentially leading to toxic effects. Menthol may prove a valuable lead structure for the synthesis of drugs that target multiple receptors involved with a number of pharmacological effects." As taken from Farco JA and Grundmann O, 2013. *Mini Rev Med Chem.* 13(1), 124-31. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23061635>

In a review of the potential toxicity of terpenic derivatives, including menthol, the CHMP concluded that the data confirms the concerns that suppositories containing terpenic derivatives can induce neurological disorders, especially convulsions, in children less than 30 months (EMA, 2012).

"BACKGROUND: Activation of TRPM8 and TRPA1 receptors generates cold and cold pain sensations, respectively, and is presumably important in clinical pain manifestations, such as cold hyperalgesia. This study investigated the interaction between TRPM8 and TRPA1 receptors through stimulation of glabrous human skin (volar forearm) by topical administration of 40% L-menthol and 10% trans-cinnamaldehyde (CA), individually and in combination. METHODS: Sensory manifestations were assessed in 10 healthy volunteers via a platform of 11 quantitative sensory (thermal and mechanical stimuli) and vasomotor tests (skin temperature, perfusion and axon-reflex-flare) in a double-blinded randomized crossover design. RESULTS: Cold pain threshold was increased ($p < 0.01$, cold allodynia) by L-menthol alone and L-menthol + CA in combination but unaffected by CA. Mechanical pain threshold was significantly decreased (mechanical hyperalgesia) by all three substances ($p < 0.01$), with a significant intergroup difference found between CA alone and the less decreased L-menthol + CA ($p < 0.05$). Application of CA alone and L-menthol + CA in combination showed an increase in skin temperature and perfusion significantly larger than that induced by L-menthol alone ($p < 0.05$). An axon-reflex-flare was present after CA administration, but was significantly reduced upon addition of L-menthol ($p < 0.01$). CONCLUSION: This study elucidates the potential of L-menthol as a counter-irritant to secondary neurogenic inflammation and provides evidence of an intricate interplay between cold receptors TRPA1 and TRPM8, warranting further investigation of the neural coding of cold pain perception." As taken from Olsen RV et al. 2014. *Eur. J. Pain* 18(9), 1333-42. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/24664788>

"A single exposure to menthol can, depending on concentration, enhance both cool sensations and encourage body heat storage. This study tested whether there is an habituation in either response after repeated-daily exposures. Twenty-two participants were assigned to one of three spray groups: Control (CON; $n=6$), 0.05% l-menthol (M0.05%; $n=8$), and 0.2% l-menthol (M0.2%; $n=8$). On Monday (20°C, 50% rh) participants were sprayed with 100mL of solution and undertook 40min of cycling at 45% of their peak power (Ex1), from Tuesday to Thursday (30°C, 50% rh) they were sprayed twice daily whilst resting (R1 to R6), Friday was a repeat of Monday (Ex2). Thermal sensation (TS), thermal comfort, perceived exertion, irritation, rectal and skin temperature (Tsk), skin blood flow (SkBF) and sweat rate were monitored. A two-way ANOVA ($\alpha=0.05$) compared responses from the beginning (Ex1, R1) and end (Ex2, R5) of the testing week. M0.2% induced significantly ($P<0.05$) cooler TS at the beginning of the week (Ex1, R1) compared to the end (Ex2, R5), indicating habituation of TS; this was not observed in M0.05%. No other perceptual or

physiological responses habituated. 0.2% Menthol caused a heat storage response, mediated by vasoconstriction, at the beginning and end of the week, suggesting the habituation of TS occurred in a pathway specific to sensation. In summary, the cooling influence of 0.2% menthol habituates after repeated-daily exposures, but with no habituation in heat storage.” As taken from Jason Gillis D et al. 2015. *Physiol. Behav.* 139, 511-8. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25484357>

“L-menthol stimulates cutaneous thermoreceptors and induces cool sensations improving thermal comfort, but has been linked to heat storage responses; this could increase risk of heat illness during self-paced exercise in the heat. Therefore, L-menthol application could lead to a discrepancy between behavioral and autonomic thermoregulatory drivers. Eight male participants volunteered. They were familiarized and then completed two trials in hot conditions (33.5 °C, 33% relative humidity) where their t-shirt was sprayed with CONTROL-SPRAY or MENTHOL-SPRAY after 10 km (i.e., when they were hot and uncomfortable) of a 16.1-km cycling time trial (TT). Thermal perception [thermal sensation (TS) and comfort (TC)], thermal responses [rectal temperature (T_{rec}), skin temperature (T_{skin})], perceived exertion (RPE), heart rate, pacing (power output), and TT completion time were measured. MENTHOL-SPRAY made participants feel cooler and more comfortable and resulted in lower RPE (i.e., less exertion) yet performance was unchanged [TT completion: CONTROL-SPRAY 32.4 (2.9) and MENTHOL-SPRAY 32.7 (3.0) min]. T_{rec} rate of increase was 1.40 (0.60) and 1.45 (0.40) °C/h after CONTROL-SPRAY and MENTHOL-SPRAY application, which were not different. Spraying L-menthol toward the end of self-paced exercise in the heat improved perception, but did not alter performance and did not increase heat illness risk.” As taken from Barwood MJ et al. 2015. *Scand. J. Med. Sci. Sports* 25(Suppl. 1), 211-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25943672>

“Topical high-concentration L-menthol is the only established human experimental pain model to study mechanisms underlying cold hyperalgesia. We aimed at investigating the combinatorial effect of cold stimuli and topical L-menthol on cold pain and secondary mechanical hyperalgesia. Analogue to the heat-capsaicin model on skin sensitization, we proposed that cold/menthol enhances or prolong L-menthol-evoked sensitization. Topical 40% L-menthol or vehicle was applied (20 minutes) on the volar forearms of 20 healthy females and males (age, 28.7 ± 0.6 years). Cold stimulation of 5°C for 5 minutes was then applied to the treated area 3 times with 40-minute intervals. Cold detection threshold and pain, mechanical hyperalgesia (pinprick), static and dynamic mechanical allodynia (von Frey and brush), skin blood flow (laser speckle), and temperature (thermocamera) were assessed. Cold detection threshold and cold pain threshold (CPT) increased after L-menthol and remained high after the cold rekindling cycles (P < 0.001). L-menthol evoked secondary hyperalgesia to pinprick (P < 0.001) particularly in females (P < 0.05) and also induced secondary allodynia to von Frey and brush (P < 0.001). Application of cold stimuli kept these areas enlarged with a higher response in females to brush after the third cold cycle (P < 0.05). Skin blood flow increased after L-menthol (P < 0.001) and stayed stable after cold cycles. Repeated application of cold on skin treated by L-menthol facilitated and prolonged L-menthol-induced cold pain and hyperalgesia. This model may prove beneficial for testing analgesic compounds when a sufficient duration of time is needed to see drug effects on CPT or mechanical hypersensitivity.” As taken from Andersen HH et al. 2015. *Pain* 156(5), 880-9. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25719613>

“PURPOSE: The study investigated the effect of a non-thermal cooling agent, L-menthol, on exercise at a fixed subjective rating of perceived exertion (RPE) in a hot environment. METHOD: Eight male participants completed two trials at an exercise intensity between 'hard' and 'very hard', equating to 16 on the RPE scale at ~35 °C. Participants were instructed to continually adjust their power output to maintain an RPE of 16 throughout the exercise trial, stopping once power output had fallen by 30%. In a randomized crossover design, either L-menthol or placebo mouthwash was administered prior to exercise and at 10 min intervals. Power output, [Formula: see text]O₂, heart rate, core and skin temperature was monitored, alongside thermal sensation and thermal comfort. Isokinetic peak power sprints were conducted prior to and immediately after the fixed RPE trial.

RESULTS: Exercise time was greater ($23:23 \pm 3:36$ vs. $21:44 \pm 2:32$ min; $P = 0.049$) and average power output increased (173 ± 24 vs. 167 ± 24 W; $P = 0.044$) in the L-menthol condition. Peak isokinetic sprint power declined from pre-post trial in the L-menthol I (9.0%; $P = 0.015$) but not in the placebo condition (3.4%; $P = 0.275$). Thermal sensation was lower in the L-menthol condition ($P = 0.036$), despite no changes in skin or core temperature ($P > 0.05$). CONCLUSION: These results indicate that a non-thermal cooling mouth rinse lowered thermal sensation, resulting in an elevated work rate, which extended exercise time in the heat at a fixed RPE." As taken from Flood TR et al. 2017. Eur. J. Appl. Physiol. 117(7), 1501-1512. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28508114>

"Fluid ingestion is necessary for life, and thirst sensations are a prime motivator to drink. There is evidence of the influence of oropharyngeal stimulation on thirst and water intake in both animals and humans, but how those oral sensory cues impact thirst and ultimately the amount of liquid ingested is not well understood. We investigated which sensory trait(s) of a beverage influence the thirst quenching efficacy of ingested liquids and the perceived amount ingested. We deprived healthy individuals of liquid and food overnight (> 12 hours) to make them thirsty. After asking them to drink a fixed volume (400 mL) of an experimental beverage presenting one or two specific sensory traits, we determined the volume ingested of additional plain, 'still', room temperature water to assess their residual thirst and, by extension, the thirst-quenching properties of the experimental beverage. In a second study, participants were asked to drink the experimental beverages from an opaque container through a straw and estimate the volume ingested. We found that among several oro-sensory traits, the perceptions of coldness, induced either by cold water (thermally) or by l-menthol (chemically), and the feeling of oral carbonation, strongly enhance the thirst quenching properties of a beverage in water-deprived humans (additional water intake after the 400 ml experimental beverage was reduced by up to 50%). When blinded to the volume of liquid consumed, individual's estimation of ingested volume is increased ($\sim 22\%$) by perceived oral cold and carbonation, raising the idea that cold and perhaps CO₂ induced-irritation sensations are included in how we normally encode water in the mouth and how we estimate the quantity of volume swallowed. These findings have implications for addressing inadequate hydration state in populations such as the elderly." As taken from Peyrot des Gachons C 2016. PLoS One 11(9), e0162261. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27685093>

"The constituents of essential oils are widely found in foods and aromatic plants giving characteristic odor and flavor. However, pharmacological studies evidence its therapeutic potential for the treatment of several diseases and promising use as compounds with analgesic-like action. Considering that pain affects a significant part of the world population and the need for the development of new analgesics, this review reports on the current studies of essential oils' chemical constituents with analgesic-like activity, including a description of their mechanisms of action and chemical aspects." As taken from de Cássia da Silveira E Sá R et al. 2017. Int. J. Mol. Sci. 18(12), E2392. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29232831>

"Major depression disorder, also known as depression, with a significant and persistent low mood as the main clinical features, is the main type of mood disorders. L-menthol (LM), the main active ingredient of mint, has been considered as safe and healthy natural ingredient by the Food and Drug Administration in the USA. In this study, LM (40 mg/kg, i.g.) produced antidepressant-like effect in the forced swimming test (FST) in mice. The sub-effective dose (5 mg/kg, i.g.) of LM combined with the sub-effective dose of fluoxetine (5 mg/kg, i.p.) or reboxetine (2.5 mg/kg, i.p.) could significantly shorten the immobility time in the FST. Pretreatment with ondansetron (a highly selective 5-HT₃ receptor antagonist, 8 mg/kg, i.p.), bicuculline [a competitive γ -aminobutyric acid (GABA) antagonist, 4 mg/kg, i.p.] and haloperidol (a non-selective D₂ receptor antagonist, 0.2 mg/kg, i.p.) significantly reversed the antidepressant-like effect of LM (40 mg/kg, i.g.). In contrast, prazosin (a α 1-adrenoceptor antagonist, 1 mg/kg, i.p.) and N-methyl-D-aspartic acid (an agonist at the glutamate site, 75 mg/kg, i.p.) did not eliminate the antidepressant-like effect of LM. All of these above indicated that LM is able to induce an antidepressant-like effect mediated by the modification of 5-HT₃ergic, GABA_Aergic and DAergic systems in the FST. LM might be used as combination

therapy in depressed patients and is a potential antidepressant.” As taken from Wang W et al. 2019. Cogn. Neurodyn. 13(2), 191-200. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30956723>

“Temperature plays a critical role in the sensation of airflow in the nasal mucosa. Neural activities of the ethmoidal nerve, a trigeminal afferent, responding to airflow are suppressed by warm airflow, whereas cold airflow enhances the ethmoidal nerve activities, which is mimicked by application of menthol, a cold-sensitive TRPM8 receptor agonist. However, it has been an open issue how menthol modulates the spatiotemporal profiles of neural activities of somatosensory cortical neurons. In this study, we assessed neural responses to an air puff stimulation (100 ms) to the nasal cavity in the absence or presence of l-menthol using an optical imaging technique with a voltage-sensitive dye in the primary cortex (S1) of urethane-anesthetized rats. A weak air puff application (15 psi) without l-menthol induced neural excitation in a part of the contralateral S1. The air puff stimulation with l-menthol significantly increased the optical signal intensity, expanded the activated area, and shortened the latency, compared to those in the absence of l-menthol. These results suggest that activation of cold-sensitive TRPM8 receptors sharpens airflow sensation in the nasal cavity and expands the receptive field, especially toward the pharynx, which may contribute to enhanced flavor perception.” As taken from Fujita S et al. 2019. Neurosci. Lett. 696, 87092. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30553866>

“Menthol, after topical application, causes a feeling of coolness due to stimulation of 'cold' receptors by inhibiting Ca⁺⁺ currents of neuronal membranes. It may also yield analgesic properties via kappa-opioid receptor agonism.”

As taken from PubChem.

“The effects of inhalation of L-menthol, D-isomenthol and D-neomenthol, upon nasal resistance and sensation to airflow were investigated in 40 subjects. L-menthol caused a highly significant enhancement of nasal sensation of airflow but despite their great similarity in structure and a similar peppermint smell the isomers D-isomenthol and D-neomenthol had no effect on nasal sensation of airflow. These findings show that L-menthol has a specific pharmacological action on nasal sensory nerve endings which is not related to its peppermint smell. It seems that menthol acts upon trigeminal sensory nerve endings within the nose (Eccles et al, 1988).”

As taken from EMA, 2020.

“Repeated sensory exposure through frequent consumption of certain foods leads to changes in the processing of sensory cues. We investigated the influence of frequent minty chewing gum consumption on the neurobehavioral processing of intranasal trigeminal and olfactory stimuli. fMRI responses to a trigeminal minty odor (L-menthol), a non-trigeminal minty odor (L-carvone), and a non-trigeminal non-minty odor (strawberry) were assessed in 29 young adults (mean age = 23 years, SD = 4.3 years; mean BMI = 22.7, SD = 1.9; thirteen females), who were divided into a minty chewing gum high frequency (N = 18) and a low infrequency (N = 11) groups based on their self-reported frequency of chewing gum consumption. Participants' performance for odor lateralization, ratings for perceived odor intensity, pleasantness, familiarity, and pungency were also collected. Compared to the low frequency group, the high frequency group rated L-menthol as more familiar and performed better during the L-menthol lateralization test. The high frequency group compared to low frequency group showed stronger activation in trigeminal and somatosensory brain regions during perception of trigeminal component. These findings suggest an association between frequent minty odor exposure and increased neurobehavioral sensitivity to intranasal trigeminal stimulation.” As taken from Han P et al. 2020. Brain Res. 1730, 146663. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31931000/>

“(–)-menthol, a major form of menthol, is one of the most commonly used chemicals. Many studies have demonstrated that (–)-menthol produces analgesic action through peripheral mechanisms which are mainly mediated by activation of TRPM8. Moreover, intrathecal injection of menthol induces analgesia as well. However, the central actions and mechanisms of (–)-menthol remain

unclear. Here, we have investigated the action of (-)-menthol on excitatory synaptic transmission in spinal lamina II layer which plays a pivotal role in modulating nociceptive transmission from the periphery by using patch-clamp technique in mice spinal cord. We found that (-)-menthol increased miniature excitatory postsynaptic current frequency. The frequency increases which (-)-menthol induced were in a dose-dependent manner (EC_{50} : 0.1079 mM). However, neither genetic knockout nor pharmacological inhibition of TRPM8 could block (-)-menthol-induced effects entirely. Furthermore, this increase was also impaired by TRPA1 antagonist HC030031, but abolished utterly by co-application of TRPM8 and TRPA1 antagonist. Our results indicate that (-)-menthol increases the excitatory synaptic transmission by activating either TRPA1 or TRPM8 channels in spinal lamina II layer.” As taken from Luo Y et al. 2019. Biochem. Biophys. Res. Commun. 516(3), 825-830. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31262448/>

“Various essential oils from plants and fragrance components such as monoterpenes have been discovered to reduce spontaneous movements in mice; thus, it has been made clear that the odor itself has the sedative activity. In the present study, we examined the sedative activity of the odors of fragrance components added to eye drops; l-menthol, d-camphor, phenylethyl alcohol, and geraniol, which are often used as refreshers or preservatives. Each fragrance component was administered by the inhalation route to mice, and the sedative effects were evaluated using an open field test. The results showed that four components administered via inhalation to mice significantly decreased the amount of spontaneous motor activity in a dose-dependent manner, indicating that all four components have a sedative effect. The optimal concentrations at which l-menthol, d-camphor, phenylethyl alcohol, and geraniol showed the highest sedative activity were 4×10^{-2} mg per cage, 4×10^{-4} mg per cage, 4×10^{-2} mg per cage, and both 4×10^{-4} and 4×10^{-2} mg per cage, respectively. The AUC graph of geraniol was represented as a W-shaped curve, suggesting that the sedative action of geraniol was biphasic. The present finding demonstrates a new perspective on a possible pharmacological property of eye drop additives used with no expected pharmacological functions.” As taken from Oshima T et al. 2021. J. Nat. Med. 75(2), 319-325. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33389551/>

“Similar to psychostimulants, the peripheral administration of menthol promotes mouse motor activity, and the neurotransmitter dopamine has been suggested to be involved in this effect. The present study aimed to elucidate the effects of l-menthol on parts of the central nervous system that are involved in motor effects. The subcutaneous administration of l-menthol significantly increased the number of c-Fos-like immunoreactive nuclei in the dorsal striatum of the mice, and motor activity was promoted. It also increased the extracellular dopamine level in the dorsal striatum of the mice. These observations indicated that after subcutaneous administration, l-menthol enhances dopamine-mediated neurotransmission, and activates neuronal activity in the dorsal striatum, thereby promoting motor activity in mice.” As taken from Umezu T. et al. (2021). Available at <https://pubmed.ncbi.nlm.nih.gov/34847183/>

6.4. Other organ systems, dependent on the properties of the substance

“Menthol, the main active constituent of peppermint oil, exerts gut spasmolytic effects, although its mechanism of action remains unclear. We investigated the effects of menthol on gastric emptying and spontaneous- or evoked- mechanical activity of whole murine stomach. Gastric emptying was calculated after i.p. administration of menthol (50mg/Kg). Responses induced by menthol on gastric intraluminal pressure and evoked-cholinergic contractions were analyzed in vitro. Menthol decreased the gastric emptying rate. In vitro, menthol (0.3-30 mM) produced a concentration-dependent relaxation of whole stomach, that was significantly reduced by tetrodotoxin or ω -conotoxin GVIA. The gastric relaxant responses were not affected by Nw-nitro-L-arginine methyl ester, inhibitor of nitric oxide-synthase, apamin or [Lys1,Pro2,5,Arg3,4,Tyr6] vasoactive intestinal peptide (VIP)(7-28), a VIP receptor antagonist, but they were significantly antagonized by atropine or guanethidine, a blocker of adrenergic neurotransmission. The joint application of atropine and guanethidine did not produce any additive effects on menthol effects. Phentolamine, an α -

adrenoceptor antagonist, but not propranolol, a β -adrenoceptor antagonist, significantly reduced menthol responses and the contemporary administration of both adrenergic antagonists did not produce additive effects. Menthol (1-100 μ M) produced a reduction of the electrically-evoked cholinergic contractions, which was prevented by guanethidine. Menthol did not affect the contractions induced by carbachol. In conclusion, menthol in mouse, is able to reduce the rate of gastric emptying and to relax the stomach in vitro. The latter effect appears due, almost in part, to neural mechanisms, with involvement of α -adrenoceptors leading to reduction of tonic ongoing release of acetylcholine." As taken from Amato A et al. 2013. Eur. J. Pharmacol. 718(1-3), 131-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24041922>

"BACKGROUND: The topical antispasmodic agent l-menthol is useful for inhibiting gastric peristalsis during diagnostic upper gastrointestinal endoscopy. However, it remains unclear whether l-menthol is similarly effective during therapeutic endoscopy, thereby improving treatment outcomes in a clinical setting. METHODS: A total of 83 patients scheduled to undergo endoscopic treatment at 8 Japanese referral centers were randomly assigned to receive l-menthol or placebo. The degree of gastric peristalsis (peristaltic score: grade 1-5) was assessed by an independent committee. The primary outcome was the proportion of subjects in whom no or mild peristalsis (grade 1 or 2) was maintained throughout endoscopic treatment. Secondary outcomes were the duration of sustained response and the incidence of adverse drug reactions. RESULTS: The proportion of patients with no or mild peristalsis was significantly higher in the l-menthol group (85.4 %, 95 % confidence intervals 70.8-94.4: 35/41 subjects) than in the placebo group (39.0 %, 24.2-55: 16/41; $P < 0.001$). The sustained response rates in the l-menthol and the placebo were, respectively, 90.0 and 39.6 % 30 min post-dose, and 79.9 and 35.7 % at the completion of the resection. The sustained response rates were significantly higher in the l-menthol group than in the placebo group ($P < 0.001$, log-rank test). The incidence of adverse drug reactions did not differ significantly between the two groups ($P = 1.000$). CONCLUSIONS: During gastric endoscopic submucosal dissection, spraying l-menthol on the gastric mucosa significantly suppressed peristalsis, with minimal adverse drug reactions as compared with placebo. l-menthol solution might be useful for therapeutic endoscopy." As taken from Fujishiro M et al. 2014. J. Gastroenterol. 49(3), 446-54. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23800946>

"BACKGROUND: Menthol reduces intestinal motility in animal studies, an effect that is probably mediated by transient receptor potential channels. Peppermint oil (PO), with menthol as a major constituent, is widely used as a spasmolytic agent in irritable bowel syndrome. In the current study, we investigated the effect of acute PO administration on intragastric pressure (IGP) profiles and gastric sensorimotor functions in health. METHODS: Healthy volunteers underwent IGP measurement before and during continuous intragastric infusion of a nutrient drink ($n = 13$), and gastric barostat studies ($n = 13$). A single capsule of PO (182 mg) or placebo was administered during the studies in a randomized controlled crossover design. Throughout the studies, healthy volunteers scored 11 epigastric symptoms on a visual analogue scale (VAS); satiation was scored on a 6-point Likert scale during intragastric infusion. KEY RESULTS: During fasting, IGP and motility index (MI) of the proximal stomach decreased significantly after PO administration compared with placebo ($P < 0.0001$ and <0.05 , respectively). In contrast, during intragastric infusion of the nutrient drink, no significant differences were detected between PO and placebo in IGP profiles, MI, satiation scores, and epigastric symptoms. The maximum infused volume, gastric compliance or sensitivity to balloon distention did not differ between both treatment arms. However, reduced appetite scores were seen during fasting after PO treatment, as compared with placebo ($P = 0.01$). Postprandial VAS scores were similar between PO and placebo. CONCLUSIONS & INFERENCES: Peppermint oil reduces IGP, proximal phasic contractility, and appetite, with negligible effects on gastric sensitivity, tone, accommodation, and nutrient tolerance in health." As

taken from Papathanasopoulos A et al. 2013. Neurogastroenterol. Motil. 25(4), e263-71. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23489975>

"Rats receiving repeated oral doses of 800 mg/kg bw (-)-menthol for one to seven days had increased activities of hepatic microsomal cytochrome C and NADPH-cytochrome P450 reductase. There was no effect on two other P450 enzyme complexes, cytochrome b₅ and NADH-cytochrome C reductase. Rat liver microsomes readily convert (-)-menthol to para-menthane-3,8-diol in the presence of NADPH and oxygen. This activity was significantly greater in microsomes obtained from phenobarbital-induced microsomal preparations than from controls, whereas 3-methylcholanthrene-induced microsomes failed to convert (-)-menthol to 1-menthane-3,8-diol in the presence of NADPH and oxygen."

As taken from IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

"L-Menthol enhances transdermal drug penetration."

"L-Menthol was not a potent in vitro inhibitor of MROD (CYP1A2) activity (IC₅₀: >300 µM) and of EROD (CYP1A1) activity (IC₅₀: >400 µM); it did inhibit PROD (CYP2B1) activity (IC₅₀: 10.6 µM), but was much less potent than beta-ionon which had an IC₅₀ of 0.03 µM."

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

"Based on ethnopharmacological indications that *Mentha* species may be used in the treatment of gastrointestinal diseases, this study aimed to characterize the gastroprotective mechanisms of menthol (ME), the major compound of the essential oil from species of the genus *Mentha*. The gastroprotective action of ME was analyzed in gastric ulcers that were induced by ethanol or indomethacin in Wistar male rats. The mechanisms responsible for the gastroprotective effect were assessed by analyzing the amount of mucus secreted, involvement of non-protein sulfhydryl (NP-SH) compounds, involvement of calcium ion channels and NO/cGMP/K(+)ATP pathway, gastric antisecretory activity and the prostaglandin E₂ (PGE₂) production. The anti-diarrheal activity and acute toxicity of ME were also evaluated. Oral treatment with ME (50mg/kg) offered 88.62% and 72.62% of gastroprotection against ethanol and indomethacin, respectively. There was an increased amount of mucus and PGE₂ production. The gastroprotective activity of ME involved NP-SH compounds and the stimulation of K(+)ATP channels, but not the activation of calcium ion channels or the production of NO. The oral administration of ME induced an antisecretory effect as it decreased the H(+) concentration in gastric juice. ME displayed anti-diarrheal and antiperistaltic activity. There were no signs of toxicity in the biochemical analyses performed in the rats' serum. These results demonstrated that ME provides gastroprotective and anti-diarrheal activities with no toxicity in rats." As taken from Rozza AL et al. 2013. Chem. Biol. Interact. 206(2), 272-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24121185>

"The aim of this research was to investigate the anti-apoptotic, antioxidant and anti-inflammatory properties of menthol against ethanol-induced gastric ulcers in rats. Wistar rats were orally treated with vehicle, carbenoxolone (100 mg/kg) or menthol (50 mg/kg) and then treated with ethanol to induce gastric ulcers. After euthanasia, stomach samples were prepared for histological slides and biochemical analyses. Immunohistochemical analyses of the cytoprotective and anti-apoptotic heat-shock protein-70 (HSP-70) and the apoptotic Bax protein were performed. The neutrophils were manually counted. The activity of the myeloperoxidase (MPO) was measured. To determine the level of antioxidant functions, the levels of glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and superoxide dismutase (SOD) were measured using ELISA. The levels of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and the anti-inflammatory cytokine interleukin-10 (IL-10) were assessed using ELISA kits. The menthol treated group presented 92% gastroprotection compared to the vehicle-treated group. An increased immunolabeled area was observed for HSP-70, and a decreased immunolabeled area

was observed for the Bax protein in the menthol treated group. Menthol treatment induced a decrease in the activity of MPO and SOD, and the protein levels of GSH, GSH-Px and GR were increased. There was also a decrease in the levels of TNF- α and IL-6 and an increase in the level of IL-10. In conclusion, oral treatment with menthol displayed a gastroprotective activity through anti-apoptotic, antioxidant and anti-inflammatory mechanisms.” As taken from Rozza AL et al. 2014. PLoS One 9(1), e86686. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24466200>

“Dry eye syndrome is a painful condition caused by inadequate or altered tear film on the ocular surface. Primary afferent cool cells innervating the cornea regulate the ocular fluid status by increasing reflex tearing in response to evaporative cooling and hyperosmoticity. It has been proposed that activation of corneal cool cells via a transient receptor potential melastatin 8 (TRPM8) channel agonist may represent a potential therapeutic intervention to treat dry eye. This study examined the effect of dry eye on the response properties of corneal cool cells and the ability of the TRPM8 agonist menthol to modify these properties. A unilateral dry eye condition was created in rats by removing the left lacrimal gland. Lacrimal gland removal reduced tears in the dry eye to 35% compared with the contralateral eye and increased the number of spontaneous blinks in the dry eye by over 300%. Extracellular single-unit recordings were performed 8-10 wk following surgery in the trigeminal ganglion of dry eye animals and age-matched controls. Responses of corneal cool cells to cooling were examined after the application of menthol (10 μ M-1.0 mM) to the ocular surface. The peak frequency of discharge to cooling was higher and the cooling threshold was warmer in dry eye animals compared with controls. The dry condition also altered the neuronal sensitivity to menthol, causing desensitization to cold-evoked responses at concentrations that produced facilitation in control animals. The menthol-induced desensitization of corneal cool cells would likely result in reduced tearing, a deleterious effect in individuals with dry eye.” As taken from Kurose M and Meng ID. 2013. J. Neurophysiol. 110(2), 495-504. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23636717>

“AIMS: To examine the effects of RQ-00434739, a novel selective TRPM8 antagonist, on deep body temperature (DBT) and normal bladder sensory function and overactivity and its associated facilitation of mechanosensitive primary bladder single-unit afferent activities (SAAs) induced by intravesical l-menthol or prostaglandin E2 (PGE2) instillation in rats. MAIN METHODS: The effect of RQ-00434739 on DBT was evaluated using intravenous administration of RQ-00434739 (1 mg/kg) or its vehicle under urethane anaesthesia. Cystometry (CMG) was performed on conscious and freely moving rats. SAAs were measured from the left L6 dorsal root under urethane anaesthesia, and the fibers were grouped as A δ - or C-fiber based on their conduction velocity. For both CMG and SAA measurements, after baseline recording with saline instillation, further recording was performed with intravesical l-menthol (6 mM) or PGE2 (60 μ M) instillation after pretreatment with intravenous RQ-00434739 (1 mg/kg) or its vehicle. KEY FINDINGS: RQ-00434739 did not significantly affect DBT. In CMG measurements, RQ-00434739 administration increased mean voided volume. Both l-menthol and PGE2 instillation decreased mean voided volume following vehicle pretreatment, whereas such effects were not observed following RQ-00434739 pretreatment. In SAA measurements, either l-menthol or PGE2 instillations increased SAAs of C-fibers, but not SAAs of A δ -fibers, in the presence of vehicle. RQ-00434739 pretreatment significantly inhibited the l-menthol- and PGE2-induced activation of C-fiber SAAs. SIGNIFICANCE: The present results demonstrate that blockade of TRPM8 channels can inhibit the pathological activation of mechanosensitive C-fibers and suggest that RQ-00434739 may be a promising therapeutic drug candidate for bladder hypersensitive disorders without affecting DBT.” As taken from Aizawa N et al. 2019. Life Sci. 218, 89-95. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30580018>

“In order to investigate the effects of peppermint oil and its most abundant metabolite and active constituent ((-)- menthol) on 5-HT₃ receptors (serotonin receptor subtype) three different in vitro models were used : [14C]guanidium influx into N1E-115 cells which express 5-HT₃ receptors, isotonic contractions of the isolated rat ileum and equilibrium competition binding studies using a radioactively labelled 5-HT₃ receptor antagonist ([3H]GR65630) (3-(5-methyl-1H-imidazol-4-yl)-1-

(1-methyl-1H-indol-3-yl)-1-propanone). Both peppermint oil and (-)-menthol inhibited [¹⁴C]guanidinium influx through 5-HT₃ receptor channels as well as serotonin induced contractions of the ileum induced by serotonin. Neither the peppermint oil nor (-)-menthol, however, was able to displace [3H]GR65630 from 5-HT₃ binding sites. It may be concluded that peppermint oil and (-)-menthol exert their antiemetic effect at least partly by acting on the 5-HT₃ receptor ion-channel complex, probably by binding to a modulatory site distinct from the serotonin binding site (Heimes et al, 2011)."

As taken from EMA, 2020

"Introduction: In randomized controlled trials, L-menthol inhibits gastrointestinal peristalsis during endoscopy. Our goal was to quantitatively synthesize the available evidence to evaluate the efficacy and safety of L-menthol for gastrointestinal endoscopy. Methods: We comprehensively searched for relevant studies published up to January 2020 in PubMed, EMBASE, Web of Science, and Cochrane Library. The main outcomes consisted of the proportion of no peristalsis, proportion of no or mild peristalsis, adenoma detection rate, and adverse events. Results: Eight randomized controlled trials analyzing 1,366 subjects were included. According to the pooled data, L-menthol significantly improved the proportion of no peristalsis (odds ratio [OR] = 6.51, 95% confidence interval [CI] = 4.94-8.57, $P < 0.00001$), and the proportion of no or mild peristalsis (OR = 7.89, 95% CI = 5.03-12.39, $P < 0.00001$) compared with the placebo, whereas it was not associated with an improvement in the adenoma detection rate (OR = 1.03, 95% CI = 0.54-1.99, $P = 0.92$). Adverse events did not differ significantly between the 2 groups (OR = 1.40, 95% CI = 0.75-2.59, $P = 0.29$). Discussion: The findings of this study support the use of L-menthol to suppress gastrointestinal peristalsis during endoscopic procedure." As taken from You Q et al. 2020. Clin. Transl. Gastroenterol. 11(10), e00252. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33031198/>

"Objective: The topical antispasmodic agent l-menthol is commonly used for gastric peristalsis suppression during diagnostic upper gastrointestinal (GI) endoscopy. We evaluated the efficacy and safety of a single dose l-menthol solution in suppressing gastric peristalsis during upper GI endoscopy in Chinese patients. Methods: In this phase III, multicenter, randomized, double-blind, placebo-controlled study (ClinicalTrials.gov data- NCT03263910), 220 patients scheduled to undergo upper GI endoscopy at five Chinese referral centers received a single dose of either 160 mg of l-menthol ($n = 109$) or placebo ($n = 111$). Both treatments were sprayed endoscopically on the gastric mucosa. An independent committee evaluated the degree of gastric peristalsis (peristaltic score: grade 1-5). Results: At baseline, the proportion of patients with grade 1 peristalsis (no peristalsis) did not differ between the groups. The proportion of patients with grade 1 peristalsis post-treatment was significantly higher in the l-menthol group (40.37%, 44/109) versus the placebo group (16.22%, 18/111; $P < 0.001$); the difference between the groups was 24.15% (95% confidence interval: 12.67%-35.63%; $P < 0.001$). In the l-menthol group, 61.47% of patients had grade 1 peristalsis after endoscopy versus 24.55% in the placebo group ($P < 0.001$). The ease of intragastric examination correlated significantly with the grade of peristalsis. The incidence of adverse events was comparable between the groups ($P = 0.340$). Conclusions: During upper GI endoscopy, a single dose of l-menthol solution (160 mg) sprayed on the gastric mucosa significantly attenuated gastric peristalsis versus placebo, thereby improving the visual stability without any safety concerns." As taken from Meng F et al. 2021. Dig. Endosc. Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33527576/>

"Background and aim: l-Menthol has smooth muscle-relaxing and antiperistaltic effects. We examined its effectiveness against peristalsis resumption during endoscopic submucosal dissection (ESD) of gastric tumors. Methods: We retrospectively examined clinical data of 485 patients (501 lesions) who underwent ESD for upper gastrointestinal tumors in 2017. We included 119 patients (127 lesions) in whom peristaltic movement resumed during ESD and l-menthol was applied; 366 patients (374 lesions) without l-menthol application were used as controls. Video recordings were reviewed to determine whether l-menthol suppressed peristalsis resumption. Results: In cases with

l-menthol application, 2 (2.9%), 36 (14.3%), and 89 (71.2%) lesions were found in the upper (U), middle (M), and lower (L) regions, respectively. In the control group, the corresponding values were 66 (17.6%), 215 (57.5%), and 93 (24.9%), respectively. l-Menthol efficacy was observed in 116 of the 127 treated lesions (91.3%), over 90% of which were in the posterior wall of the U region, anterior wall and greater curvature of the M region, and anterior wall and lesser curvature of the L region. The most and least effective areas for l-menthol application were the anterior wall of gastric antrum and posterior wall of the M region, respectively. The mean time from application to peristalsis inhibition was 8.7 s. No adverse effects were observed; perforation and secondary hemorrhage were not significantly different between the groups. Conclusion: Direct l-menthol application to the submucosal layer during mucosal resection affects smooth muscles and rapidly inhibits peristalsis resumption. Clinically, l-Menthol can be used to suppress peristalsis recurrence during ESD, without adverse effects.” As taken from Ishiyama A et al. 2021. JGH Open 5(6), 653-657. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/34124381/>

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing L-menthol and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of L-menthol. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	18,000 (89-78-1)	Carmines, 2002 & Rustemeier et al., 2002
	23,400 (89-78-1)	Baker et al., 2004a
	11,991 (2216-51-5)	Roemer et al, 2014
	17300 (2216-51-5)	Stabbert et al., 2019
In vitro genotoxicity	18,000 (89-78-1)	Carmines, 2002 & Roemer et al., 2002
	23,400 (89-78-1)	Baker et al., 2004c
	2,030 (2216-51-5)	fGLH Study Report (2010)
	11,991 (2216-51-5)	Roemer et al, 2014
	17300 (2216-51-5)	Stabbert et al., 2019
In vitro cytotoxicity	18,000 (89-78-1)	Carmines, 2002 & Roemer et al., 2002
	23,400 (89-78-1)	Baker et al., 2004c
	2,030 (2216-51-5)	fGLH Study Report (2010)
	11,991 (2216-51-5)	Roemer et al, 2014
	17300 (2216-51-5)	Stabbert et al., 2019
Inhalation study	5,000 (synthetic, 2216-51-5)	Gaworski et al., 1998

	353 (Brazilian, (89-78-1))	
	18,000 (89-78-1)	Carmines, 2002 & Vanscheeuwijck et al., 2002
	23,400 (89-78-1)	Baker et al., 2004c
	11,991 (2216-51-5)	Schramke et al, 2014
Skin painting	5,000 (synthetic, 2216-51-5) 353 (Brazilian, (89-78-1))	Gaworski et al., 1999
In vivo genotoxicity	11,991 (2216-51-5)	Schramke et al, 2014

Transfer studies:

In a series of pyrolysis studies on tobacco ingredients, the intact transfer of 97.4% of menthol was reported. One extra compound at greater than 1% of total ion current was detected by gas chromatography-mass spectrometry. The authors comment that pyrolysis does not provide a robust prediction of the compounds that are formed from ingredients during cigarette smoking studies (Purkis et al. 2011).

Impregnation of cigarette filters with menthol in an alcoholic solution (10%) resulted in TPM 40.2mg/cig., tar 30.2mg/cig and nicotine 1.59mg/cig in conventional filter + menthol cigarettes. When applied to a polymeric filter TPM decreased to 7.7mg/cig., tar 6.8mg/cig and nicotine 0.1mg/cig (Shepherd et al. 1968).

When menthol was added as one of a group of ingredients at amounts ranging from 5-80mg/cig via insertion between 2 cellulose acetate filter tips, an increase in mainstream smoke components tar, CO and B[a]P was observed, whereas a decrease was observed for cyanide, nitrogen oxide, hydrogen sulfide and ammonia (Matsushita 1980).

Cigarette smoking is established as a substantial contributor to risks for cancer, cardiovascular and respiratory diseases. Less is known about the potential of cigarette composition to affect smoking risks. The use of cigarette flavoring ingredients such as menthol is currently of worldwide public health and regulatory interest. The unique conditions of menthol inhalation exposure that occur coincident with that of the complex cigarette smoke aerosol require specialized studies to support an assessment of its safety in cigarette flavoring applications. The present state of knowledge is sufficient to support an assessment of the safety of the use of menthol in cigarettes. Scientific, smoking behavioral and epidemiological data available through mid-2009 is critically reviewed and a broad convergence of findings supports a judgment that menthol employed as a cigarette tobacco flavoring ingredient does not meaningfully affect the inherent toxicity of cigarette smoke or the human risks that attend smoking. There remains a need for well-designed studies of the potential of menthol to affect smoking initiation, cessation and addiction in order to differentiate any independent effects of menthol in cigarettes from those imposed by socioeconomic, environmental and peer influences on these complex human behaviors. As taken from Heck D.J. Food and Chemical Toxicology 48 (2010) S1-S38.

Menthol applied via solution of menthol in chloroform to the filters of 4 different cigarette brands, at four concentrations resulted in mainstream smoke transfer rate of 22-26% (Mitchell et al. 1963).

Spraying 1.63g of menthol (3.6% included in α -cyclodextrine) onto 100g tobacco resulted in transfer to mainstream smoke of 0.05mg/cig or 8.5%. Spraying 2.5g menthol (6.4% included in α -cyclodextrine) onto 100g tobacco resulted in a transfer to mainstream smoke rate of 8.8% (Robb et al. 1964).

When known amounts of menthol were smoked onto Cambridge filter pads, the transfer to mainstream smoke ranged from 0.21mg/cig to 0.5mg/cig (Lyerly 1967).

Transfer of menthol (3.6% included in α -cyclodextrine) to mainstream smoke was estimated at 16-23% when sprayed onto tobacco at an amount equivalent to 0.6%. Spraying a 1% solution of

menthol (4.6% included in tri-o-thymotid) onto tobacco resulted in transfer to mainstream smoke estimated at 33-43%. When a 2.5% solution (menthol 6.42% included in α -cyclodextrine) was sprayed transfer was estimated at 8.1-10% (Bavley 1969).

Injection of 1.1 μ Ci ¹⁴C-Menthol into non-filtered cigarettes resulted in 1% transfer to mainstream smoke (gas phase), and 27.9% transfer to mainstream smoke (TPM) (Jenkins et al. 1970).

When 3.6mg menthol was applied in a solution of menthol (80%) and ethanol (20%) to cigarette filter, transfer of menthol to mainstream smoke decreased from 14.7% to 10.6% when the cigarette was aged for 11 days. After 11 days the TPM had decreased from 38mg to 32mg (Badgett 1970). When the same solution was applied to carbon and non-carbon filter cigarettes, transfer to mainstream smoke was 14.7% for the non-carbon and 1.1% for the carbon filter product (no ageing). TPM of the carbon filter cigarette decreased to 33mg from 38mg (non-carbon) (Badgett 1971).

When menthol was applied to 9 samples of cigarettes at levels ranging from 0.013-3.2mg/cig, transfer to mainstream smoke ranged from 18.7% to 31.0% (Brozinski et al. 1972).

In a cigarette containing 1.88mg menthol added to the filter the transfer to mainstream smoke decreased from 49% after 3 days storage, to 31% after 28 days storage. When the menthol was added to the tobacco to a level of 2.34mg, delivery in mainstream smoke decreased from 20% after 3 days to 19% after 28 days storage (Curran 1972).

Transfer of menthol to mainstream smoke was highest (approximately 30%) when most of the menthol was on the filter following application of 2.7mg on the finished cigarette. High transfer was observed (approximately 20%) when most of the menthol was applied to the tobacco. The transfer rate was approximately 16% when 70% of the menthol was applied to the tobacco or 60% on the filter (Riehl et al. 1973).

Studies have shown that 98.9% of benzaldehyde was transferred to mainstream smoke. This transfer was indicated by smoke chemistry from ¹⁴C-labelled compounds. The percentage transfer predicted by pyrolysis was 99.5 (Jenkins et al, 1970).

Investigation of the influence of filter additives on the mainstream smoke transfer rate of menthol, when added to tobacco at 2mg/cig, demonstrated an increase from the control with 5% and 8% triacetin filters and a decrease with triethylene glycol triacetate, glycerol and 1,2-Propanediol filters (Curran 1975).

When applied in combination with sucrose to activated carbon filter cigarettes and compared with non filter cigarettes, the mainstream smoke transfer of menthol increased from 0.39mg/cig (no filter) to 0.46mg/cig (filter) when sucrose was present at 5%. 3% sucrose solution resulted in menthol transfer of 0.42mg/cig (no filter) and 0.55mg/cig (filter) (Tigglebeck 1976).

Application of menthol (released from *Mentha arvensis*) at 10% to tobacco by mixing Virginia tobacco with dried mint leaves containing 1.56% menthol resulted in delivery of 16% in mainstream smoke (BAT 1978).

When menthol (released from Poly[α -styryl γ -menthyl carbonate]) was applied to tobacco at 2.2% per weight by spraying of a cyclohexane solution containing the flavour precursor, release of menthol in mainstream smoke was 1.34% of the polymer before storage and 1.39% after storage. When applied at 1.7%, 1.74% and 2.37% per weight tobacco, release of menthol in mainstream smoke was 1.72%, 2.65% and 2.37% of the polymer respectively (Grubbs et al. 1978).

When menthol was added as one of a group of ingredients at amounts ranging from 5-80mg/cig via insertion between 2 cellulose acetate filter tips, pyrolysis resulted in its release (Matsushita 1980).

When menthol was applied to 85mm full flavour menthol American blend cigarettes at 9 different amounts ranging from 0.12-1.05% via top dressing with menthol at a constant rate, transfer to mainstream smoke ranged from 9.7% to 16.7%. Tar levels ranged from 17.3-18.1mg/cig and

nicotine ranged from 1.23-1.33mg/cig. Application of the same 9 formulations to full flavour low tar cigarettes resulted in mainstream smoke transfers ranging from 7.3% to 12.5%, tar levels of 8.3-8.9mg/cig and nicotine range of 0.71-0.76mg/cig. Application to ultra low tar menthol product resulted in transfers ranging from 3.7% to 8.1% and tar and nicotine levels ranging from 5.7-6.4mg/cig and 0.5-0.54mg/cig respectively (Perfetti 1985).

Application to cigarette plug wrap of 5.9mg menthol solubilized in ethanol and mixed with triacetin resulted in puff by puff transfer ranging from 0.04mg to 0.15mg menthol. The transfer rate in all puffs was 11.5%. Under different storage conditions menthol delivery ranged from 4.4-12.4% (Nichols et al. 1987).

When investigated as one of a group of flavour constituents' transfer to mainstream smoke at different levels of ventilation, all constituents including menthol decreased with increasing ventilation. CO and tar deliveries also decreased with increasing ventilation (Jing 1999).

"Menthol has long been an important flavorant in tobacco products, and both its historical and present uses are topics of increasing debate. Menthol can exist in eight different stereoisomeric forms (as four enantiomeric pairs) that possess different sensory properties. As regards use in tobacco products, the open scientific literature and available industry documents focus on the D-menthol and L-menthol enantiomeric pair, and in particular on L-menthol, but are ambiguous about the actual importance of D-menthol in tobacco products. This study provides the first openly available measurements regarding the stereoisomeric forms of menthol as found in selected United States sub-brands of smokeless tobacco (SLT), cigarettes, and cigarette smoke. Gas chromatography/mass spectrometry (GC/MS) was applied using a "chiral" GC column to separate and determine the forms of menthol present in headspace air above various samples of United States sub-brands of SLT, cigarette filler material, and cigarette smoke particulate matter. Additional GC $\times$ GC/Time-of-flight mass spectrometry measurements were also made. The dominant form of menthol by far in any of the samples was L-menthol. For the selected cigarettes and SLT products tested from the U.S. market, the only form of menthol found was L-menthol. Other forms may be present in products that were not tested. No evidence was found of thermal racemization upon smoking of L-menthol to a d+l mixture" (Chen et al. 2011).

"The 2009 Family Smoking Prevention and Tobacco Control Act empowered the U.S. Food and Drug Administration to study "the impact of the use of menthol in cigarettes on the public health, including such use among children, African Americans, Hispanics and other racial and ethnic minorities," and develop recommendations. Current scientific evidence comparing human exposures between menthol and nonmenthol smokers shows mixed results. This is largely because of the many differences between commercial menthol and nonmenthol cigarettes other than their menthol content. We conducted an innovative study using two types of test cigarettes: a commercial nonmenthol brand that we mentholated at four different levels, and Camel Crush, a commercial cigarette containing a small capsule in the filter that releases menthol solution into the filter when crushed. Cigarettes were machine-smoked at each of the menthol levels investigated, and the total particulate matter (TPM) was collected on a quartz fiber filter pad and analyzed by gas chromatography/mass spectrometry for menthol, nicotine, tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), cotinine, and quinoline. The mainstream smoke was also monitored continuously in real time on a puff-by-puff basis for seven gas-phase constituents (acetaldehyde, acetonitrile, acrylonitrile, benzene, 1,3-butadiene, isoprene, and 2,5-dimethylfuran), using a proton transfer reaction-mass spectrometer. Average yields (in micrograms/cigarette) for the analytes were determined. Menthol in the TPM samples increased linearly with applied menthol concentration, but the amounts of nicotine along with the target TSNAs, PAHs, cotinine, and quinoline in the cigarettes remained essentially unchanged. Similarly, yields of the targeted volatile organic compounds (VOCs) in whole smoke from the mentholated nonmenthol cigarettes that were measured in real-time were largely unaffected by their menthol levels. In the Camel Crush cigarettes, however, the VOC yields appeared to increase in the presence of menthol, especially in the gas phase. Although we succeeded in characterizing key mainstream smoke constituents in

cigarettes that differ only in menthol content, further study is needed to definitively answer whether menthol affects exposure to selected cigarette constituents and thereby influences harm” (Gordon et al. 2011).

Cigarette smoking is established as a substantial contributor to risks for cancer, cardiovascular and respiratory diseases. Less is known about the potential of cigarette composition to affect smoking risks. The use of cigarette flavoring ingredients such as menthol is currently of worldwide public health and regulatory interest. The unique conditions of menthol inhalation exposure that occur coincident with that of the complex cigarette smoke aerosol require specialized studies to support an assessment of its safety in cigarette flavoring applications. The present state of knowledge is sufficient to support an assessment of the safety of the use of menthol in cigarettes. Scientific, smoking behavioral and epidemiological data available through mid-2009 is critically reviewed and a broad convergence of findings supports a judgment that menthol employed as a cigarette tobacco flavoring ingredient does not meaningfully affect the inherent toxicity of cigarette smoke or the human risks that attend smoking. There remains a need for well-designed studies of the potential of menthol to affect smoking initiation, cessation and addiction in order to differentiate any independent effects of menthol in cigarettes from those imposed by socioeconomic, environmental and peer influences on these complex human behaviors. As taken from Heck D.J. Food and Chemical Toxicology 48 (2010) S1-S38.

A brief report claimed that the application of condensed vapour from mentholated cigarettes, to mice skin, gave no greater tumour yield than did that from of non-mentholated cigarettes (BIBRA, 1990).

‘Menthol (2-isopropyl-5-methyl-cyclohexan-1-ol) is used in food, pharmaceutical, and tobacco products. Despite its long usage history and GRAS status, scientific literature on effects of cigarette mentholation is limited. Because African-American men have high lung cancer rates and predominantly smoke mentholated cigarettes, and because menthol's cooling effect might affect puffing and smoke inhalation, possible adverse effects of cigarette mentholation have been suggested. We review the evidence on the effects of mentholation on smokers, and we also identify areas for further study. Five large epidemiological studies provide no evidence that cigarette mentholation increases lung cancer risk. Mentholation cannot explain the higher risk for lung cancer in African-American male smokers, who also predominantly smoke mentholated cigarettes. Limited data on other cancers also suggest no risk from mentholation. The scientific literature suggests that cigarette mentholation does not increase puff number or puff volume of smoked cigarettes, and has little or no effect on heart rate, blood pressure, uptake of carbon monoxide, tar intake or retention, or blood cotinine concentration. Mentholation has little effect on other smoke constituents, and no apparent effect on nicotine absorption, airway patency and smoking initiation, dependency or cessation. Any toxicological effects of cigarette mentholation on adult smokers are probably quite limited’ (Werley et al., 2007)

“Since the 1920s, menthol has been added to cigarettes and used as a characterizing flavor. The health effects of cigarette smoking are well documented, however the health effects of menthol cigarettes as compared to non-menthol cigarettes is less well studied. This review discusses menthol's effects on 1) biomarkers of tobacco smoke exposure, 2) toxicity and cellular effects, 3) lung function and respiration, 4) pulmonary and/or vascular function, 5) allergic reactions and inflammation, and 6) tobacco-related diseases. It is concluded that menthol is a biologically active compound that has effects by itself and in conjunction with nicotine, however much of the data on the other areas of interest are inconclusive and firm conclusions cannot be drawn”. (Hoffman 2011)

“BACKGROUND: US mentholated cigarette sales have increased considerably over 50 years. Preference for mentholated cigarettes is markedly higher in Black people. While menthol itself is not genotoxic or carcinogenic, its acute respiratory effects might affect inhalation of cigarette smoke. This possibility seems consistent with the higher lung cancer risk in Black men, despite Black people smoking less and starting smoking later than White people. Despite experimental

data suggesting similar carcinogenicity of mentholated and non-mentholated cigarettes, the lack of convincing evidence that mentholation increases puffing, inhalation or smoke uptake, and the similarity of lung cancer rates in Black and White females, a review of cigarette mentholation and lung cancer is timely given current regulatory interest in the topic. METHODS: Epidemiological studies comparing lung cancer risk in mentholated and non-mentholated cigarette smokers were identified from MedLine and other sources. Study details were extracted and strengths and weaknesses assessed. Relative risk estimates were extracted, or derived, for ever mentholated use and for long-term use, overall and by gender, race, and current/ever smoking, and meta-analyses conducted. RESULTS: Eight generally good quality studies were identified, with valid cases and controls, and appropriate adjustment for age, gender, race and smoking. The studies afforded good power to detect possible effects. However, only one study presented results by histological type, none adjusted for occupation or diet, and some provided no results by length of mentholated cigarette use. The data do not suggest any effect of mentholation on lung cancer risk. Adjusted relative risk estimates for ever use vary from 0.81 to 1.12, giving a combined estimate of 0.93 (95% confidence interval 0.84-1.02, $n = 8$), with no increase in males (1.01, 0.84-1.22, $n = 5$), females (0.80, 0.67-0.95, $n = 5$), White people (0.87, 0.75-1.03, $n = 4$) or Black people (0.90, 0.73-1.10, $n = 4$). Estimates for current and ever smokers are similar. The combined estimate for long-term use (0.95, 0.80-1.13, $n = 4$) again suggests no effect of mentholation. CONCLUSION: Higher lung cancer rates in Black males cannot be due to their greater preference for mentholated cigarettes. While some study weaknesses exist, the epidemiological evidence is consistent with mentholation having no effect on the lung carcinogenicity of cigarettes" (Lee 2011).

"The US Food and Drug Administration is assessing whether menthol should be banned as an additive to cigarettes. An important part of this determination concerns the health effects of mentholated relative to non-mentholated cigarettes. We examined the ecologic association between sales of mentholated cigarettes for the period 1950-2007, menthol preference by race and sex, and incidence rates of four tobacco-related cancers during 1973-2007. Total sales of mentholated cigarettes (market share) increased from about 3% in 1950 to slightly less than 30% in 1980 and remained fairly stable thereafter. Additional data show consistently that, compared to White smokers, Black smokers favor mentholated cigarettes by roughly a 3-fold margin. Differences in the incidence of lung cancer, squamous cell cancer of the esophagus, oropharyngeal cancer, and laryngeal cancer by race and sex and trends over a 35-year period, during which menthol sales were relatively stable and during which Black smokers were much more likely to smoke mentholated cigarettes compared to Whites, are not consistent with a large contribution of menthol, over and above the effect of smoking per se" (Kabat et al, 2012).

"A survey of selected mainstream smoke constituents from commercially marketed US cigarettes was conducted in 2009. The US cigarette market was segmented into thirteen (13) strata based on Cambridge Filter Method (CFM) "tar" category and cigarette design parameters. Menthol and non-menthol cigarettes were included. Sixty-one (61) cigarette brand styles were chosen to represent the market. Another thirty-four (34) brand styles of interest were included in the survey along with a Kentucky 3R4F reference cigarette. Twenty mainstream smoke constituents were evaluated using the Health Canada smoking regimen. By weighting the results of the 61 brand styles using the number of brand styles represented by each stratum, the mainstream smoke constituent means and medians of the US cigarette market were estimated. For nicotine, catechol, hydroquinone, benzo(a)pyrene and formaldehyde the mean yields increased with increasing "tar" yields. Constituent yields for the ultra-low "tar" and low "tar" cigarettes were not significantly different for most other analytes as ventilation blocking defeated any filter air dilution design features. In contrast, normalization per mg nicotine provided an inverse ranking of cigarette yields per CFM "tar" categories. Menthol cigarette mean constituent yields were observed to be within the range of the non-menthol cigarettes of similar "tar" categories". As taken from Bodnar JA et al. 2012. Regul. Toxicol. Pharmacol. 64(1), 35-42. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22683394>

“One-third of smokers primarily use menthol cigarettes and usage of these cigarettes leads to elevated serum nicotine levels and more difficulty quitting in standard treatment programmes. Previous brain imaging studies demonstrate that smoking (without regard to cigarette type) leads to up-regulation of $\beta(2)^*$ -containing nicotinic acetylcholine receptors (nAChRs). We sought to determine if menthol cigarette usage results in greater nAChR up-regulation than non-menthol cigarette usage. Altogether, 114 participants (22 menthol cigarette smokers, 41 non-menthol cigarette smokers and 51 non-smokers) underwent positron emission tomography scanning using the $\alpha(4)\beta(2)^*$ nAChR radioligand 2-[(18)F]fluoro-A-85380 (2-FA). In comparing menthol to non-menthol cigarette smokers, an overall test of 2-FA total volume of distribution values revealed a significant between-group difference, resulting from menthol smokers having 9-28% higher $\alpha(4)\beta(2)^*$ nAChR densities than non-menthol smokers across regions. In comparing the entire group of smokers to non-smokers, an overall test revealed a significant between-group difference, resulting from smokers having higher $\alpha(4)\beta(2)^*$ nAChR levels in all regions studied (36-42%) other than thalamus (3%). Study results demonstrate that menthol smokers have greater up-regulation of nAChRs than non-menthol smokers. This difference is presumably related to higher nicotine exposure in menthol smokers, although other mechanisms for menthol influencing receptor density are possible. These results provide additional information about the severity of menthol cigarette use and may help explain why these smokers have more trouble quitting in standard treatment programmes.” As taken from Brody AL et al. 2013. *Int J Neuropsychopharmacol.* 16(5), 957-66. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23171716>

“Purpose: Menthol in cigarettes has been suggested to inhibit metabolism of nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The objective of this study was to investigate the glucuronide metabolite ratios (MR) for nicotine (NICGLUC/NIC), cotinine (COTGLUC/COT), trans 3'-hydroxy cotinine (3OHCOTGLUC/3OHCOT). 4-methylnitrosamino-1-(3-pyridyl)-1-butanol (NNAL - NNALGLUC/NNAL); and the ratio of trans 3'-hydroxy cotinine to cotinine (3OHCOT/COT) between adult menthol and non-menthol smokers (AS). Methods: The data was from the Total Exposure Study (TES), a stratified, multi-center, cross-sectional study that included 3,585 AS and 1,077 non-smokers. Daily urinary excretion of nicotine and five metabolites, NNAL and NNAL glucuronides, and serum cotinine were measured in the AS. The analysis included 1044 menthol (448 African-Americans, AA) and 2297 non-menthol (161 AA) AS. Results: Smoking mentholated cigarettes did not decrease any of the MR. Race was the most important significant main effect for all the MRs. AAs exhibited statistically significantly lower NICGLUC/NIC, COTGLUC/COT, NNALGLUC/NNAL and 3OHCOT/COT, but higher 3OHCOTGLUC/3OHCOT compared to Whites. Age, liver function, alcoholic beverages, etc., were some of the other significant effects for some MRs. Menthol was not a statistically significant effect, e.g. the adjusted mean NNALGLUC/NNAL between menthol and non-menthol AS was 2.93 vs. 2.80 ($p>0.05$, AA) and 3.38 vs. 3.35 ($p>0.05$, Whites). The models only explained 2.6-12.6% of the MR variability. Number of cigarettes was the most important factor affecting serum cotinine levels. Conclusions: Menthol does not inhibit the metabolism of nicotine or NNK. The daily exposure of related constituents is primarily influenced by number of cigarettes smoked per day”. As taken from Sarkar M et al. 2012. *Drug Metab. Letts* 6(3), 198-206. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23140558>

“Menthol can reduce sensory irritation and it has been hypothesised that this could result in smokers of mentholated cigarettes taking larger puffs and deeper post-puff inhalations thereby obtaining higher exposures to smoke constituents than smokers of non-mentholated cigarettes. The aim of our study was to use part-filter analysis methodology to assess the effects of cigarette menthol loading on regular and occasional smokers of mentholated cigarettes. We measured mouth level exposure to tar and nicotine and investigated the effects of mentholation on smokers' sensory perceptions such as cooling and irritation. Test cigarettes were produced containing no menthol and different loadings of synthetic and natural l-menthol at 1 and 4mg ISO tar yields. A target of 100 smokers of menthol cigarettes and 100 smokers who predominantly smoked non-menthol cigarettes from both 1 and 4mg ISO tar yield categories were recruited in Poland and Japan. Each subject was required to smoke the test cigarette types of their usual ISO tar yield.

There were positive relationships between menthol loading and the perceived 'strength of menthol taste' and 'cooling' effect. However, we did not see marked menthol-induced reductions in perceived irritation or menthol-induced increases in mouth level exposure to tar and nicotine". As taken from Ashley M et al. 2012. Reg. Tox. Pharmacol. 63, 381-390. PubMed, 2013. Available at <http://www.ncbi.nlm.nih.gov/pubmed/22634246?dopt=AbstractPlus>

"INTRODUCTION: Cigarette flavorings, with the exception of menthol, have been banned in the United States under the Family Smoking Prevention and Tobacco Control Act. Given the large number of menthol cigarette smokers in the United States, we investigated whether cigarette type (nonmenthol or menthol) is associated with peripheral artery disease (PAD). METHODS: The authors studied 5,973 adults, 40 years of age and older, who participated in the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2004. Smoking status and cigarette type were derived from self-reported questionnaires. PAD was defined as an ankle-brachial blood pressure index <0.9 in at least 1 leg. RESULTS: Fifty percent of participants were never-smokers compared to 31%, 14%, and 5% of former, current nonmenthol, and current menthol cigarette smokers, respectively. The weighted prevalence of PAD in the study population was 5%. After multivariable adjustment, the odds ratios for PAD were 1.44 (95% CI: 0.97, 2.15), 3.65 (95% CI: 1.57, 8.50), and 2.51 (95% CI: 1.09, 5.80) comparing former, current nonmenthol cigarette smokers, and current menthol cigarette smokers to never-smokers. The associations between smoking and PAD were similar for smokers of nonmenthol and menthol cigarettes (p value for heterogeneity = .59). CONCLUSIONS: In a representative sample of the U.S. population, current use of both menthol and nonmenthol cigarettes was associated with increased prevalence of PAD, with no difference in risk between cigarette types." As taken from Jones MR et al. 2013. Nicotine Tob Res. 15(7), 1183-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23212436>

"BACKGROUND: Cigarette smoke is harmful to human health at both cellular and genetic levels. Recently, a unique bioassay for smoke cytotoxicity using air pollution-sensitive plant cells (tobacco) has been proposed. METHODS: Model plant cells (tobacco Bel-W3 cells) and human cells (alveolar epithelial A549 cells) suspended in fresh culture media were exposed to cigarette smoke sampled after lighting the tip of cigarettes (with vs. without menthol capsules) which were attached to a glass pipe connected to the cell-containing plastic tubes. Control cultures were also assessed. RESULTS: After exposing tobacco plant cells to cigarette smoke, cell death occurred in a dose-dependent manner. Cell death was significantly enhanced by mentholated smoke, while menthol alone was shown to be inert suggesting that menthol synergistically contributes to the enhancement of cell death, initiated by smoke-associated compounds. The enhanced toxicity of mentholated smoke was confirmed in human alveolar epithelial A549 cells. CONCLUSIONS: Cigarette smoke cytotoxicity leading to cell death assessed in plant and human model cells was enhanced by menthol. Further research into these findings is encouraged." As taken from Noriyasu A et al. 2013. Tob. Induc. Dis. 11(1), 18. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24001273>

"OBJECTIVE: The Food and Drug Administration is considering regulation of menthol cigarettes. While persons with mental distress are known to smoke cigarettes at high rates, little is known about their use of menthol. The authors examined the association of psychological distress and menthol use in a national sample of adult smokers. METHODS: Data were from the 2008 and 2009 National Survey on Drug Use and Health. Past month smokers (N=24,157) were categorised for menthol or non-menthol use. Psychological distress was categorised as none/mild, moderate or severe on the Kessler six-item scale. RESULTS: The prevalence of menthol use was higher among individuals with severe psychological distress, women, young adults, African-Americans, Native Hawaiians/Pacific Islanders, persons with fewer years of education and lower income, and the unmarried and uninsured. In a multivariate model controlling for socio-demographic factors, smoking intensity and time to first cigarette, smokers with severe (adjusted OR (AOR) 1.23, 95% CI 1.04 to 1.46, p=0.02) but not moderate (AOR 1.03, 95% CI 0.92 to 1.15, p=0.58) psychological distress were significantly more likely to smoke menthols compared with smokers with none/mild distress. CONCLUSION: An elevated prevalence of menthol use was found among persons with

severe psychological distress, suggesting another group that could potentially benefit from the regulation of menthol cigarettes.” As taken from Hickman NJ et al. 2014. Tob. Control. 23(1), 7-13. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22821797>

Addition of menthol to cigarettes may be associated with increased initiation of smoking. The potential mechanisms underlying this association are not known. Menthol, likely due to its effects on cold-sensing peripheral sensory neurons, is known to inhibit the sensation of irritation elicited by respiratory irritants. However, it remains unclear whether menthol modulates cigarette smoke irritancy and nicotine absorption during initial exposures to cigarettes, thereby facilitating smoking initiation. Using plethysmography in a C57Bl/6J mouse model, we examined the effects of L-menthol, the menthol isomer added to cigarettes, on the respiratory sensory irritation response to primary smoke irritants (acrolein and cyclohexanone) and smoke of Kentucky reference 2R4 cigarettes. We also studied L-menthol's effect on blood levels of the nicotine metabolite, cotinine, immediately after exposure to cigarette smoke. L-menthol suppressed the irritation response to acrolein with an apparent IC(50) of 4 ppm. Suppression was observed even at acrolein levels well above those necessary to produce a maximal response. Cigarette smoke, at exposure levels of 10 mg/m(3) or higher, caused an immediate and marked sensory irritation response in mice. This response was significantly suppressed by L-menthol even at smoke concentrations as high as 300 mg/m(3). Counterirritation by L-menthol was abolished by treatment with a selective inhibitor of Transient Receptor Potential Melastatin 8 (TRPM8), the neuronal cold/menthol receptor. Inclusion of menthol in the cigarette smoke resulted in roughly a 1.5-fold increase in plasma cotinine levels over those observed in mice exposed to smoke without added menthol. These findings document that, L-menthol, through TRPM8, is a strong suppressor of respiratory irritation responses, even during highly noxious exposures to cigarette smoke or smoke irritants, and increases blood cotinine. Therefore, L-menthol, as a cigarette additive, may promote smoking initiation and nicotine addiction.” As taken from Ha MA et al. 2015. PLoS One 10(2), e0117128. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25679525>

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Laevo-Menthol was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Laevo-Menthol when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	23407	JTI KB Study Report(s)
In vitro cytotoxicity	23407	JTI KB Study Report(s)

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing l-Menthol was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	33,000	Logic (2019a)

		Labstat International Inc. (2022a)
In vitro genotoxicity	33,000	Logic (2019a)
		Labstat International Inc. (2022b)
In vitro cytotoxicity	33,000	Logic (2019a)
		Labstat International Inc. (2022b)
In vivo genotoxicity	7,500	Logic (2019a)
Inhalation study	7,500	Logic (2019a)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing l-Menthol was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	Tested level	Reference
Aerosol chemistry	34.6 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	34.6 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro cytotoxicity	34.6 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	34.6 mg/(tobacco portion; 310 mg)	Logic (2019b)
Inhalation study	34.6 mg/(tobacco portion; 310 mg)	Logic (2019b)

Aerosol from heated tobacco stick(s) containing l-menthol was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	16.1	Labstat International Inc. (2020a)
		Labstat International Inc. (2021a)
In vitro genotoxicity	16.1	Labstat International Inc. (2020b)
		Labstat International Inc. (2021b)
In vitro cytotoxicity	16.1	Labstat International Inc. (2020b)
		Labstat International Inc. (2021b)

“Tobacco products containing flavorings, such as electronic nicotine delivery devices (ENDS) or e-cigarettes, cigars/cigarillos, waterpipes, and heat-not-burn devices (iQOS) are continuously

evolving. In addition to increasing the exposure of teenagers and adults to nicotine containing flavoring products and flavoring enhancers, chances of nicotine addiction through chronic use and abuse also increase. These flavorings are believed to be safe for ingestion, but little information is available about their effects on the lungs. In this review, we have discussed the in vitro and in vivo data on toxicity of flavoring chemicals in lung cells. We have further discussed the common flavoring agents, such as diacetyl and menthol, currently available detection methods, and the toxicological mechanisms associated with oxidative stress, inflammation, mucociliary clearance, and DNA damage in cells, mice, and humans. Finally, we present potential biomarkers that could be utilized for future risk assessment. This review provides crucial parameters important for evaluation of risk associated with flavouring agents and flavoring enhancers used in tobacco products and ENDS. Future studies can be designed to address the potential toxicity of inhaled flavorings and their biomarkers in users as well as in chronic exposure studies.” As taken from Kaur G et al. 2018. *Toxicology Letters* 288, 143-155. PubMed, 2018, available at

<https://www.ncbi.nlm.nih.gov/pubmed/29481849>

The lowest observed genotoxic effect level (LOGEL) for 1R6F was observed at an exposure of 1 puff +S9 and 0.66 to 1.33 puffs –S9, for p-HTP Menthol, 16 to 24 puffs +S9 and 24 puffs –S9 and p-HTP Regular 8 to 16 puffs +S9 and 16 puffs –S9. Indicated using an ECMN3 comparison, the 1R6F smoke demonstrated much greater potency than the p-HTP aerosols. The ECMN3 analysis also highlighted that the p-HTP Regular aerosol was 2.3-fold (+S9) and 1.7-fold (–S9) more potent than the p- HTP Menthol aerosol under the conditions of the test.

The Ames bacterial reverse mutation test was carried out in two bacterial strains, TA98 and TA100 (both +S9 and –S9), demonstrated in combination to be responsive to HPHCs present in cigarette smoke (Rudd et al., 2020). In both strains, both in the presence and absence of S9, 1R6F smoke induced significant increases in revertant colonies with increasing numbers of puffs, and over a smaller puff-wise exposure range than tested with the p-HTPs. The p-HTP aerosols, however, did not induce significant increases in revertant colonies in either strain (+S9) under the conditions of test. Following the formation of nonrevertant pinpoint colonies upon the first exposure replicate with p- HTP Menthol, indicating toxicity, the exposure range was reduced from 81 to 189 to 18–90 puffs. The non-revertant pinpoint colonies were not included in the slope analysis. As taken from Chapman F. et. al. *Toxicol In Vitro*. 2023. Available at: <https://pubmed.ncbi.nlm.nih.gov/36372310/>

The p-HTP Menthol aerosol bPBS did not appear to induce any DNA damage via double strand breaks above background levels, however, there was a significant signal at the top tested concentration following 24 h only for the p-HTP Regular sample, indicating possible low level secondary DNA damage. As taken from Chapman F. et. al. *Toxicol In Vitro*. 2023. Available at: <https://pubmed.ncbi.nlm.nih.gov/36372310/>

In the NRU cytotoxicity assessment following 1R6F whole smoke or p-HTP whole aerosol exposures, cytotoxicity was significant (>EC20 achieved) and clear dose responses were observed to all three test articles (Fig. 3), however, the number of puffs required to induce EC50 was on average 38-fold higher for the p-HTP aerosols compared to 1R6F smoke exposure. As taken from Chapman F et. al. *Toxicology In Vitro*. 2023. Available at: <https://pubmed.ncbi.nlm.nih.gov/36372310/>

Although significant increases in the slope of the RWD50 response with increasing bPBS puffs/ml concentration were observed in response to treatment with all three test articles, this response was reduced by around 60-fold on comparison of both p-HTP Menthol and p-HTP Regular aerosol bPBS to 1R6F smoke bPBS. In addition to this, the dose range over which this response was observed was 15-fold wider for the p-HTPs (0–0.45puffs/ml) compared to 1R6F (0–0.03puffs/ml). Therefore, 1R6F smoke bPBS was found to be a much more potent inhibitor of wound healing than either p-HTP aerosol bPBS. Additionally, in this endpoint, p-HTP Menthol and p-HTP Regular aerosol bPBS did not appear to demonstrate differences to each other in effects. As taken from

10. Ecotoxicity

10.1. Environmental fate

L-Menthol's production and use in liqueurs, confectionary, perfumes, cosmetic products and pharmaceuticals such as cough drops and nasal decongestants may result in its release to the environment through various waste streams. It is the main component of peppermint oils and cornmint oils obtained from *Mentha piperita* and *Mentha arvensis*. If released to air, a vapor pressure of 0.064 mm Hg at 25 deg C indicates L-menthol will exist solely as a vapor in the ambient atmosphere. Vapor-phase L-menthol will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 16 hours. If released to soil, L-menthol is expected to have low mobility based upon an estimated Koc of 1,500. Volatilization from moist soil surfaces is expected to be an important fate process based upon an estimated Henry's Law constant of 1.5×10^{-5} atm-cu m/mole. L-Menthol is not expected to volatilize from dry soil surfaces based upon its vapor pressure. A 0% of the theoretical BOD was measured for L-menthol using an activated sludge inoculum over a 4 week incubation period. If released into water, L-menthol is expected to adsorb to suspended solids and sediment in the water column based upon the estimated Koc. Volatilization from water surfaces is expected to be an important fate process based upon this compound's estimated Henry's Law constant. Estimated volatilization half-lives for a model river and model lake are 2 and 18 days, respectively. The potential for bioconcentration is considered low based upon BCF values in the range of 1 to 15 measured in carp. Occupational exposure to L-menthol may occur through inhalation and dermal contact with this compound at workplaces where L-menthol is produced or used. The general population may be exposed to L-menthol through dermal contact with cosmetic products and ingestion of products containing L-menthol. (SRC) . **PEER REVIEWED**

L-Menthol's production and use in liqueurs, confectionary, perfumes, cosmetic products and pharmaceuticals such as cough drops and nasal decongestants(1,2) may result in its release to the environment through various waste streams(SRC). [(1) Budvari S; Merck Index, 12th ed, Whitehouse Station, NJ Merck & Co. p. 933 (1996) (2) Wagner FS; Kirk-Othmer's Encycl Chem Tech 4th ed. Kroschwitz JI ed. NY,NY: John Wiley & Sons 1: 133 (1991)] **PEER REVIEWED**

TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 1,500(SRC), determined from a log Kow of 3.3(2) and a regression-derived equation(3), indicates that L-menthol is expected to have low mobility in soil(SRC). Volatilization of L-menthol from moist soil surfaces is expected to be an important fate process(SRC) given an estimated Henry's Law constant of 1.5×10^{-5} atm-cu m/mole(SRC), using a fragment constant estimation method(4). L-

Menthol is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 0.064 mm Hg(5). A 0% of the theoretical BOD was measured for L-menthol using an activated sludge inoculum over a 4 week incubation period(2). [(1) Swann RL et al; Res Rev 85: 23 (1983) (2) Chemicals Inspection and Testing Institute; Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1 (1992) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 4-9 (1990) (4) Meylan WM, Howard PH; Environ Toxicol Chem 10: 1283-93 (1991) (5) Perry RH, Green D; Perry's Chemical Handbook 6th ed. N, NY: McGraw-Hill (1984)] **PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 1,500(SRC), determined from a log Kow of 3.3(2) and a regression-derived equation(3), indicates that L-menthol is expected to adsorb to suspended solids and sediment in water(SRC). Volatilization from water surfaces is expected(3) based upon an estimated Henry's Law constant of 1.5×10^{-5} atm-cu m/mole(SRC), developed using a fragment constant estimation method(4). Volatilization half-lives for a model river and model lake are 2 and 18 days, respectively(SRC), using an estimation method(3). According to a classification scheme(5), BCF values of 1-15 measured in carp(2) suggest bioconcentration in aquatic organisms is low(SRC). A 0% of the theoretical BOD was measured for L-menthol using an activated sludge inoculum over a 4 week incubation period(2). [(1) Swann RL et al; Res Rev 85: 23 (1983) (2) Chemicals Inspection and Testing Institute. Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1 (1992) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 4-9, 5-4, 5-10, 15-1 to 15-29 (1990) (4) Meylan WM, Howard PH; Environ Toxicol Chem 10: 1283-93 (1991) (5) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), L-menthol, which has a vapor pressure of 0.064 mm Hg at 25 deg C(2), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase L-menthol is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 16 hours(SRC) from its estimated rate constant of 2.4×10^{-11} cu cm/molecule-sec(3). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Perry RH, Green D; Perry's Chemical Handbook 6th ed. NY, NY: McGraw-Hill (1984) (3) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993)] **PEER REVIEWED**

A 0% of the theoretical BOD was measured for L-menthol using an activated sludge inoculum over a 4 week incubation period(1) [(1) Chemicals Inspection and Testing Institute; Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1 (1992)] **PEER REVIEWED**

The rate constant for the vapor-phase reaction of L-menthol with photochemically-produced hydroxyl radicals has been estimated as 2.4×10^{-11} cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 16 hours at an atmospheric concentration of 5×10^5 hydroxyl radicals per cu cm(1). L-Menthol is not expected to undergo hydrolysis in the environment due to the lack of hydrolyzable functional groups(2) nor to directly photolyze due to the lack of absorption in the environmental UV spectrum. [(1) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 7-4, 7-5 (1990)] **PEER REVIEWED**

BCF values of 1-15 were measured for carp exposed to 0.02-0.2 mg/l of L-menthol over a 6 week incubation period(1). According to a classification scheme(2), these BCF values suggest the potential for bioconcentration in aquatic organisms is low(SRC). [(1) Chemicals Inspection and Testing Institute. Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1 (1992)(1) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

The Koc of L-menthol is estimated as approximately 1,500(SRC), using a log Kow of 3.3(1) and a regression-derived equation(2). According to a classification scheme(3), this estimated Koc value suggests that L-menthol is expected to have low mobility in soil(SRC). [(1) Chemicals Inspection and Testing Institute; Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1 (1992) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 4-9 (1990) (3) Swann RL et al; Res Rev 85: 23 (1983)] **PEER REVIEWED**

The Henry's Law constant for L-menthol is estimated as 1.5×10^{-5} atm-cu m/mole(SRC) using a fragment constant estimation method(1). This Henry's Law constant indicates that L-menthol is expected to volatilize from water surfaces(2). Based on this Henry's Law constant, the volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 3 m/sec)(2) is estimated as approximately 2 days(SRC). The volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec)(2) is estimated as approximately 18 days(SRC). L-Menthol's Henry's Law constant(1,SRC) indicates that volatilization from moist soil surfaces may occur(SRC). L-Menthol is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 0.064 mm Hg at 25 deg C(3). [(1) Meylan WM, Howard PH; Environ Toxicol Chem 10: 1283-93 (1991) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (3) Perry RH, Green D; Perry's Chemical Handbook 6th ed. NY,NY: McGraw-Hill (1984)] **PEER REVIEWED**

As taken from HSDB, 2003.

The Ecological Categorization Results from the Canadian Domestic Substances List state that cyclohexanol, 5-methyl-2-(1-methylethyl)-[1R-(1a,2,5a)] (CAS RN 2216-51-5) is persistent in the environment:

Media of concern leading to Categorization	Water-Soil
Experimental Biodegradation half-life (days)	Not Available
Experimental Biodegradation result (%)	0
Reference	CHEMICALS INSPECTION AND TESTING INSTITUTE.; BIODEGRADATION AND BIOACCUMULATION DATA OF EXISTING CHEMICALS BASED ON THE

(biodegradation result)	CSCL JAPAN.; JAPAN CHEMICAL INDUSTRY ECOLOGY - TOXICOLOGY AND INFORMATION CENTER.ISBN 4-89074-101-1.; 1992
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.3314
TOPKAT probability of biodegradation	0
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	0.4441

ROBUST STUDY SUMMARY - Persistence

Item	Yes	No
Reference: Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan, 1992. Chemicals Inspection and Testing Institute (Ed.). Published by: Japan Chemical Industry Ecology-Toxicology & Information Center.		
<u>Test Substance</u> (CAS # and name): The list of the substances is attached (see file on N drive)		
Chemical composition of the substance (including purity, by-products)		X
<u>Method</u>		
References	X	
OECD, EU, national, or other standard method?	X	
Justification of the method/protocol if not a standard method was used	Not applicable	
<u>Test design / conditions</u>		
Study type (photodegradation, hydrolysis, biodegradation, other – <u>specify, do not assess</u>): Biodegradation		
Test type (aerobic or anaerobic - <u>specify, do not assess</u>):		

Test medium (air, water, soil, sediment - <u>specify, do not assess</u>):		
Information on stability of the substance in the media of concern is reported?	X	
Controls (positive or negative) Both	X	
Number of replicates (including controls)		X
Temperature (specify) 25±1 °C	X	
Duration of the experiment (specify) 14 or 28 days	X	
For photodegradation only		
Light source (specify):	Not applicable	
Light spectrum and relative intensity based on sunlight intensity:	Not applicable	
For hydrolysis only		
Measured concentrations reported?	Not applicable	
Basic water properties (pH, hardness, etc.)	Not applicable	
For biodegradation only		
Ready or inherent biodegradation (specify): Both types (see file on N drive)	X	
Innoculum (concentration and source)	X	
Results		
Endpoints: half-life (preferred); degradation, %; etc. (<u>do not assess this item</u>): % biodegradation by BOD or TOC (see file on N drive)		
Information on breakdown products (<u>do not assess this item</u>)		X

Overall score: 8/10 = 80%
EC Reliability code: 1
Reliability category (high, satisfactory, low): high
Comments:

Data accessed June 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

EPISuite provides the following information:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	1.52E-005 atm-m3/mole (1.54E+000 Pa-m3/mole)
Group Method:	2.56E-005 atm-m3/mole (2.60E+000 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 3.630E-006 atm-m3/mole (3.678E-001 Pa-m3/mole) VP: 0.00767 mm Hg (source: MPBPVP) WS: 435 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	3.19 (exp database)
Log Kaw used:	-3.207 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	6.397
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.8319

Biowin2 (Non-Linear Model) :	0.8707
Biowin3 (Ultimate Survey Model):	3.0138 (weeks)
Biowin4 (Primary Survey Model) :	3.7517 (days)
Biowin5 (MITI Linear Model) :	0.4554
Biowin6 (MITI Non-Linear Model):	0.3314
Biowin7 (Anaerobic Linear Model):	0.3226
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	29.3 Pa (0.22 mm Hg)
Log Koa (Koawin est):	6.397
Kp (particle/gas partition coef. (m ³ /ug)):	1.02E-007
Mackay model:	6.12E-007
Octanol/air (Koa) model:	

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	3.69E-006
Mackay model:	8.18E-006
Octanol/air (Koa) model:	4.9E-005

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	24.0849 E-12 cm ³ /molecule-sec
Half-Life =	0.444 Days (12-hr day; 1.5E6 OH/cm ³)
Half-Life =	5.329 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 5.94E-006 (Junge-Pankow, Mackay avg) 4.9E-005 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	88.15 L/kg (MCI method)
Log Koc:	1.945 (MCI method)
Koc :	189.7 L/kg (Kow method)
Log Koc:	2.278 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 2.56E-005 atm-m³/mole (estimated by Group SAR Method)

Half-Life from Model River:	29.87 hours (1.244 days)
Half-Life from Model Lake:	430.6 hours (17.94 days)

Removal In Wastewater Treatment:

Total removal:	8.92 percent
Total biodegradation:	0.14 percent
Total sludge adsorption:	7.48 percent
Total to Air:	1.30 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.2	10.7	1000
Water	27.8	360	1000
Soil	70.9	720	1000
Sediment	0.15	3.24e+003	0

Persistence Time: 417 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1a,2,5a)] (CAS RN 2216-51-5) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	18.9
Experimental result iT (mg/l)	18.9
Test species iT (Latin)	Pimephales promelas

Test species iT (Common)	Fathead minnow
Final EndPoint iT	LC50
Exposure duration iT (hours)	96
Reference iT	Center for Lake Superior Environmental Stud., Univ.of Wisconsin-Superior, Superior, WI I:355
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	22.3
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	5.844
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	30.648
Toxicity to fish (LC50 in mg/l) as predicted by Aster	8.907547
Toxicity to fish (LC50 in mg/l) as predicted by PNN	38.16727
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	0.629
Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	0.646
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	5.84E+000

Data accessed June 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

ACUTE/PROLONGED TOXICITY TO FISH

Type:	flow through

Species:	Pimephales promelas (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l
LC50:	18.9
EC50:	18.4
Limit test:	no
Analytical monitoring:	yes
Method:	other
Year:	1985
GLP:	no
Test substance:	other TS: purity 99%
Method:	Fish (30 d old; mean length 17.6 mm; mean weight 0.079 g) exposed in Lake Superior water; 5 TS concentrations in the range of 4.39 to 24.6 mg/l tested (plus control); number of dead fish recorded every 24 h; observations of fish behaviour and body morphology at regular intervals; TS analysis by GLC
Result:	Affected fish lost schooling behaviour, were hyperactive and underreactive to external stimuli. They had increased respiration, were darkly colored and lost equilibrium prior to death. The 96-h samples were omitted due to unrealistic analytical results.
Test condition:	24.4 degrees C; dissolved oxygen 6.8 mg/l; hardness 44.5 mg CaCO3/l; alkalinity 44.5 mg CaCO3/l; pH 7.7
Reliability:	(1) valid without restriction. Test procedure comparable to standard method and in accordance with general accepted scientific standards; detailed documentation of test procedure and test conditions
Flag:	Critical study for SIDS endpoint 21.12.2001 (21)
Type:	Static
Species:	Brachydanio rerio (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l
LC0:	13.2

LC50:	15.6
LC100:	18.4
Limit test:	no
Analytical monitoring:	yes
Method:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year:	1992
GLP:	yes
Test substance:	other TS: 99.9 %
Remark:	LC50-value: geom. mean of LC0 and L C100. LC50 can be calculated with probit analysis. In this test the LC50 obtained with probit analysis is identical to the geometric mean. All LC values are based on measured concentrations
Result:	RESULTS: EXPOSED - Nominal/measured concentrations: nominal (mg/l) 7.8 11 16 22 31 measured (mg/l) (0 h) 6.6 9.5 13.8 19.8 27.5 (mg/l) (24 h) 6.7 8.9 13.5 18.6 25.9 (mg/l) (48 h) 6.0 8.7 13.3 18.2 (mg/l) (72 h) 5.5 8.4 12.9 17.1 (mg/l) (96 h) 5.2 8.0 12.5 - Effect data (Mortality): Mortality, visible abnormalities of fishes - Concentration / response curve: There were no dead fishes in tanks with concentration: 7.8, 11 and 16 mg/l. 22 mg/l hours (h) 0 2 24 48 72 96 Mortality (%) 0 0 50 50 100 31 mg/l Mortality (%) 0 100 - Effect concentration vs. test substance solubility: Undissolved substance particles remained on the water surface of all test media. - Other effects: Temperature degradation during testing process was higher than 1 °C. RESULTS: CONTROL: No dead fish - Number/percentage of animals showing adverse effects: 7.8 mg/l hours (h) 2 24 48 72 96 7.8 mg/l - - - - -

	11 mg/l 100%A 100%A 100%A 100%A 100%A 16 mg/l 100%A 100%A 80%A 80%A 100%A 20%B 20%B 22 mg/l 100%A 50%B 50%B 10%B 10%B - Nature of adverse effects: A: slow and inactive swimming behaviour B: loss of equilibrium (uncontrolled movements) Reliability: (2) valid with restrictions Guideline Study; effective concentrations decreased below 80% of the nominal during the test period
Flag:	Critical study for SIDS endpoint 05.03.2003 (22)
Type:	other: Static or semi-static
Species:	Oryzias latipes (Fish, fresh water)
Exposure period:	48 hour(s)
Unit:	mg/l
LC50:	26
Method:	other: according to JIS K 0102-1986-71 (Japanese Industrial Standard)
Year:	1992
Test substance:	no data
Remark:	TS unclear. The reference notes both CAS -No. 2216-51-5 and 15356-70-4
Test condition:	25 +- 2 degrees C; 10 fish/level
Reliability:	(2) valid with restrictions Test procedure according to guideline without detailed documentation
Flag:	Critical study for SIDS endpoint 07.03.2002 (6)

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type:	static
Species:	Daphnia magna (Crustacea)

Exposure period:	48 hour(s)
Unit:	mg/l
EC0:	11.35
EC50:	26.6
EC100:	92.35
Analytical monitoring:	yes
Method:	Directive 92/69/EEC, C.2
Year:	2002
GLP:	yes
Test substance:	other TS: purity 99.985 % according to H & R GmbH
Method:	Method for chemical analysis: GC analysis Limit of quantitation: 0.5 mg/l Recovery rate: 102% ± 5% Sampling schedule of chemical analysis Stock solution: at 0 h Control: at 0 and 48 h Test concentrations: at 0, 24 and 48 h
Result:	The results are expressed in terms of nominal concentrations (at 24 h), and in terms of mean measured concentrations (at 48 h). Measured concentrations ranged from 93.1 - 104 % of nominal values at 0 h, and from 81.3 - 91.6 % of nominal values at 48 h, respectively. After 24 h EC0 was 25 mg/l, EC50 37.7 (lower/upper 95% confidence limit: 18.8/75.6) mg/l and EC100 100 mg/l After 48 h EC0 was 11.35 mg/l, EC50 26.6 (14.7/48.2) mg/l and EC100 92.35 mg/l Highest test concentration resulting in 0 % immobilisation (EC0 48 h):11.35 mg/l Lowest test substance concentration resulting in 100 % immobilization (EC100 48 h): 92.35 mg/l
Test condition:	- A population of parthenogenetic females of synchronized age structure is maintained since more than 15 years in the test facility under constant temperature conditions (20 +/- 1 °C) at a 16/8 h light-dark photoperiod (illumination: < 1000 lux) - The culture water (so-called 'M4 medium') is partly renewed once a week. The Daphnia are exclusively fed with unicellular green algae (<i>Desmodesmus subspicatus</i>) ad libitum - Mortalities of parent Daphnia during the culture period are recorded daily in a semi-quantitative way. The neonates are separated from their parent Daphnia by filtration prior to the acute test Culture and dilution water: - Reconstituted water (so-called 'M4 medium', originally described in Water Research 24 (9): 1157 - 1167), prepared according to the recommendations of Bundesgesundheitsamt Berlin. This standard dilution water is used for both, the maintenance of the test animals and the preparation of stock and test solutions of the test substance - The total hardness of the dilution water, measured at test start, was 13.8°dH (= 246.3 mg/l CaCO₃) Test substance: - The test substance was pulverized. - A stock solution was prepared to give

	the desired series of test concentrations. To achieve this 119.9 mg of the test substance were added to 1 litre of dilution water and treated for 1 hour in an ultrasonic bath and afterwards stirred for 24 hours on a magnetic stirrer. Finally undissolved particles of the test substance were removed by filtration using a folded filter of pore size 7 - 12 µm. Exposure conditions: - Test vessels: holding 10 neonates in 20 ml of test medium - Experimental design: 6 test concentrations plus 1 control 10 neonates per vessel, 2 replicates per concentration/control, no feeding during the exposure period, static system - Method of initiation: neonates were placed in prepared media - Photoperiod: 16 h light/8 h dark - Temperature: 20 +/- 1 °C - Aeration: none - Test concentration/s (nominal): 3.2, 6.3, 12.5, 25, 50 and 100 mg/l - Method of administration: stock solution - Medium renewal: none - Duration of exposure: 48 h - Criteria of effects: The criterion of adverse effects used in this study was the substance-induced alteration of the normal mobility behaviour and the loss of locomotory actions of the neonates, observed at 24 and 48 h
Reliability:	(1) valid without restriction Guideline study in accordance with the OECD principles of GLP
Flag:	Critical study for SIDS endpoint 21.02.2003 (23)

TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species:	Scenedesmus subspicatus (Algae)
Endpoint:	growth rate
Exposure period:	72 hour(s)
Unit:	mg/l
NOEC:	9.65
LOEC:	18.75
EC50:	21.4
Analytical monitoring:	yes
Method:	Directive 92/69/EEC, C.3
Year:	2002

GLP:	yes
Test substance:	other TS: purity 99.985 % according to H & R GmbH
Method:	Chemical analysis method: GC analysis
Limit of quantitation:	0.5 mg/l
Recovery rate:	105 % $\pm$ 5 % Sampling schedule of chemical analysis - Stock solution: at 0 h - Control: at 0 and 72 h - Test concentrations: at 0 and 72 h Expression of results Cell density measurements in the test and control cultures are tabulated according to the concentration of test substance and the time of measurement. Growth curves are plotted for each test concentration and control. The area under the growth curve [b] is calculated for each test culture. The growth rate [r] is calculated for each test culture. The percentage inhibition of both, growth [b] and growth rate [r], is calculated for each test concentration. If possible, EC 50 values for both, growth [b] and growth rate [r], are calculated by probit analysis. If possible, NOEC and LOEC of both, growth [b] and growth rate [r], are determined by a multisample comparison (according to DUNNETT 1955, 1964). Result: b: growth r: growth rate Results (72 h)[mg/l]: EbC50: 20 ErC50: 21.4 NOEC [b]: 9.65 LOEC [b]: 18.75 NOEC [r]: 9.65 LOEC [r]: 18.75 All results are expressed in terms of mean measured concentrations. Measured concentrations ranged from 92.0 - 102.5 % of nominal values at 0 h, and from 88.0 - 105 % of nominal values at 72 h, respectively.
Test condition:	Test species: Name: <i>Desmodesmus subspicatus</i>; former name: <i>Scenedesmus subspicatus</i> Source: Non-axenic strain of the test species obtained from 'The Collection of Algal Cultures' of the Institute of Plant Physiology at the University of Göttingen (Germany) - Maintenance of stock cultures: Exponentially-growing stock cultures are maintained in the test facility under constant temperature conditions (23 $\pm$ 2°C) at a light intensity in the range 60 - 120 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (measured in the range 400 to 700 nm using a spherical quantum flux meter). The nutrient medium (according to BRINGMANN & KÜHN 1977) is renewed once a week. Cell density measurements are made using a microcell counter -

	Preparation of pre-cultures: Pre-cultures are set up three days before the start of a test. They are grown under identical exposure conditions as the stock cultures, except from the use of a different nutrient medium (annex 1) - Test cultures: The algal inocula for a test are taken from an exponentially growing pre-culture and are mixed with the nutrient medium (annex 1) to make up to a final cell density of about 10000 cells/ml in the test medium. Pretreatment of the test substance - The test substance was pulverized - A stock solution was prepared to give the desired series of test concentrations. To achieve this 124.9 mg of the test substance were added to 1 litre of dilution water and treated for 1 h in an ultrasonic bath and afterwards stirred for 24 h on a magnetic stirrer Exposure conditions - Test vessels: 300 ml Erlenmeyer flasks with stoppers - Culturing apparatus: light chamber in which a temperature in the range 21 °C to 25 °C can be maintained at +/- 2 °C, and continuous uniform illumination is provided in the spectral range 400 to 700 nm - Light intensity: at the average of the test solutions, a light intensity in the range 60 to 120 µE m⁻² s⁻¹ - Cell density measurements: microcell counter
Experimental design:	- 4 Test concentrations plus 1 control, 3 replicates per concentration, 6 replicates per control, initial cell density in the test cultures approximately 10000 cells/ml additionally highest test concentration without algae – Test concentration/s (nominal): 5.0, 10, 20 and 40 mg/l- Method of administration: stock solution - Duration of exposure: 72 h - Criteria of effects: The criteria of adverse effects used in this study were the substance-induced inhibition of growth [b] and growth rate [r], respectively, of the algal population.
Reliability:	(1) valid without restriction Guideline study in accordance with the OECD principles of GLP
Flag:	Critical study for SIDS endpoint 21.02.2003 (24)

TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type:	aquatic
Species:	activated sludge
Unit:	mg/l
EC10:	51
EC50:	237
Analytical monitoring:	no
Method:	ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year:	1992
GLP:	yes
Test	other TS: 99.9 %

substance:	
Remark:	direct weight
Reliability:	(2) valid with restrictions Guideline Study, incubation period not reported
Flag:	Critical study for SIDS endpoint 07.03.2002 (25)
Species:	aerobic microorganisms
Exposure period:	48 hour(s)
Method:	Test of antimicrobial activity of the essential oil of Calamintha nepeta and its main constituents against bacteria and fungi (Listeria monocytogenes, Bacillus cereus, Salmonella veneziana, Salmonella paratyphi B, Salmonella typhimulium, Fusarium moniliforme, Botrytis cinerea, Aspergillus niger, Pyricularia oryzae). Bacteria and fungi cultures were placed on the surface of a culture medium. 20 µl menthol solution (dissolved 1:1 in Tween 80) were placed on paper disks which were placed in the petri dishes and incubated at 37 degrees C.
Result:	Menthol showed no activity against any of the tested microorganisms.
Reliability:	(3) invalid Unsuitable test system. Tween 80 was present at a high concentration. 26.07.2002 (26)

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

Record for (1R,2S,5R)-5-Methyl-2-(1-methylethyl)cyclohexanol (CAS RN 2216-51-5):

Spec. Name	Sci.	Exp. Type	Media Type	Resp. Site	Endpoint BCF	Trend	Effect	Conc (ug/L)	Stat. Signif.
Spec. Common Name		Chem. Anal.	Loc	Obs. Dur. (Days)		Eff %	Effect Meas.	Appl. Rate	Sig. Level
Xenopus laevis		S	FW		LOEL	INC	PHY	F 1 mM	ASIG
African Clawed Frog		U	LAB	0.001		136	EPYR/		<0.01
Pimephales promelas		F	FW		LC50		MOR	A 18900 ug/L	
Fathead Minnow		M	LAB	4			MORT		

As taken from the EPA ECOTOX database.

“BACKGROUND: Dengue fever virus transmitted by *Aedes aegypti* causes lethal mortalities of human beings, and, because of the lack of any vaccine, management of this vector, especially with phytochemicals, is essential. In the present investigation, the structure-activity relationship of monoterpenes and their acetyl derivatives was studied to identify structural features that are responsible for mosquitocidal activity. RESULTS: Derivatization of monoterpenes (eugenol, geraniol, linalool, L-menthol and terpenole) followed by structure-activity relationship studies identified all five acetyl derivatives as having enhanced mosquitocidal activity against fourth-instar larvae of *Aedes aegypti*. Acetylation of the hydroxyl group in general increased activity in comparison with hydroxyl compounds. Based on LC50 values (ppm), the activities could be placed in the following order: eugenyl acetate (50.2) > linalyl acetate (119.7) > terpinyl acetate (287.1) > menthyl acetate (308.4) > geranyl acetate (325.5), as compared with monoterpenoids: eugenol (82.8) > linalool (242.6) > terpineol (331.7) > L-menthol (365.8) > geraniol (415.0). In eugenyl acetate, the presence of an aromatic ring and a side chain with an allylic double bond makes it most effective. CONCLUSION: Bioactive functional groups identified in the study may contribute to the understanding of larvicidal activity of acetyl derivatives and may help in the development of ecofriendly mosquito larvicidal compounds.” As taken from Pandey SK et al. 2013. Pest. Manag. Sci. 69(11), 1235-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23908002>

ECOSAR Version 1.11 reports the following aquatic toxicity data for CAS RN 2216-51-5:

Values used to Generate ECOSAR Profile

Log Kow: 3.381 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 490 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Fish	96-hr	LC50	7.379
Neutral Organics :	Daphnid	48-hr	LC50	4.760
Neutral Organics :	Green Algae	96-hr	EC50	6.008
Neutral Organics :	Fish		ChV	0.838
Neutral Organics :	Daphnid		ChV	0.662
Neutral Organics :	Green Algae		ChV	2.090

Neutral Organics :	Fish (SW)	96-hr	LC50	9.367
Neutral Organics :	Mysid	96-hr	LC50	2.731
Neutral Organics :	Fish (SW)		ChV	2.363
Neutral Organics :	Mysid (SW)		ChV	0.157

“Menthol is a common compound in pharmaceutical and commercial products and a popular additive to cigarettes. The molecular targets of menthol remain poorly defined. In this study we show an effect of menthol on the $\alpha 7$ subunit of the nicotinic acetylcholine (nACh) receptor function. Using a two-electrode voltage-clamp technique, menthol was found to reversibly inhibit $\alpha 7$ -nACh receptors heterologously expressed in *Xenopus* oocytes. Inhibition by menthol was not dependent on the membrane potential and did not involve endogenous $\text{Ca}(2+)$ -dependent $\text{Cl}(-)$ channels, since menthol inhibition remained unchanged by intracellular injection of the $\text{Ca}(2+)$ chelator BAPTA and perfusion with $\text{Ca}(2+)$ -free bathing solution containing $\text{Ba}(2+)$. Furthermore, increasing ACh concentrations did not reverse menthol inhibition and the specific binding of $[(125)\text{I}]$ α -bungarotoxin was not attenuated by menthol. Studies of $\alpha 7$ - nACh receptors endogenously expressed in neural cells demonstrate that menthol attenuates $\alpha 7$ mediated $\text{Ca}(2+)$ transients in the cell body and neurite. In conclusion, our results suggest that menthol inhibits $\alpha 7$ -nACh receptors in a noncompetitive manner.” As taken from Ashoor A et al. 2013. PLoS One 8(7), e67674. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23935840>

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

TOX. TO OTHER NON MAMM. TERR. SPECIES

Species:	other: larvae of <i>Ostrinia nubilalis</i> (Lepidoptera: Pyralidae; European corn borer)
Endpoint:	other: survival and growth
Exposure period:	6 day(s)
Year:	1999
GLP:	no
Test substance:	other TS: l-Menthol, laboratory chemical
Method:	Two different chemical application methods conducted: dripping the solution onto the solidified artificial diet (on-diet test) or mixing the solution in the diet before it solidified (in-diet test). In each test cup 1 neonate larva was placed on the diet. On-diet test: 199 larvae tested at 6 doses In-diet test: 160 larvae tested at 5 doses

Result:	On-diet test: LC50 = 2.35 mg per cup (1st-instar). In-diet test: LC50 = 17.4 mg per cup (2nd-instar)
Test condition:	25 +- 2 degrees C; photoperiod 14:10 (light:dark) hours
Reliability:	(3) invalid No conclusion from environmental concentrations to effects possible 22.12.2001 (27)
Species:	other: Aspergillus flavus
Exposure period:	5 day(s)
Year:	1998
GLP:	no
Test substance:	other TS: menthol from Mentha piperita
Method:	Maize grain protection assay against A. flavus
Result:	Total inhibition of A. flavus
Test condition:	Maize grains immersed in essential oil, dried, and sprayed with fungal spore suspension
Reliability:	(3) invalid No conclusion from environmental concentrations to effects possible 22.12.2001 (28)

As taken from OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>

Record for (1R,2S,5R)-5-Methyl-2-(1-methylethyl)cyclohexanol (CAS RN 2216-51-5):

Spec. Sci. Name Spec. Name Common Name	Resp. Type Exp. Dur. (Days)	Media Type Test Loc.	Exp. Site Chem. Anal.	Dose# Res. Sample Unit	Endpoint BAF/BCF	Effect Effect Meas.	Signif. Sig. Level	Dose Dose Stat. Meth.
Flowers, Trees, Shrubs, Ferns								
Triticum aestivum	COL	FLT	CM	2	LOEL	MPH	ASIG	A 0.5 AI %/wt sd

Bread Wheat	7	LAB	U			LGTH	0.05	
Triticum aestivum	COL	FLT	CM	2	NOEL	REP	ANOSIG	A 0.5 AI %/wt sd
Bread Wheat	7	LAB	U			GERM	0.05	
Insects/Spiders								
Anopheles tessellatus		NONE	DA	6	ED50	MOR		F 0.54 (0.46- 1.04) ug/ml
Mosquito	0.042	LAB	U			TKNO		
Culex quinquefasciatus		NONE	DA	6	ED50	MOR		F 0.50 (0.41- 0.62) ug/ml
Southern House Mosquito	0.042	LAB	U			TKNO		
Aedes aegypti		FLT	EN	6	ED50	MOR		F > 20 g/L
Yellow Fever Mosquito	0.042	LAB	U			TKNO		
Musca domestica		NONE	FU	>=5	LC50	MOR		F 3.6 (2.5- 5.2) ug/cm3
House Fly	0.583	LAB	U			MORT		
Tribolium castaneum		NONE	FU	>=5	LC50	MOR		F > 1780 ug/cm3
Rust-Red Flour Beetle	1	LAB	U			MORT		

Diabrotica undecimpunctata ssp. howardi		UKS	DA		LC50	MOR		F 89.8 (81.1-99.5) ug/g soil
Southern Corn Rootworm	2	LAB	U			MORT		
Musca domestica		NONE	TP	>=5	LC50	MOR		F 193 (171-217) ug/org
House Fly	1	LAB	U			MORT		
Culex quinquefasciatus		NONE	DA	6	LC50	MOR		F 0.50 (0.32-0.76) ug/ml
Southern House Mosquito	1	LAB	U			MORT		
Anopheles tessellatus		NONE	DA	6	LC50	MOR		F 0.36 (0.24-0.45) ug/ml
Mosquito	1	LAB	U			MORT		
Ostrinia nubilalis			FD	7	LC50	MOR		F 2.35 (1.59-3.60) mg/eu
European Corn Borer	6	LAB	U	LV		MORT		
Ostrinia nubilalis			FD	6	LC50	MOR		F 17.4 (14.4-20.8) ppm
European Corn Borer	6	LAB	U	LV		MORT		

Tribolium castaneum		NONE	EN	2	LC50	MOR		F > 500 ug/cm2
Rust-Red Flour Beetle	1	LAB	U			MORT		
Sitophilus oryzae		NONE	EN	2	LC50	MOR		F 221.68 (161.95-307.76) ug/cm2
Rice Weevil	1	LAB	U			MORT		
Tribolium castaneum		FLT	EN	13	LC50	MOR		F > 100 mg/L
Rust-Red Flour Beetle	1	LAB	U			MORT		
Sitophilus oryzae		FLT	EN	13	LC50	MOR		F 61.75 (52.46-73.56) ug/cm2
Rice Weevil	1	LAB	U			MORT		
Aedes aegypti		FLT	EN	6	LC50	MOR		F > 20 g/L
Yellow Fever Mosquito	1	LAB	U			MORT		
Spodoptera littoralis		FLT	EN	>=6	LC50	MOR		F 9.36 (7.44-11.53) mg/L
Egyptian Cotton Leafworm	1	LAB	U			MORT		
Tribolium castaneum			TP		LD99	MOR		F 1545.4 (1211-2405)

								ug/mg
Rust-Red Flour Beetle	2	LAB	U			MORT		
Rhyzopertha dominica			TP		LD99	MOR		F 1393.7 (1228.7-1694.3) ug/mg
Lesser Grain Borer	2	LAB	U			MORT		
Sitophilus oryzae			TP		LD99	MOR		F 1068.5 (901.9-1388.6) ug/mg
Rice Weevil	2	LAB	U			MORT		
Callosobruchus maculatus			TP		LD99	MOR		F 823.5 (620.4-1397.1) ug/mg
Cowpea Weevil	2	LAB	U			MORT		
Tribolium castaneum		FLT	EN		LD99	MOR		F 28.1 (19.4-84.0) mg/L media
Rust-Red Flour Beetle	2	LAB	U			MORT		
Rhyzopertha dominica		FLT	EN		LD99	MOR		F 7.9 (6.7-12.9) mg/L media
Lesser Grain Borer	2	LAB	U			MORT		
Sitophilus oryzae		FLT	EN		LD99	MOR		F 7.9 (6.7-12.8)

								mg/L media
Rice Weevil	2	LAB	U			MORT		
Callosobruchus maculatus		FLT	EN		LD99	MOR		F 8.4 (7.3- 14.1) mg/L media
Cowpea Weevil	2	LAB	U			MORT		
Tribolium castaneum		FLT	EN	2	LOEL	AVO	ASIG	F 20 ug/ml
Rust-Red Flour Beetle	0.125	LAB	U			CHEM	0.05	
Rhyzopertha dominica		FLT	EN	2	LOEL	AVO	ASIG	F 20 ug/ml
Lesser Grain Borer	0.125	LAB	U			CHEM	0.05	
Sitophilus oryzae		FLT	EN	2	LOEL	AVO	ASIG	F 20 ug/ml
Rice Weevil	0.125	LAB	U			CHEM	0.05	
Callosobruchus maculatus		FLT	EN	2	LOEL	AVO	ASIG	F 20 ug/ml
Cowpea Weevil	0.125	LAB	U			CHEM	0.05	
Apis sp.	HO	NONE	EN	3	LOEL	BEH	SIG	F 60 g/eu
Bee	90	FIELDA	U			HONY	<0.05	
Ostrinia nubilalis	WO		FD	7	LOEL	DVP	SIG/	F 0.0002 mg/eu

European Corn Borer	6	LAB	U	PU		PUPA	0.05/	
Ostrinia nubilalis			FD	6	LOEL	DVP	SIG/	F 1 ppm
European Corn Borer		LAB	U	LV		STGE	0.05/	
Ostrinia nubilalis			FD	7	LOEL	DVP	SIG	F 20 mg/eu
European Corn Borer	(0 - 31.5)	LAB	U			STGE	0.05	
Ostrinia nubilalis			FD	7	LOEL	DVP	ASIG	F 0.0002 mg/eu
European Corn Borer		LAB	U			EMRG	0.05	
Ostrinia nubilalis			FD	6	LOEL	DVP	SIG	F 10 ppm
European Corn Borer	(19.43 - 29.1)	LAB	U			PUPA	0.05	
Ostrinia nubilalis			FD	6	LOEL	DVP	SIG	F 10 ppm
European Corn Borer	(30.53 - 42.1)	LAB	U			EMRG	0.05	
Peridroma saucia	MC	NONE	FD	6	LOEL	ENZ	SIG	F 0.025 %
Variegated Cutworm	2.5	LAB	U			P450	<0.05	
Ostrinia nubilalis			FD	7	LOEL	GRO	SIG	F 20 mg/eu
European Corn		LAB	U			WGHT	0.05	

Borer								
Spodoptera littoralis		NONE	TP	2	LOEL	MOR	ASIG	F 1/ (NR/-NR/) mg/org
Egyptian Cotton Leafworm	1	LAB	U	LV		MORT	0.05	
Apis sp.	HO	NONE	EN	3	NOEL	BEH	NOSIG	F 30 g/eu
Bee	90	FIELDA	U			HONY	<0.05	
Apis sp.	NOC	NONE	EN	2	NOEL	BEH	ANOSIG	F 30 g/eu
Bee	90	FIELDA	U			HONY	<0.05	
Apis sp.	NOC	NONE	EN	2	NOEL	BEH	ANOSIG	F 30 g/eu
Bee	90	FIELDA	U			HONY	<0.05	
Apis sp.	HO	NONE	EN	2	NOEL	BEH	ANOSIG	F 60 g/eu
Bee	90	FIELDA	U			HONY	<0.05	
Ostrinia nubilalis	WO	NAT	FD	8	NOEL	DVP	NOSIG/	F 1000 ppm
European Corn Borer	6	LAB	U	PU		PUPA	0.05/	
Ostrinia nubilalis			FD	7	NOEL	DVP	NOSIG	F 2 mg/eu
European Corn Borer	(0 - 31.5)	LAB	U			STGE	0.05	
Ostrinia nubilalis			FD	7	NOEL	DVP	ANOSIG	F 1000 ppm

European Corn Borer	12	LAB	U			STGE	0.05	
Ostrinia nubilalis			FD	7	NOEL	DVP	ANOSIG	F 1000 ppm
European Corn Borer	30	LAB	U			STGE	0.05	
Ostrinia nubilalis			FD	7	NOEL	DVP	ANOSIG	F 1000 ppm
European Corn Borer	42	LAB	U			EMRG	0.05	
Ostrinia nubilalis			FD	6	NOEL	DVP	NOSIG	F 1000 ppm
European Corn Borer	11	LAB	U			STGE	0.05	
Ostrinia nubilalis			FD	6	NOEL	DVP	NOSIG	F 1 ppm
European Corn Borer	(19.43 - 29.1)	LAB	U			PUPA	0.05	
Ostrinia nubilalis			FD	6	NOEL	DVP	NOSIG	F 1 ppm
European Corn Borer	(30.53 - 42.1)	LAB	U			EMRG	0.05	
Peridroma saucia	MC	NONE	FD	6	NOEL	ENZ	NOSIG	F 0.010 %
Variegated Cutworm	2.5	LAB	U			P450	<0.05	
Ostrinia nubilalis			FD	7	NOEL	GRO	NOSIG	F 2 mg/eu
European Corn Borer		LAB	U			WGHT	0.05	

Ostrinia nubilalis			FD	7	NOEL	GRO	ANOSIG	F 1000 ppm
European Corn Borer		LAB	U			WGHT	0.05	
Apis sp.		NONE	EN	2	NOEL	REP	ANOSIG	F 60 g/eu
Bee	34	FIELDA	U			SBRD	<0.05	
Apis sp.		NONE	EN	3	NOEL	REP	ANOSIG	F 60 g/eu
Bee	34	FIELDA	U			SBRD	<0.05	
Apis sp.		NONE	EN	2	NOEL	REP	ANOSIG	F 30 g/eu
Bee	34	FIELDA	U			SBRD	<0.05	
Apis sp.		NONE	EN	2	NOEL	REP	ANOSIG	F 30 g/eu
Bee	34	FIELDA	U			SBRD	<0.05	
Ostrinia nubilalis			FD	6		GRO	MULT	F (1-10000) ppm
European Corn Borer		LAB	U			WGHT	0.05	
Molluscs								
Theba pisana		FLT	EN	12	LC50	MOR		F > 100 mg/L
Land Snail	1	LAB	U			MORT		
Theba pisana		NONE	TP	13	LD50	MOR		F 99.44 (57.97-138.32)

								ug/org
Land Snail	1	LAB	U			MORT		
Deroceras reticulatum		FLT	CM	2	NR-LETH	MOR		A 0.5 AI %
Grey Field Slug	1	LAB	U			MORT		

As taken from the EPA ECOTOX database.

“Housefly (*Musca domestica* L.), one of the most common insects in human settlements, has been associated as vectors for various food-borne pathogens, causing food spoilage and disease transmission. The control of housefly was attempted using plant monoterpenes; menthone, menthol, menthyl acetate, limonene, citral and 1,8-cineole, against different life stages of housefly. Bioefficacy against housefly adults revealed highest repellent activity by menthol (95.6 percent) and menthone (83.3 percent). Against housefly larvae, menthol with an LC90 of 0.02µl/cm(2) in contact toxicity assay and menthone with a LC90 value of 5.4µl/L in fumigation assay were found to be most effective control agent. With respect to pupicidal activity, superior performance was shown by menthol, citral and 1,8-cineole in contact toxicity assay and citral and 1,8-cineole in fumigation assay. Limonene was found to be the poorest performer in all the assays. Overall, highest efficacy observed for menthol and menthone in various bioassays was in agreement with the results of essential oil activity obtained previously. Significant activity of monoterpenes against various life stages of housefly demonstrates their potential as excellent insecticides with prospects of monoterpenes being developed into eco-friendly and acceptable products for housefly control.” As taken from Kumar P et al. 2014. Ecotoxicol. Environ. Saf. 100, 1-6. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24433784>

“The nematocidal activity and chemical characterization of aqueous extracts and essential oils of three mint species, namely, *Mentha × piperita*, *Mentha spicata*, and *Mentha pulegium*, were investigated. The phytochemical analysis of the essential oils was performed by means of GC-MS, whereas the aqueous extracts were analyzed by LC-MS. The most abundant terpenes were isomenthone, menthone, menthol, pulegone, and carvone, and the water extracts yielded mainly chlorogenic acid, salvianolic acid B, luteolin-7-O-rutinoside, and rosmarinic acid. The water extracts exhibited significant nematocidal activity against *Meloidogyne incognita*, and the EC50/72h values were calculated at 1005, 745, and 300 mg/L for *M. × piperita*, *M. pulegium*, and *M. spicata*, respectively. Only the essential oil from *M. spicata* showed a nematocidal activity with an EC50/72h of 358 mg/L. Interestingly, menthofuran and carvone showed EC50/48h values of 127 and 730 mg/L, respectively. On the other hand, salicylic acid, isolated in the aqueous extracts, exhibited EC50 values at 24 and 48 h of 298 ± 92 and 288 ± 79 mg/L, respectively.” As taken from Caboni P et al. 2013. J. Agric. Food Chem. 61(41), 9784-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24050256>

“The screening for repellency against the booklouse, *Liposcelis bostrychophila* (Badonnel), and the red flour beetle, *Tribolium castaneum* (Herbst), from 14 Chinese medicinal herbs showed that the essential oils of *Curcuma longa* L., *Epimedium pubescens* Maximowicz, *Lindera aggregate* (Sims) Kostermans, *Nardostachys chinensis* Battandier, *Schizonepeta tenuifolia* Briquet, *Zanthoxylum schinifolium* Sieber et Zuccarini, and *Z. officinale* Roscoe exhibited strong repellency against *L. bostrychophila* and *T. castaneum*. A total of 35 components of the essential oil of *E. pubescens* were identified by gas chromatography and gas chromatography-mass spectrometry. Beta-Eudesmol (14.89%), alpha-pinene (13.38%), borneol (9.56%), (R)-carvone (7.89%), and menthol (7.45%) were the main components of the essential oil of *E. pubescens*. From the essential oil of *E.*

pubescens, four monoterpenoids and one sesquiterpenoid were isolated by bioassay-guided fractionation. The compounds were identified alpha-pinene, borneol, menthol, carvone, and beta-eudesmol. (R)-carvone, menthol, borneol, and beta-eudesmol were strongly repellent against *L. bostrychophila* at concentration of 8.5 nl/cm² after 2 h exposure whereas alpha-pinene exhibited moderate repellency. (R)-carvone exhibited stronger repellency against the booklouse than the positive control, N, N-diethyl-3-methylbenzamide (DEET). Moreover, (R)-carvone also possessed stronger repellency against *T. castaneum* than DEET. The other four constituents, menthol, borneol, and beta-eudesmol also showed repellency against the red flour beetles but weaker than DEET at lower concentrations." As taken from Liang Y et al. 2013. J. Econ. Entomol. 106(1), 513-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23448068>

"BACKGROUND: The toxicity of red pine needle hydrodistillate (RPN-HD), 19 RPN-HD constituents and another 12 structurally related compounds and the control efficacy of four experimental spray formulations containing RPN-HD (0.5, 1, 2 and 3% sprays) to adult *Dermatophagoides farinae* were evaluated. RESULTS: RPN-HD (24 h LC₅₀, 68.33 µg cm⁻²) was toxic to mites. Menthol was the most toxic compound (12.69 µg cm⁻²), and the toxicity of this compound and benzyl benzoate did not differ significantly. High toxicity was also produced by α-terpineol, bornyl acetate, geranyl acetate, thymol, linalyl acetate, terpinyl acetate, citral, linalool and camphor (18.79-36.51 µg cm⁻²). These compounds were more toxic than either deet or dibutyl phthalate. In vapour-phase mortality tests, these compounds were consistently more toxic in closed versus open containers, indicating that their mode of delivery was largely a result of vapour action. RPN-HD 3% experimental spray provided 95% mortality against adult *D. farinae*, whereas permethrin (cis:trans, 25:75) 2.5 g L⁻¹ spray treatment resulted in 0% mortality. CONCLUSION: In the light of global efforts to reduce the level of highly toxic synthetic acaricides in indoor environments, RPN-HD and the compounds described merit further study as potential biocides for the control of *Dermatophagoides* populations as fumigants with contact action." As taken from Lee JH et al. 2013. Pest. Manag. Sci. 69(6), 696-702. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23109302>

"The acaricidal activities of peppermint oil and menthol isomers against mites in stored food were evaluated using fumigant and contact bioassays and were compared with the activity of benzyl benzoate as a synthetic acaricide. Based on the 50% lethal dose (LD₅₀) values against *Tyrophagus putrescentiae* in the fumigant bioassay, menthol (0.96 µg/cm²) was approximately 12.18 times more effective than benzyl benzoate (11.70 µg/cm²), followed by (+)-neomenthol (1.33 µg/cm²), (-)-menthol (1.60 µg/cm²), and (+)-menthol (1.90 µg/cm²). In the filter paper bioassay, menthol (0.55 µg/cm²) was about 15.18 times more active than benzyl benzoate (8.35 µg/cm²), followed by (-)-menthol (0.84 µg/cm²), (+)-menthol (0.92 µg/cm²), and (+)-neomenthol (1.72 µg/cm²). However, (+)-isomenthol did not exhibit any acaricidal activity against *T. putrescentiae* in the fumigant and filter paper bioassays. These results indicate that peppermint oil and menthol isomers could be effective natural acaricides for managing mites in stored food." As taken from Park JH et al. 2014. J. Food Prot. 77(9), 1819-23. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25285505>

ECOSAR Version 1.11 reports the following terrestrial toxicity data for CAS RN 2216-51-5:

Values used to Generate ECOSAR Profile

Log Kow: 3.381 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 490 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Earthworm	14-day	LC50	195.365

“The first record of tracheal mites, *Acarapis woodi*, in Japan was made in 2010. These mites have since caused serious damage to the colonies of Japanese honey bees, *Apis cerana japonica*. In the present study, to control the mites on Japanese honey bees with l-menthol, an agent used for European honey bees, *Apis mellifera*, we investigated (1) the seasonality of menthol efficacy, (2) the overwintering mortality of menthol-treated colonies, and (3) the menthol residue in honey under field conditions in cooperation with private beekeepers of Japanese honey bees. Seasonal menthol efficacy was tested by applying 30 g of l-menthol for 1 month in different seasons. Mite prevalence was measured by dissecting the honey bee thorax. Overwintering mortality was monitored during winter after checking the mite prevalence in autumn, and was compared with that of untreated colonies reported in our previous study. The residual level of menthol in honey was measured by GC-MS. The results showed that the menthol-treated colonies had a smaller rate of increase in mite prevalence than the untreated colonies. The effects of menthol were highest in March and April. The winter mortality was depressed by menthol treatment. Honey samples extracted from the menthol-treated colonies included 0.4 ppm of menthol residue on average. Our findings suggest that menthol treatment is effective for controlling the tracheal mites on Japanese honey bees.” As taken from Maeda T and Sakamoto Y. 2016. Exp. Appl. Acarol. 70(3), 299-308. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27497591>

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1a,2,5a)] (CAS RN 2216-51-5) is not bioaccumulative in the environment:

Empirical Log Kow	3.4
Reference (Log Kow)	EPISUITE KOWWIN v1.67
Log Kow predicted by KowWin	3.38
Log BAF T2MTL predicted by Gobas	0.48298117522285
Log BCF 5% T2LTL predicted by Gobas	0.483800290754957
Log BCF Max predicted by OASIS	2.73513873517688
Log BCF predicted by BCFWIN	1.756

Experimental BCF (log)	0.891708849089096
Reason for pivotal value	Average Vertebrate BCF
Test species BCF/BAF (Latin)	Cyprinus carpio
Test species BCF/BAF (Common)	Common carp
Mean water concentration (ug/l)	20
Mean water concentration (comment)	Below water solubility by a factor of 5
Water concentration: Measured/Nominal/Uncertain	Measured
RadioLabel	No
Exposure duration (days)	42
Exposure duration (comment)	Sufficient exposure duration for 80% steady state; "Reported steady state"
Exposure type	Flow-through
Mean temperature (deg. Celcius)	22
Exposure route	Water (but fed)
Exposure media	Fresh Water
Mean measured lipid content (%)	4.7
Tissue analysed	Whole body
Calculation method	Cb/Cw

Comment (BAF/BCF)	Average BCF; Apparent measured water concentrations; Reported "Steady state"
Reference (BAF/BCF)	Chemicals Evaluation and Research Institute. 1992. Biodegradation and Bioconcentration of Existing Chemical Substances under the Chemical Substances Control Law. http://qsar.cerij.or.jp/cgi-bin/DEGACC/index.cgi?e

Data accessed June 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

EPISuite provides the following information:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	1.772 (BCF = 59.12 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-0.1483 days (HL = 0.7108 days)
Log BCF Arnot-Gobas method (upper trophic):	1.368 (BCF = 23.36)
Log BAF Arnot-Gobas method (upper trophic):	1.368 (BAF = 23.36)
log Kow used:	3.19 (expkow database)

11. References

- 2021). Informationssystem für Gefährliche Stoffe. Public Version 05/2021. Record for menthol (CAS RNs 89-78-1, 15356-60-2, 2216-51-5, 15356-70-4 and 1490-40-6). Available via <https://www.echemportal.org/echemportal/substance-search>
- Abdallah SJ et al. (2019). Updates in opioid and nonopioid treatment for chronic breathlessness. Curr. Opin. Support. Palliat. Care 13(3), 167-173. DOI: 10.1097/SPC.0000000000000449. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31335450/>
- AICIS (2017). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme. Inventory Multi-Tiered Assessment and Prioritisation (IMAP) Tier I. Health record for cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1.alpha.,2.beta.,5.alpha.)]- (CAS RN 2216-51-5). Dated 27 October 2021. Available at <https://www.industrialchemicals.gov.au/chemical-information/search-assessments?assessmentcasnumber=2216-51-5>
- AICIS (Undated). Australian Government Department of Health. Australian Inventory of Industrial Chemical. Available at <https://www.industrialchemicals.gov.au/search-inventory>
- Aizawa N et al. (2019). RQ-00434739, a novel TRPM8 antagonist, inhibits prostaglandin E2-induced hyperactivity of the primary bladder afferent nerves in rats. Life Sci. 218, 89-95. DOI: 10.1016/j.lfs.2018.12.031. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30580018?dopt=AbstractPlus>

- Amato A et al. F (2013). Tetrodotoxin-dependent effects of menthol on mouse gastric motor function. *Eur. J. Pharmacol.* 718(1-3), 131-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24041922>
- Andersen HH et al. (2015). Cold and L-menthol-induced sensitization in healthy volunteers--a cold hypersensitivity analogue to the heat/capsaicin model. *Pain* 156(5), 880-9. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25719613>
- Api AM (2022) RIFM fragrance ingredient safety assessment, menthol, CAS Registry Number 89-78-1. *Food Chem Toxicol.* 2022 May;163 Suppl 1:112927. doi: 10.1016/j.fct.2022.112927. Epub 2022 Mar 19. Available at Argoff CE (2013). Topical analgesics in the management of acute and chronic pain. *Mayo Clin. Proc.* 88(2), 195-205. PubMed, 2014 available at <https://pubmed.ncbi.nlm.nih.gov/35318088/>
- Arunkumar S et al. (2018). Effect of terpenes on transdermal iontophoretic delivery of diclofenac potassium under constant voltage. *Pharm. Dev. Technol.* 23(8), 806-814. DOI: 10.1080/10837450.2017.1369110. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/28814142>
- Ashley M et al. (2012). Lack of effect of menthol level and type on smokers' estimated mouth level exposures to tar and nicotine and perceived sensory characteristics of cigarette smoke. *Reg. Tox. Pharmacol.* 63, 381-390. PubMed, 2013. Available at <http://www.ncbi.nlm.nih.gov/pubmed/22634246>
- Ashoor A et al. (2013). Menthol binding and inhibition of $\alpha 7$ -nicotinic acetylcholine receptors. *PLoS One* 8(7), e67674. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23935840>
- Badgett C E and J S Osmalov (1970). Tabakrauchfilter und mit ihm versehener Rauchartikel; Patent (German): 1962452 (July 1970).
- Badgett C E and J S Osmalov (1971). Flavor-releasing smoking article and method of making the same; Patent (US): 3,603,319 (Sept 1971).
- Baibars M et al. (2012). Menthol toxicity: an unusual cause of coma. *Case Rep. Med.* 2012, 187039. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23251165>
- Baker R and Bishop L. (2004). The pyrolysis of tobacco ingredients. *J. Anal. Appl. Pyrolysis* 71, 223-311
- Baker R et al. (2004a). The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives. *Food and Chemical Toxicology* 42s, S3-S37.
- Baker R et al. (2004c). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food and Chemical Toxicology* 42s, S53-S83.
- Barwood MJ et al. (2015). Relieving thermal discomfort: Effects of sprayed L-menthol on perception, performance, and time trial cycling in the heat. *Scand. J. Med. Sci. Sports* 25(Suppl. 1), 211-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25943672>
- BAT (1978). British-American Tobacco Co Ltd: Flavouring Tobacco; Research Disclosure 16836 (Apr 1978) p 8.
- Bavley A and E W Robb (1969). Rauchwaren und Verfahren, Rauchwaren Aroma zu verleihen; Patent (German): 1517302 (Sept 1969).
- Belsito D et al (2008). A toxicologic and dermatologic assessment of cyclic and non-cyclic terpene alcohols when used as fragrance ingredients. *Food and Cosmetics Toxicology*, 46, S1-S71.
- Benowitz NL, Herrera B, Jacob P (2004). Mentholated cigarette smoking inhibits nicotine metabolism. *J. Pharmacol Exp Ther* 319:1208-15.
- BIBRA, 1990, Menthol
- Bodnar JA et al. (2012). Mainstream smoke chemistry analysis of samples from the 2009 US cigarette market. *Regul. Toxicol. Pharmacol.* 64(1), 35-42. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22683394>

- Brody AL et al. (2013). Up-regulation of nicotinic acetylcholine receptors in menthol cigarette smokers. *Int. J. Neuropsychopharmacol.* 16(5), 957-66. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23171716>
- Brozinski M et al. (1972). Untersuchungen über die Verteilung des Menthols auf Tabak, Filter und Rauch von Mentholcigaretten; *Beitr. Tabakforsch.* 6 (1972) 124-130.
- Bruusgaard-Mouritsen MA et al.(2020).Natural ingredients in cosmetic products-A suggestion for a screening series for skin allergy. *Contact Dermatitis* 83(4), 251-270. DOI: 10.1111/cod.13550. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32248558/>
- Caboni P et al. (2013). Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incognita*. *J. Agric. Food Chem.* 61(41), 9784-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24050256>
- Camarasa G & Alomar A (1978). Menthol dermatitis from cigarettes.*Contact Dermatitis*, 4, 169-170 [cited in SIAR, 2003].
- Carmines E (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1. Cigarette design, testing approach, and review of results. *Food and Chemical Toxicology*, 40, 77-91.
- CCRIS (2001). Record for (-)-menthol. CCRIS record no. 3728. Last revision date 28 March 2001 (records no longer being updated after 2011). Available at <https://www.toxinfo.io/>
- Charles River Laboratories. (2022). Assessment of Oral Irritation Potential in the EpiGingival™ 3D Tissue Model using Nicotine Pouches
- Cheang WS et al. (2013). Menthol relaxes rat aortae, mesenteric and coronary arteries by inhibiting calcium influx. *Eur. J. Pharmacol.* 702, 79-84. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23380688>
- ChemIDplus. Records for l-menthol (CAS RN 2216-51-5). Available via PubChem
- ChemSpider. Record for L-(-)-menthol (CAS RN 2216-51-5) and for L-(-)-Menthol (CAS 89-78-1) Undated. Available at <https://www.chemspider.com/Chemical-Structure.15803.html>
- Chen et al. (2011). The stereoisomers of menthol in selected tobacco products. A brief report. *Nicotine and Tobacco Research*, 13, 741-745 Available at <http://www.ncbi.nlm.nih.gov/pubmed/21447840>
- Chen Y et al. (2013). Effect of unsaturated menthol analogues on the in vitro penetration of 5-fluorouracil through rat skin. *Int. J. Pharm.* 443(1-2), 120-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23333756>
- Choi HY et al. (2017). Improvement of the pharmacological activity of menthol via enzymatic β -anomer-selective glycosylation. *AMB Express* 7(1), 167. DOI: 10.1186/s13568-017-0468-0. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28853018>
- CosIng. Cosmetic substances and ingredients database. Record for menthol (CAS RNs 1490-04-6, 2216-51-5, 89-78-1, 15356-70-4). Undated.Available at <https://ec.europa.eu/growth/tools-databases/cosing/>
- CPID (undated). Consumer Product Information Database. Record for menthol, L- (CAS RN 2216-51-5). Accessed June 2021. Available at <https://www.whatsinproducts.com/>
- Curran J G (1972). Delivery of menthol from cigarettes containing either a mentholated filter or mentholated tobacco; *Tobacco Science* 16 (1972) 40-42.
- Curran J G (1975). Effect of certain liquid filter additives on menthol delivery; *Tobacco Science* 19 (1975) 69-70.
- Danish EPA (2016). Survey of allergenic substances in products targeted children – toys and cosmetic products. Survey of Chemical Substances in Consumer Products No. 148, 2016. Available at: <http://www2.mst.dk/Udgiv/publications/2016/08/978-87-93529-00-7.pdf>
- de Cássia da Silveira E Sá R et al.(2017).Analgesic-Like Activity of Essential Oil Constituents: An Update. *Int. J. Mol. Sci.* 18(12), E2392. DOI: 10.3390/ijms18122392. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29232831>
- Doull et al. (1994). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at <http://legacy.library.ucsf.edu/tid/thy03c00>

- Doull et al. (1998). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at <http://legacy.library.ucsf.edu/tid/wzp67e00>
- ECHA (2023). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 24 May 2023. Available at: <https://echa.europa.eu/information-on-chemicals/cl-inventory-database>
- ECHA (undated). European Chemicals Agency. Information on Chemicals. Record for L-menthol and menthol. Available at: <https://echa.europa.eu/information-on-chemicals/registered-substances>
- ECOSAR (undated). Record for cyclohexanol, 5-methyl-2-(1-methylethyl)-, 1R-(1 α ,2 β ,5 α)- (CAS RN 2216-51-5). (ECOSAR content has not been updated since 2012, version 1.11.) Available to download, through EPISuite, at <https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface>
- Effah F. et. al. Pulmonary effects of e-liquid flavors: a systematic review. J Toxicol Environ Health B Crit Rev. 2022; 25(7): 343–371. DOI: 10.1080/10937404.2022.2124563. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9590402/>
- EFSA (2011) Scientific Opinion on Flavouring Group Evaluation 9, Revision 3 (FGE.09Rev3): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 251 EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). EFSA Journal 9, 2396.
- EFSA (2015). Scientific opinion on flavouring group evaluation 9, revision 6 (FGE.09Rev6): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). EFSA Journal 13(9), 4243. Available at <https://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4243/epdf>
- EFSA (2016). Safety and efficacy of secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols from chemical group 8 when used as flavourings for all animal species. EFSA Journal 14(6), 4475. Available at <https://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4475/epdf>
- EMA (2012). European Medicines Agency. Assessment report for suppositories containing terpenic derivatives. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Terpenic_31/WC500122526.pdf
- EMA (2020). European Medicines Agency. Committee on Herbal Medicinal Products (HMPC). Assessment report on Mentha x piperita L., folium and aetheroleum. Final – Revision 1. EMA/HMPC/522409/2013. 15 January 2020. Available at https://www.ema.europa.eu/en/documents/herbal-report/assessment-report-mentha-x-piperita-l-folium-aetheroleum-revision-1_en.pdf
- EPA ECOTOX Database. Record for (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexanol. Accessed January 2015. Available at: https://cfpub.epa.gov/ecotox/quick_query.htm
- EPISuite (undated). Record for cyclohexanol, 5-methyl-2-(1-methylethyl)-, 1R-(1.alpha, 2.beta, 5.alpha)- (CAS 2216-51-5). Accessed January 2015. (EPISuite content has not been updated since 2012, version 4.11.) EPISuite is available to download at EPISuite (2017). Record for cyclohexanol, 5-methyl-2-(1-methylethyl)-, 1R-(1.alpha, 2.beta, 5.alpha)- (CAS 2216-51-5). EPISuite version 4.11. Last updated June 2017. EPISuite is available to download at <https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411>
- Farco JA and Grundmann O (2013). Menthol--pharmacology of an important naturally medicinal "cool". Mini Rev. Med. Chem. 13(1), 124-31. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23061635>

- Faridi U et al. (2016). Repurposing L-Menthol for Systems Medicine and Cancer Therapeutics? L-Menthol Induces Apoptosis through Caspase 10 and by Suppressing HSP90. OMICS 20(1), 53-64. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26760959>
- FDA (2022). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 13 October 2023. Available at <https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances>
- FDA (2023a). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR). Title 21. Current as of 19 May 2023. Available at <https://www.ecfr.gov/cgi-bin/ECFR?page=browse>
- FDA (2023b). US Food and Drug Administration. Inactive Ingredient Database. Data through 1 April 2023. Available at <https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>
- Feng X et al. (2019). Pharmacokinetics behaviors of l-menthol after inhalation and intravenous injection in rats and its inhibition effects on CYP450 enzymes in rat liver microsomes. Xenobiotica 49(10), 1183-1191. DOI: 10.1080/00498254.2018.1537531. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/30654691/>
- fGLH Study Report (2010).
- Flood TR et al. (2017). Oral L-menthol reduces thermal sensation, increases work-rate and extends time to exhaustion, in the heat at a fixed rating of perceived exertion. Eur. J. Appl. Physiol. 117(7), 1501-1512. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28508114>
- Fujishiro M et al. (2014). Efficacy of spraying l-menthol solution during endoscopic treatment of early gastric cancer: a phase III, multicenter, randomized, double-blind, placebo-controlled study. J. Gastroenterol. 49(3), 446-54. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23800946>
- Fujita S et al. (2019). Menthol-induced facilitation of cerebrocortical excitatory propagation induced by air puff stimulation of the nasal cavity in the rat: An optical imaging study. Neurosci. Lett. 696, 87092. DOI: 10.1016/j.neulet.2018.12.018. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30553866>
- Gaworski CL et al. (1997), Food Chem Toxicol. 1997 Jul;35(7):683-92, available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9301652&query_hl=4&itool=pubmed_DocSum
- Gaworski CL et al. (1998). Toxicologic evaluation of flavor ingredients added to cigarette smoke: 13-week inhalation exposures in rats. Inhalation Toxicology, 10, 357.
- Gaworski CL et al. (1999), Toxicology. 1999 Nov 29;139(1-2):1-17, available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=10614684&query_hl=131&itool=pubmed_docsum
- Gilissen L et al. (2018). Allergic contact dermatitis caused by topical herbal remedies: importance of patch testing with the patients' own products. Contact Dermatitis 78(3), 177-184. DOI: 10.1111/cod.12939. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29214642>
- Gillis DJ et al. (2015). Influence of repeated daily menthol exposure on human temperature regulation and perception. Physiol. Behav. 139, 511-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25484357>
- Gillissen A et al. (2013). A multi-centre, randomised, double-blind, placebo-controlled clinical trial on the efficacy and tolerability of GeloMyrtol® forte in acute bronchitis. Drug Res. (Stuttg.) 63(1), 19-27. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23447044>
- Giovino GA et al. (2015). Differential trends in cigarette smoking in the USA: is menthol slowing progress? Tob. Control. 24(1), 28-37. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/23997070>

- Gordon SM et al. (2011). Effect of cigarette menthol content on mainstream smoke emissions. *Chem Research in Toxicol* 24, 1744-1753. Available at PUBMED <http://www.ncbi.nlm.nih.gov/pubmed/21888394>
- Grubbs J H et al.(1978). alpha-Substituted vinyl menthyl carbonates; Patent (US): 4,127,601 (Nov 1978).
- GSBL (2021). Record for (-)-menthol (CAS RN 2216-51-5). GSBLPUBLIC 2021. Version 3.4.3. Last updated February 2021. Accessed June 2021. Available via <https://www.gsbl.de/index.html>
- Gusain P et al. (2017). Chirality-Dependent Interaction of d- and l-Menthol with Biomembrane Models. *Membranes (Basel)* 7(4), E69. DOI: 10.3390/membranes7040069. PubMed, 2018 available at
- Ha MA et al.(2015). Menthol attenuates respiratory irritation and elevates blood cotinine in cigarette smoke exposed mice. *PLoS One* 10(2), e0117128. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25679525>
- Hall RL and Oser BL (1965). Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. III. GRAS substances. *Food Technology* 19(2), 151-197. Available at [https://www.femaflavor.org/sites/default/files/3_GRAS_Substances\(2001-3124\)_0.pdf](https://www.femaflavor.org/sites/default/files/3_GRAS_Substances(2001-3124)_0.pdf)
- Han P et al. (2020). Frequent minty chewing gum use is associated with increased trigeminal sensitivity: An fMRI study. *Brain Res.* 1730, 146663. DOI: 10.1016/j.brainres.2020.146663. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31931000/>
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Record for l-menthol (CAS RN 2216-51-5). Last updated 4 May 2021. Accessed June 2021. Available at <http://webprod.hc-sc.gc.ca/nhp/bdipsn/ingredReq.do?id=7210&lang=eng>
- Heck D.J. A review and assessment of menthol employed as a cigarette flavoring ingredient. *Food and Chemical Toxicology* 48 (2010) S1-S38.
- Hickman NJ et al. (2014). Menthol use among smokers with psychological distress: findings from the 2008 and 2009 National Survey on Drug Use and Health. *Tob. Control* 23(1), 7-13. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22821797>
- Highstein B & Zeligmann J (1951). *J. Am. Med. Assoc.* 146, 816
- Hoffman AC (2011). The health effects of menthol cigarettes as compared to non-menthol cigarettes. *Tobacco induced Diseases*, 9 Suppl 1: S7 Available at PUBMED <http://www.ncbi.nlm.nih.gov/pubmed/21624153>
- HSDB (2003). Record for (L)-menthol. Hazardous Substances Data Bank Number: 5662. Last Revision Date: 14 February 2003.. Available at: <https://www.toxinfo.io/>
- Hu X. and Li M. (2021).Menthol promotes cytokine secretion in human bronchial epithelial BEAS-2B cells by activating transient receptor potential melastatin 8 (TRPM8)Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.2021 Jul;37(7):577-584.Available at <https://pubmed.ncbi.nlm.nih.gov/34140067/>
- IFRA (undated). International Fragrance Association.IFRA Transparency List. Accessed June 2021. Available at <https://ifrafragrance.org/priorities/ingredients/ifra-transparency-listIGS> (
- IPCS INCHEM WHO FOOD ADDITIVES SERIES: 42 available at <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>
- Ishiyama A et al. (2021). Effect of spraying l-menthol on peristalsis resumption during endoscopic submucosal dissection of gastric tumors. *JGH Open* 5(6), 653-657. DOI: 10.1002/jgh3.12549. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/34124381/>
- Jason Gillis D et al.(2015).Influence of repeated daily menthol exposure on human temperature regulation and perception. *Physiol. Behav.* 139, 511-8. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25484357>

- Jenkins R W Jr et al.(1970).Cigarette smoke formation studies: II. Smoke distribution and mainstream pyrolytic composition of added 14C-menthol (U). Beitr. Tabakforsch. Int. 5, 299-301.
- Jenner P M et al.(1964).Food and Cosmetics Toxicology, 2, 327 [cited in BIBRA, 1990].
- Jing Y & Xian K (1999). The effects of different levels of filter ventilation on the deliveries of flavor constituents in mainstream smoke; CORESTA, Meeting of the smoke and technology study groups, 5-9th September 1999, Innsbruck, Austria.
- Jones MR et al. (2013). Smoking, menthol cigarettes, and peripheral artery disease in U.S. adults. Nicotine Tob Res. 15(7), 1183-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23212436>
- Journigan VB and Zaveri NT (2013). TRPM8 ion channel ligands for new therapeutic applications and as probes to study menthol pharmacology. Life Sci. 92(8-9), 425-37. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23159643>
- JTI KB Study Report (s)
- JTI Study Report (s).
- Kabat GC, Shivappa N & Hebert JR (2012). Mentholated cigarettes and smoking-related cancers revisited: An ecologic examination. Regulatory and Applied Toxicology 63, 132-139. Available at PUBMED <http://www.ncbi.nlm.nih.gov/pubmed/22429780>
- Kabbani N (2013). Not so Cool? Menthol's discovered actions on the nicotinic receptor and its implications for nicotine addiction. Front. Pharmacol. 4, 95. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23898298>
- Kamatou GP et al. (2013). Menthol: a simple monoterpene with remarkable biological properties. Phytochemistry 96, 15-25. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24054028>
- Kanezaki M et al. (2020). Effect of olfactory stimulation by L-menthol on laboratory-induced dyspnea in COPD. Chest 157(6), 1455-1465. DOI: 10.1016/j.chest.2019.12.028. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/31981567/>
- Kashima H and Hayashi N (2013). Facial skin blood flow responses to irritant stimuli in the oral cavity.Auton. Neurosci.174(1-2), 61-5. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23265982>
- Kaur G et al.(2018).Mechanisms of toxicity and biomarkers of flavoring and flavor enhancing chemicals in emerging tobacco and non-tobacco products. Toxicology Letters 288, 143-155. DOI: 10.1016/j.toxlet.2018.02.025. PubMed, 2018, available at <https://www.ncbi.nlm.nih.gov/pubmed/29481849>
- Kawasaki H et al. (2013). Inhibition by menthol and its related chemicals of compound action potentials in frog sciatic nerves. Life Sci. 92(6-7), 359-67. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23352972>
- Khan IA and Abourashed EA (2010). Leung's Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. Third Edition. John Wiley & Sons, Inc., Hoboken, New Jersey.
- Kim S and Lee JY (2018).L-Menthol attenuates the magnitude of cold-induced vasodilation on the extremities of young females. J. Physiol. Anthropol. 37(1), 14. DOI: 10.1186/s40101-018-0174-x. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29739454>
- Kligman A M (1975). Report to RIFM, 12 February.
- Kong G et al. (2013). Menthol cigarette and marijuana use among adolescents. Nicotine Tob. Res. 15(12), 2094-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23884319>
- Kozlovich S et al. (2019). Role of L- and d-menthol in the glucuronidation and detoxification of the major lung carcinogen, NNAL. Drug Metab. Dispos. 47(12), 1388-1396. DOI: 10.1124/dmd.119.088351. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31578206/>

- Kumar P et al. (2014). Biocontrol potential of essential oil monoterpenes against housefly, *Musca domestica* (Diptera: Muscidae). *Ecotoxicol. Environ. Saf.* 100, 1-6. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24433784>
- Kurose M and Meng ID (2013). Dry eye modifies the thermal and menthol responses in rat corneal primary afferent cool cells. *J. Neurophysiol.* 110(2), 495-504. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23636717>
- Labstat International Inc. (2020). Determination of Mutagenic, Cytotoxic and Genotoxic Responses of Smokeless Products and 1R6F Cigarette Using Ames, NRU and in vitro Micronucleus Assays
- Labstat International Inc. (2020a) Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- Labstat International Inc. (2020b) Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products. Biological Activity Test Report(s).
- Labstat International Inc. (2021). Characterization of E-cigarette Aerosol. Analytical Test Report.
- Labstat International Inc. (2021a). Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- Labstat International Inc. (2021b). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products. Biological Activity Test Report(s).
- Labstat International Inc. (2022). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Collected Matter (ACM) and Mainstream Gas Vapor Phase (GVP) of Electronic Cigarette Products. Biological Activity Test Report.
- LactMed (2021). Drugs and Lactation Database. Record for peppermint. Last updated 18 January 2021. Accessed June 2021. Available at <https://www.ncbi.nlm.nih.gov/books/NBK501851/>
- Lau BK et al. (2014). Menthol enhances phasic and tonic GABAA receptor-mediated currents in midbrain periaqueductal grey neurons. *Br. J. Pharmacol.* 171(11), 2803-13. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/24460753>
- Laude E A et al. (1994). The anti-tussive action of menthol and other aromatic vapours in conscious guinea-pigs. *Am. J. Respir. Crit. Care Med.*, 149(S), A187 [cited in Tamaoki et al., 1995].
- Le Bourhis B & Soenen A-M (1973). Studies on the psychotropic action of some aromatic compounds used in food. *Food and Cosmetics Toxicology*, 11, 1-9 [in French with English abstract].
- Lee JH et al. (2013). Contact and fumigant toxicity of *Pinus densiflora* needle hydrodistillate constituents and related compounds and efficacy of spray formulations containing the oil to *Dermatophagoides farinae*. *Pest. Manag. Sci.* 69(6), 696-702. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23109302>
- Lee PN (2011). Systematic review of the epidemiological evidence comparing lung cancer risk in smokers of mentholated and unmentholated cigarettes. *BMC Pulmonary Medicine* 11, 18. Available at <http://www.ncbi.nlm.nih.gov/pubmed/21501470>
- Liang Y et al. (2013). Evaluation of repellency of some Chinese medicinal herbs essential oils against *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* 106(1), 513-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23448068>
- Liu Y et al. (2018). L-Menthol alleviates cigarette smoke extract induced lung injury in rats by inhibiting oxidative stress and inflammation via nuclear factor kappa B, p38 MAPK and

Nrf2 signalling pathways. RSC Adv. 8, 9353-9363. DOI: 10.1039/C8RA00160J. Available at <http://pubs.rsc.org/en/content/articlehtml/2018/ra/c8ra00160j>

- Logic (2019a). G.5. Nonclinical Evaluation Summary - Logic Power (PMTA) and G.5. Nonclinical Evaluation Summary - Logic Pro (PMTA)
- Logic (2019b). G.5. Nonclinical Evaluation Summary - Logic Vapeleaf (PMTA)
- Lu S et al. (2013). Somatosensory profiling of intra-oral capsaicin and menthol in healthy subjects. Eur. J. Oral Sci. 121(1), 29-35. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23331421>
- Luke E (1962). Lancet, i, 110 [cited in BIBRA, 1990 for Any other relevant toxicity section].
- Luo Y et al. (2019). (-)-Menthol increases excitatory transmission by activating both TRPM8 and TRPA1 channels in mouse spinal lamina II layer. Biochem. Biophys. Res. Commun. 516(3), 825-830. DOI: 10.1016/j.bbrc.2019.06.135. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31262448/>
- Lyster L A (1967). Direct vapor chromatographic determination of menthol, propylene glycol, nicotine and Triacetin in cigarette smoke; Tob. Sci. 11 (1967) 49-51.
- Macht D I (1939). Arch. Int. Pharmacodyn., 63, 43 [cited in BIBRA, 1990 for Any other relevant toxicity section].
- Maeda T and Sakamoto Y. (2016). Field application of menthol for Japanese honey bees, *Apis cerana japonica* (Hymenoptera: Apidae), to control tracheal mites, *Acarapis woodi* (Acari: Tarsonemidae). Exp. Appl. Acarol. 70(3), 299-308. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27497591>
- Matsushita H & Shinozaki M (1980). Flavourant composition for tobacco, method for its production and tobacco product containing said composition. Patent (European): EP 0 014 105 A2 (Aug 1980).
- Meng F et al. (2021). Antiperistaltic effect and safety of l-menthol oral solution on gastric mucosa for upper gastrointestinal endoscopy in Chinese patients: Phase III, multicenter, randomized, double-blind, placebo-controlled study. Dig. Endosc. Epub ahead of print. DOI: 10.1111/den.13941. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33527576/>
- Miller-Holt et al. (2022). In vitro evaluation of mutagenic, cytotoxic, genotoxic and oral irritation potential of nicotine pouch products. Toxicology Reports, 9, 1316-1324 PubMed, 2022 available at <https://pubag.nal.usda.gov/catalog/7806205>
- Millqvist E et al. (2013). Inhalation of menthol reduces capsaicin cough sensitivity and influences inspiratory flows in chronic cough. Resp. Med. 107, 433-438. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23266255>
- Mitchell B C et al (1963). The chromatographic determination of menthol in cigarettes and cigarette smoke; Tobacco Science 7, 64-67.
- Morris AM et al. (2021). Effects of e-cigarette flavoring chemicals on human macrophages and bronchial epithelial cells. International Journal of Environmental Research and Public Health 18, 11107. (10.3390/ijerph182111107). Available at Monnot AD et al. (2019). Skin sensitization induction risk assessment of common ingredients in commercially available cleansing conditioners. Dermatitis 30(2), 116-128. DOI: 10.1097/DER.0000000000000445. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/34769627/>
- Morton C et al. (1995). Contact sensitivity to menthol and peppermint in patients with intra-oral symptoms. Contact Dermatitis, 32, 281-284.
- Muscat JE et al (2012) Menthol smoking in relation to time to first cigarette and cotinine: Results from a community-based study. Regulatory Toxicology and Pharmacology 63, 166-170. Available at <http://www.ncbi.nlm.nih.gov/pubmed?term=Muscat+JE>
- Nagai N et al. (2019). Combination with l-menthol enhances transdermal penetration of indomethacin solid nanoparticles. Int. J. Mol. Sci. 20(15), 3644. DOI: 10.3390/ijms20153644. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31349657/>

- Nakagawa S, Tagami H & Aiba S (2009). Erythema multiforme-like generalized contact dermatitis to l-menthol contained in anti-inflammatory medical compresses as an ingredient. *Contact Dermatitis* 61, 178-179.
- Nichols W et al. (1987). Matrix entrapment of flavorings for smoking articles; Patent (European): EP 0 223 454 A2 (May 1987).
- Nonnemaker J et al. (2013). Initiation with menthol cigarettes and youth smoking uptake. *Addiction* 108(1), 171-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22862154>
- Noriyasu A et al. (2013). Menthol-enhanced cytotoxicity of cigarette smoke demonstrated in two bioassay models. *Tob. Induc. Dis.* 11(1), 18. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24001273>
- OECD (undated). Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1à,2 ,5à)]- (CAS RN 2216-51-5). Available at <http://webnet.oecd.org/CCRWeb/Search.aspx>
- OECD-SIDS (2006), MENTHOLS (2216-51-5, 15356-60-2, 89-78-1, 1490-04-6) available at <http://www.inchem.org/documents/sids/sids/menthols.pdf>
- Ökolab Test Report (2021). Determination of Mutagenic, Cytotoxic and Genotoxic Responses of Smokeless Products and 1R6F Cigarette Using Ames, NRU and in vitro Micronucleus Assay
- Oliver AJ et al. (2013). Flavored and nonflavored smokeless tobacco products: rate, pattern of use, and effects. *Nicotine Tob. Res.* 15(1), 88-92. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22529222>
- Olsen RV et al. (2014). Somatosensory and vasomotor manifestations of individual and combined stimulation of TRPM8 and TRPA1 using topical L-menthol and trans-cinnamaldehyde in healthy volunteers. *Eur. J. Pain* 18(9), 1333-42. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/24664788>
- Oshima T et al. (2021). Sedative effects of l-menthol, d-camphor, phenylethyl alcohol, and geraniol. *J. Nat. Med.* 75(2), 319-325. DOI: 10.1007/s11418-020-01470-1. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33389551/>
- Pan R et al. (2012). Central mechanisms of menthol-induced analgesia. *J. Pharmacol. Exp. Ther.* 343(3), 661-72. PubMed, 2013 available at <https://www.ncbi.nlm.nih.gov/pubmed/22951274>
- Pandey SK et al. (2013). Structure-activity relationships of monoterpenes and acetyl derivatives against *Aedes aegypti* (Diptera: Culicidae) larvae. *Pest. Manag. Sci.* 69(11), 1235-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23908002>
- Papathanasopoulos A et al. (2013). Effect of acute peppermint oil administration on gastric sensorimotor function and nutrient tolerance in health. *Neurogastroenterol. Motil.* 25(4), e263-71. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23489975>
- Parashar G et al. (2017). – (-) Menthol Induces Reversal of Promoter Hypermethylation and Associated Up-Regulation of the FANCF Gene in the SiHa Cell Line. *Asian Pac. J. Cancer Prev.* 18(5), 1365-1370. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28612587>
- Park JH et al. (2014). Acaricidal activity of constituents derived from peppermint oil against *Tyrophagus putrescentiae*. *J. Food Prot.* 77(10), 1819-23. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25285505>
- Pereira EJ et al. (2013). The effect of inhaled menthol on upper airway resistance in humans: A randomized controlled crossover study. *Can. Resp. J.* 20, e1-e4. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23457678>
- Perfetti T A & Gordin H H (1985). Just noticeable difference studies of mentholated cigarette products; *Tob. Sci.* 29 (1985) 57-67.

- Peyrot des Gachons C (2016). Oral Cooling and Carbonation Increase the Perception of Drinking and Thirst Quenching in Thirsty Adults. PLoS One 11(9), e0162261. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27685093>
- Philip Morris USA Internal Report (2001). Pyrolysis GC/MS Analysis of Menthol. Request 20011725. Scan PO10725A.D.
- Plevkova J et al. (1985). The role of trigeminal nasal TRPM8-expressing afferent neurons in the antitussive effects of menthol. J. Appl. Physiol. 115(2), 268-74. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23640596>
- Posadzki P et al. (2012). Adverse effects of aromatherapy: a systematic review of case reports and case series. Int. J. Risk Saf. Med. 24(3), 147-61. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22936057>
- PubChem (2023). Record for l-menthol (CAS RN 2216-51-5). Created 16 September 2004. Modified 20 May 2023. Available at <https://pubchem.ncbi.nlm.nih.gov/compound/16666>
- PubChem (2023). REcord for Menthol (CAS RN 89-78-1). Created 25 March 2005. Last modified 20 May 2023. Available at: <https://pubchem.ncbi.nlm.nih.gov/compound/1254>
- Purkis SW, Mueller C and Intorp M (2011). The fate of ingredients in and impact on cigarette smoke. Food and Cosmetics Toxicology, 49, 3238-3248.
- Reitzel LR et al. (2013a). Associations of menthol use with motivation and confidence to quit smoking. Am. J. Health Behav. 37(5), 629-34. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23985285>
- Reitzel LR et al. (2013b). Race moderates the effect of menthol cigarette use on short-term smoking abstinence. Nicotine Tob. Res. 15(5), 883-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23288873>
- Resnicow K et al. (2014). Unplanned Quitting in a Triethnic Sample of U.S. Smokers. Nicotine Tob. Res. 16(6), 759-65. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/24420329>
- Rickard BP et al. (2021). E-Cigarette flavoring chemicals induce cytotoxicity in HepG2 cells. ACS Omega 6(10), 6708-6713. DOI: 10.1021/acsomega.0c05639. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33748584/>
- Riehl T F et al. (1973). Menthol distribution and transfer; Tob. Sci. 17 (1973) 10-11.
- Robb E W et al. (1964). The use of non-volatile adducts in smoke flavor; Tobacco Science 8 (1964) 3-7.
- Roemer E et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. Food and Chemical Toxicology, 40, 105-111.
- Roemer E et al., (2014). Toxicological assessment of kretek cigarettes Part 6: The impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regulatory Toxicology and Pharmacology 70; S66-80.
- Rojewski AM et al. (2014). Menthol cigarette use predicts treatment outcomes of weight-concerned smokers. Nicotine Tob. Res. 16(1), 115-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24113927>
- Rosbrook K and Green BG. (2016). Sensory effects of menthol and nicotine in an E-cigarette. Nicotine Tob. Res. 18(7), 1588-95. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26783293>
- Rozza AL et al. (2013). Effect of menthol in experimentally induced ulcers: pathways of gastroprotection. Chem. Biol. Interact. 206(2), 272-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24121185>
- Rozza AL et al. (2014). The gastroprotective effect of menthol: involvement of anti-apoptotic, antioxidant and anti-inflammatory activities. PLoS One 9(1), e86686. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24466200>
- RTECS (2017). Registry of Toxic Effects of Chemical Substances. Record for L-menthol (CAS RN 2216-51-5). Last updated April 2017.

- RTECS (2019). Registry of Toxic Effects of Chemical Substances. Record for Menthol (CAS RN 89-78-1). Last updated March 2019.
- Rustemeier K et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2. Chemical composition of mainstream smoke. Food and Chemical Toxicology, 40, 93-104.
- Sarkar M et al. (2012). Metabolism of nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in menthol and non-menthol cigarette smokers. Drug Metab. Letts 6(3), 198-206. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23140558>
- SCCS (2011). Scientific Committee on Consumer Safety. Opinion on Fragrance allergens in cosmetic products. The SCCS adopted this pre-consultation opinion at its 13th plenary meeting of 13-14 December 2011. Available at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf
- Schmeltz I & Schlotzhauer W S (1968). Benzo(a)pyrene, phenols and other products from pyrolysis of the cigarette additive dl-menthol. Nature (England) 219(5152): 370-371.
- Schramke H et al., (2014). Toxicological assessment of kretek cigarettes Part 7: The impact of ingredients added to kretek cigarettes on inhalation toxicity. Regulatory Toxicology and Pharmacology 70; S81-89.
- Shah M et al. (1996). Contact allergy in patients with oral symptoms: a study of 47 patients. American Journal of Contact Dermatitis, 7, 146-151.
- Sharp D (1978). The sensitization potential of some perfume ingredients tested using a modified Draize procedure. Toxicology, 9, 261-271.
- Shepherd T H & Gould F E (1968). Tobacco smoke filters; Patent (US): 3,470,883 (Aug 1968).
- SIAR (2003). SIDS Initial Assessment Report for SIAM 16. Menthols. UNEP Publications.
- Sidhu S. et al (2022) Unraveling the molecular mechanism of l-menthol against cervical cancer based on network pharmacology, molecular docking and in vitro analysis. Mol Divers. 2022 Apr 25. doi: 10.1007/s11030-022-10429-1. Available at <https://pubmed.ncbi.nlm.nih.gov/35467269/>
- Smith AP and Boden C (2013). Effects of chewing menthol gum on the alertness of healthy volunteers and those with an upper respiratory tract illness. Stress Health 29(2), 138-42. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22674677>
- Squier CA, Mantz MJ & Wertz PW (2011). Effect of menthol on the penetration of tobacco carcinogens and nicotine across porcine oral mucosa ex vivo. Nicotine and Tobacco Research, 12, 763-767. Available at PUBMED <http://www.ncbi.nlm.nih.gov/pubmed/20511349>
- Stabbert R et al. (2019). Assessment of priority tobacco additives per the requirements in the EU Tobacco Products Directive (2014/40/EU): Part 2: Smoke chemistry and in vitro toxicology. Regul. Toxicol. Pharmacol. 104, 163-199. DOI: 10.1016/j.yrtph.2019.03.002. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30858113>
- Strasser AA et al. (2013). The effect of menthol on cigarette smoking behaviors, biomarkers and subjective responses. Cancer Epidemiol. Biomarkers Prev. 22(3), 382-9. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23334588>
- Tamaoki J et al. (1995). Effect of menthol vapour on airway hyperresponsiveness in patients with mild asthma. Respiratory Medicine, 89, 503-504.
- Tiggelbeck D D & Manes M (1976). Mentholated cigarette filter; Patent (US): 3,972,335 (Aug 1976).
- Topp R et al. (2013a). Topical menthol, ice, peripheral blood flow, and perceived discomfort. J. Athl. Train. 48(2), 220-5. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23672386>
- Topp R et al. (2013b). The effect of either topical menthol or a placebo on functioning and knee pain among patients with knee OA. J. Geriatr. Phys. Ther. 36(2), 92-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22976810>

- Uchida Y et al. (2021). UCP1 and TRPM8 Expression in the brown fat did not affect the restriction of menthol-induced hyperthermia by estradiol in ovariectomized rats. *J. Nutr. Sci. Vitaminol. (Tokyo)* 67(2), 130-134. DOI: 10.3177/jnsv.67.130. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33952734/>
- Umezu T. et al. (2021). l-Menthol increases extracellular dopamine and c-Fos-like immunoreactivity in the dorsal striatum, and promotes ambulatory activity in mice *PLoS One*. 2021 Nov 30;16(11):e0260713doi: 10.1371/journal.pone.0260713.eCollection 2021. Available at <https://pubmed.ncbi.nlm.nih.gov/34847183/>
- US EPA (2023). Safer Chemical Ingredients List. Last updated 27 March 2023. Available at <https://www.epa.gov/saferchoice/safer-ingredients>
- US EPA 2020 CDR list (Chemical Data Reporting Rule). Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- US EPA ECOTOX Database (2021). Record for (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexanol. Available at: <https://cfpub.epa.gov/ecotox/search.cfm>
- US EPA InertFinder Database (2023). Last updated 25 April 2023. Available at <https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1>
- US EPA ToxCast. Available via US EPA CompTox Chemistry Dashboard at <https://comptox.epa.gov/dashboard>
- US EPA TSCA inventory. Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- Uter W et al. (2010). Contact allergy to fragrances: current patch test results (2005–2008) from the Information Network of Departments of Dermatology. *Contact Dermatitis*, 63, 254-261
- Valente A et al. (2015). The absorption and metabolism of a single L-menthol oral versus skin administration: Effects on thermogenesis and metabolic rate. *Food Chem. Toxicol.* 86, 262-73. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26429629>
- Vanscheeuwijck PM et al. (2002). *Food Chem Toxicol.* 2002 Jan;40(1):113-31, available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11731041&query_hl=124&itool=pubmed_docsum
- Verhulst L and Goossens A (2016). Cosmetic components causing contact urticaria: a review and update. *Contact Dermatitis* 75(6), 333-344. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27593503>
- Villanti AC et al. (2013). Flavored tobacco product use among U.S. young adults. *Am. J. Prev. Med.* 44(4), 388-91. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23498105>
- Wackowski OA et al. (2014). Young Adults' Behavioral Intentions Surrounding a Potential Menthol Cigarette Ban. *Nicotine Tob. Res.* 16(6), 876-80. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/24514070>
- Wade AG et al. (2019). Comparison of diclofenac gel, ibuprofen gel, and ibuprofen gel with levomenthol for the topical treatment of pain associated with musculoskeletal injuries. *J. Int. Med. Res.* 47(9), 4454-4468. DOI: 10.1177/0300060519859146. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31353997/>
- Wang J et al. (2010). The effect of menthol containing cigarettes on adult smokers' exposure to nicotine and carbon monoxide. *Regulatory Toxicology and Pharmacology*, 57, 24-30. Available at PUBMED <http://www.ncbi.nlm.nih.gov/pubmed/20025920>
- Wang W et al. (2019). L-menthol exhibits antidepressive-like effects mediated by the modification of 5-HTergic, GABAergic and DAergic systems. *Cogn. Neurodyn.* 13(2), 191-200. DOI: 10.1007/s11571-018-9513-1. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30956723>
- Wei E T & Seid D A (1983). AG-3-5: Chemical producing sensations of cold. *J. Pharm. Pharmacol.*, 35(2), 110-112 [cited in RTECS record for l-menthol 2216-51-5 for Addictiveness section].

- Werley et al. (2007). Possible effects on smokers of cigarette mentholation: a review of the evidence relating to key research questions. *Regul Toxicol Pharmacol.* 2007 Mar;47(2):189-203.
- Wetzel I (1943). *Arch. Kinderheilkunde*, 129, 74 [cited in BIBRA, 1990].
- WHO (2021). World Health Organization. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Menthol. Accessed June 2021. Available at <https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1519>
- Willis DN et al. (2011). Menthol attenuates respiratory irritation responses to multiple cigarette smoke irritants. *FASEB Journal* 25, 4434-4444.
- Winhusen TM et al. (2013). A tale of two stimulants: mentholated cigarettes may play a role in cocaine, but not methamphetamine, dependence. *Drug Alcohol Depend.* 133(3), 845-51. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24075226>
- Wise PM et al. (2011). The effect of menthol vapor on nasal sensitivity to chemical irritation. *Nicotine Tob. Res.* 13(10), 989-97. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/21652736>
- Xu X et al. (2011). Effect of menthol on ocular drug delivery. *Graefe's Archive for Ocular and Clinical Ophthalmology.* 249, 1503-1510. Available at <http://www.springerlink.com/content/2156121h7382477q/>
- Yang B et al. (2013). Study on pharmacokinetics and in vitro/in vivo correlation of menthol in Zhike Chuanbei Pipa dropping pills in rats. [Article in Chinese.] *Zhongguo Zhong Yao Za Zhi* 38(9), 1421-5. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23944081>
- You Q et al. (2020). L-Menthol for gastrointestinal endoscopy: A systematic review and meta-analysis. *Clin. Transl. Gastroenterol.* 11(10), e00252. DOI: 10.14309/ctg.0000000000000252. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33031198/>
- Zhang Y et al. (2014). Effects of caffeine and menthol on cognition and mood during simulated firefighting in the heat. *Appl. Ergon.* 45(3), 510-4. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23891504>
- Zuo X et al. (2013). Operant behavioral responses to orofacial cold stimuli in rats with chronic constrictive trigeminal nerve injury: effects of menthol and capsazepine. *Mol. Pain* 9, 28. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23767981>

12. Other information

- Paschke T et al. (2002). Effects of Ingredients on Cigarette Smoke Composition and Biological Activity: A Literature Overview. *Beiträge zur Tabakforschung* 20(3), 107–247. Available at <http://www.degruyter.com/view/j/cttr.2002.20.issue-3/cttr-2013-0736/cttr-2013-0736.xml?rskey=3xWHy2&result=2>
- Rodgman A (2004). Some Studies of the Effects of Additives on Cigarette Mainstream Smoke Properties. III. Ingredients Reportedly Used in Various Commercial Cigarette Products in the USA and Elsewhere. *Beiträge zur Tabakforschung* 21(2), 47–104. Available at <http://www.degruyter.com/view/j/cttr.2004.21.issue-2/cttr-2013-0771/cttr-2013-0771.xml?rskey=3xWHy2&result=4>
- Yadav N and Chandra H. (2018). Modulation of alveolar macrophage innate response in proinflammatory-, pro-oxidant-, and infection- models by mint extract and chemical constituents: Role of MAPKs. *Immunobiology* 223(1), 49-56. DOI: 10.1016/j.imbio.2017.10.015. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29031422>

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May 2023



Scientific Committee on Consumer Safety

SCCS

OPINION
on
Fragrance allergens in cosmetic products

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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Summary

Contact allergy to fragrance ingredients may develop following skin contact with a sufficient amount of these substances, often through the use of cosmetic products. Contact allergy is an altered specific reactivity in the immune system, which entails recognition of the fragrance allergen(s) in question by immune cells. Contact allergy, which *per se* is a latent condition, i.e. without visible signs or symptoms, persists lifelong. Upon each re-exposure to sufficient amounts of the allergen(s) eczema develops (allergic contact dermatitis), which typically will involve the face, the armpits and/or the hand(s). The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Around 16% of eczema patients in the European population are sensitised to fragrance ingredients. From studies performed on sectors of the population it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. The overall trend of fragrance allergy has been stable during the last 10 years, as some causes of fragrance allergy have decreased and others increased.

Most individuals with contact allergy to fragrance ingredients are aware that they cannot tolerate scented products on their skin and are often able to specifically name product categories that initiated their disease. In this context colognes, eau de toilette, deodorants and lotions are named significantly more often by fragrance allergic eczema patients than by patients without fragrance contact allergy.

Commercially available fragrances and other scented cosmetic products can provoke allergic contact dermatitis under patch test as well as simulated use conditions.

Appropriate diagnostic procedures and patient information are cornerstones in secondary prevention of contact allergy. The SCCNFP identified in 1999 a set of 26 fragrance allergens with a well-recognised potential to cause allergy, for which information should be provided to consumers about their presence in cosmetic products.

This listing has shown to be important in the clinical management of patients who are allergic to one or more of these 26 fragrance chemicals. Listing of the 26 fragrances has also been shown to be beneficial for patients with contact allergy to one or more of the fragrance chemicals, because these are identified on the ingredient listings of cosmetic products, and can thus be avoided.

The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature to identify fragrance allergens, including natural extracts, relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of (i) established fragrance allergens, (ii) likely fragrance allergens and (iii) possible fragrance allergens.

The studies since the SCCNFP Opinion on fragrance allergy in consumers confirm that the fragrance allergens identified by SCCNFP in 1999 are still relevant fragrance allergens for consumers from their exposure to cosmetic products. The review of the clinical and experimental data published since then shows that many more fragrance substances have been shown to be sensitisers in humans. Based on the clinical experience alone, 82 substances can be classified as established contact allergens in humans, 54 single chemicals and 28 natural extracts. Of these, 12 chemicals and 8 natural extracts were found to pose a high risk of sensitisation to the consumer, considering the high number of reported cases. In particular one ingredient stood out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1500 reported cases since the 1999 opinion.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking. Additionally, limited *in vivo* evidence together with Structure-Activity Relationship analysis suggests that other fragrance ingredients may also be a cause of concern with regard to their potential of causing contact allergy in humans.

The review also lists fragrance substances that can act as prehapten or prohaptens, forming new or more potent allergens by air oxidation and/or metabolic activation. Such

activation processes are of concern as they increase the risk of sensitisation and also the risk for cross reactivity between fragrance substances. In addition to known prehapten fragrance substances, the SCCS performed SAR analyses to identify fragrance substances with structural alerts that indicate that they are possible prehapten. While in the case of prohapten the possibility of becoming activated is inherent to the molecule and cannot be avoided, the activation of prehapten can be prevented by appropriate measures.

The SCCS examined available elicitation dose-response data to decide whether safe thresholds can be established for the fragrance allergens of concern, i.e. those found to pose a high risk of sensitisation to consumers. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy. As data from human dose elicitation experiments are very limited in several respects, no levels that could be considered safe for the majority of contact allergic consumers could be established for individual substances. The studies available, however, indicate that a general level of exposure of up to 0.8 µg/cm² (0.01% in cosmetic products) may be tolerated by most consumers, including these with contact allergy to fragrance allergens. The SCCS is of the opinion that this level of exposure (up to 0.01%) would suffice to prevent elicitation for the majority of allergic individuals, unless there is experimental or clinical substance-specific data allowing the derivation of individual thresholds.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.

The suggested general threshold, although limiting the problem of fragrance allergy in the consumer significantly, would not preclude that the most sensitive segment of the population may react upon exposure to these levels and does not remove the necessity for providing information to the consumer concerning the presence of the listed fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, the SCCP had recommended limiting the concentration in cosmetics to 200 ppm. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.

The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents. The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

1. Background

As a result of the public consultation on perfumery materials, which ended on 27 January 2007, there were further requests and information on important and/or frequently used allergens other than those proposed for regulation, such as farnesol, citral, linalool and hydroxyisohexyl-3-cyclohexenecarboxaldehyde. These substances were not part of the consultation, but they all belong to the 26 fragrance substances which should be labelled when present in cosmetic products under certain conditions.

The 26 fragrance substances were introduced into annex III of the Cosmetics Directive by the 7th amendment (2003/15/EC) on the basis of the SCCNFP draft opinion (SCCNFP/0017/98) published on 30 September 1999 for public consultation and the final opinion adopted by the SCCNFP during the plenary session of 8 December 1999.

Thirteen of the allergenic fragrance substances listed in this opinion have been frequently reported as well-recognised contact allergens in consumers and are thus of most concern; 11 others are less well documented. See the lists below from the opinion.

List A: *Fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognised consumer allergens.*

Common name	CAS number
Amyl cinnamal	122-40-7
Amylcinnamyl alcohol	101-85-9
Benzyl alcohol	100-51-6
Benzyl salicylate	118-58-1
Cinnamyl alcohol	104-54-1
Cinnamal	104-55-2
Citral	5392-40-5
Coumarin	91-64-5
Eugenol	97-53-0
Geraniol	106-24-1
Hydroxycitronellal	107-75-5
Hydroxymethylpentyl-cyclohexenecarboxaldehyde	31906-04-4
Isoeugenol	97-54-1

List B: *Fragrance chemicals, which are less frequently reported and thus less documented as consumer allergens.*

Common name	CAS number
Anisyl alcohol	105-13-5
Benzyl benzoate	120-51-4
Benzyl cinnamate	103-41-3
Citronellol	106-22-9
Farnesol	4602-84-0
Hexyl cinnamaldehyde	101-86-0
Lilial	80-54-6
d-Limonene	5989-27-5
Linalool	78-70-6
Methyl heptine carbonate	111-12-6
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5

Furthermore, two fragrances (natural mixtures) were added

Common name	CAS number
Oak moss	90028-68-5
Tree moss	90028-67-4

At the time there were insufficient scientific data to allow for the determination of dose-response relationships and/or thresholds for these allergens. Nevertheless, in a pragmatic administrative decision the limits of 0.01 and 0.001% were set, for rinse-off and leave-on products respectively.

Scientific information of both a general and a specific nature has been submitted to DG ENTR in order to ask the SCCS for a revision of the 26 fragrances with respect to further restrictions and possible even delisting.

2. Terms of reference

- 1. Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?*
- 2. Can the SCCS establish any threshold for their safe use based on the available scientific data?*
- 3. Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?*

3. Introduction

Fragrance ingredients

Fragrance and flavour substances are organic compounds with characteristic, usually pleasant, odours. They are ubiquitously used in perfumes and other perfumed cosmetic products, but also in detergents, fabric softeners, and other household products where fragrance may be used to mask unpleasant odours from raw materials. Flavourings are used in foods, beverages, and dental products. Fragrance substances are also used in aromatherapy and may be present in herbal products, and used as topical medicaments for their antiseptic properties.

Contact allergy to fragrance ingredients occurs when an individual has been exposed, on the skin, to a sufficient degree of fragrance contact allergens. Contact allergy is a life-long, specifically altered reactivity in the immune system. This means that once contact allergy is developed, cells in the immune system will be present which can recognise and react towards the allergen. As a consequence, symptoms, i.e. allergic contact dermatitis, may occur upon re-exposure to the fragrance allergen(s) in question. Allergic contact dermatitis is an inflammatory skin disease characterised by erythema, swelling and vesicles in the acute phase. If exposure continues it may develop into a chronic condition with scaling and painful fissures of the skin. Allergic contact dermatitis to fragrance ingredients is most often caused by cosmetic products and usually involves the face and/or hands. It may affect fitness for work and the quality of life of the individual.

Fragrance contact allergy has long been recognised as a frequent and potentially disabling problem. Prevention is possible as it is an environmental disease and if the environment is modified (e.g. by reduced use concentrations of allergens), the disease frequency and severity will decrease. Ingredient information is a cornerstone in the prevention of allergic contact dermatitis, as knowledge about the allergens which a patient has been exposed to is crucial for including the right substances in the allergy test, and for subsequent information on avoidance of re-exposure. However, the labelling rules in the Cosmetics Directive 76/768/EEC stipulated that perfume and aromatic compositions and their raw materials shall be referred to by the word “perfume” or “aroma”, rather than being labelled individually. This is the reason why the SCCNFP in their opinion SCCNFP/0017/98 (1) identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the Cosmetics Directive as individual ingredient labelling of the 26 fragrance allergens (Annex III, entries 67-92). However, safe use concentrations of these fragrances in cosmetic products had not yet been determined and much new evidence concerning fragrance allergy has been published since the 1999 opinion. The present request to review the list of recognised fragrance allergens which the consumer needs to be made aware of, to indicate thresholds for their safe use and to consider possible modification of allergens by metabolism and autoxidation, required a thorough review of all relevant scientific data. This includes both published scientific literature as well as unpublished scientific information on fragrances from the industry. The International Fragrance Association (IFRA), as representative of the fragrance industry, was contacted to provide relevant unpublished scientific data on fragrance ingredients. This information, together with the up-to-date published scientific literature, has been critically reviewed for the present SCCS opinion. The relevant data gaps are identified and recommendations for research addressing these gaps are made.

4. Clinical aspects of contact allergy to fragrance ingredients

4.1. Spectrum of reactions

Adverse reactions to fragrances in perfumes and in fragranced cosmetic products include allergic contact dermatitis, irritant contact dermatitis, photosensitivity, immediate contact reactions (contact urticaria), and pigmented contact dermatitis. Airborne and connubial contact dermatitis occur.

4.1.1. Allergic contact dermatitis

Mechanism

Allergic contact dermatitis (ACD) depends primarily on the activation of allergen-specific T-cells. In allergic contact dermatitis, a distinction is made between induction (sensitisation) and elicitation phases. A useful review is available (2).

The induction phase includes the events following initial contact with the allergen and is complete when the individual is sensitised and capable of giving a positive allergic contact dermatitis reaction.

The elicitation phase begins upon re-exposure to the allergen (challenge) and results in clinical manifestation of allergic contact dermatitis.

The entire process of the induction phase requires ca. 10 days to several weeks, whereas an elicitation phase reaction develops within 1–2 days.

Most contact allergens are small, chemically reactive compounds. As these compounds are too small to be directly immunogenic, they act as haptens; i.e. they react with higher molecular weight epidermal and/or dermal biomolecules to form immunogenic adducts. It is usually considered that the biomolecules involved are free or membrane bound proteins, which react via nucleophilic thiol, amino, and hydroxyl groups.

Dendritic cells (DCs) and the local tissue microenvironment are crucial factors in the development of ACD. Langerhans cells (LCs), as epidermal DCs, and dermal DCs are pivotal for the sensitisation and the elicitation phases of ACD. During sensitisation, DCs react with the immunogenic complexes by interaction with neighbouring keratinocytes, migration to the local draining lymph nodes and the priming of naïve T-cells. These reactions are mediated by inflammatory cytokines, chemokines and adhesion molecules. Antigen specific effector T-cells are then recruited into the skin upon contact with the same hapten (elicitation). Following their recruitment these T-cells are activated by antigen-presenting skin cells, including LCs, dermal DCs and keratinocytes, and macrophages.

Although most allergens can form hapten–carrier complexes directly, some need activation, e.g. by enzyme-induced metabolic conversion or abiotic oxidation. Such compounds are termed prohaptens and prehaptens, respectively, and are discussed in more detail in chapter 5. Well known examples of prehaptens and prohaptens are limonene and eugenol. Reduced enzyme activity in certain individuals, related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations.

Once sensitised, individuals can develop allergic contact dermatitis upon re-exposure to the contact allergen. Positive patch test reactions mimic this process of allergen-specific skin hyper-sensitivity. Skin contact induces an inflammatory reaction that is maximal within 2–3 days and, without further allergen supply, then declines.

Overview of clinical features

Perfumes and deodorants are the most frequent sources of sensitisation to fragrance ingredients in women, while aftershave products and deodorants are most often responsible in men (3). Thereafter, eczema may appear or be worsened by contact with other

fragranced products such as cosmetics, toiletries, household products, industrial contacts and flavourings.

Contact allergy to a particular product or chemical is established by means of diagnostic patch testing. When patients with suspected allergic cosmetic dermatitis are investigated, fragrances are identified as the most frequent allergens, not only in perfumes, after-shaves and deodorants, but also in other cosmetic products. Evaluation of perfume allergy may be difficult; a perfume compound may consist of ten to > 300 basic components selected from about 2500 materials.

Between 6 and 14% of patients routinely tested for suspected allergic contact dermatitis react to a standard indicator of fragrance allergy, the Fragrance Mix I (4), see also chapter 4.3.2. When tested with ten popular perfumes, 6.9% of female eczema patients proved to be allergic to them (5) and 3.2–4.2% were allergic to fragrances from perfumes present in various cosmetic products (6). The finding of a positive reaction to the Fragrance Mix I should be followed by a search for its relevance, i.e. is fragrance allergy the cause of the patient's current or previous complaints, or does it at least contribute to it? Between 50 and 65% of all positive patch test reactions to the mix are relevant. Sometimes, correlation with the clinical picture is lacking and many patients appear to tolerate perfumes and fragranced products without problems (7). This may be explained by: a) irritant (false-positive) patch test reactions to the mix; b) the absence of relevant allergens in those products; and c) the concentration being too low to elicit clinically visible allergic contact reactions. Contact allergy to fragrances often causes dermatitis of the hands (and aggravation of), face and neck, axillae and patches in areas where perfumes are dabbed on such as behind the ears, upper chest, elbow flexures and wrists. Depending on the degree of sensitivity and exposure, the severity of dermatitis may range from mild to severe with dissemination (8) [pp 158–170].

Clinical studies have shown a highly significant association between reporting a history of visible skin symptoms from using scented products and a positive patch test to the Fragrance Mix I (9). Provocation studies with perfumes and deodorants have also shown that fragrance-mix-positive eczema patients often react to use-tests with the products. Subsequent chemical analysis of such products has detected significant amounts of one or more Fragrance Mix I ingredients, confirming the relevance of positive patch tests to the Fragrance Mix I in these patients (5, 10).

Hands

Contact sensitisation may be the primary cause of hand eczema, or may be a complication of irritant or atopic hand eczema. The number of positive patch tests has been reported to correlate with the duration of hand eczema, indicating that long-standing hand eczema may often be complicated by sensitisation (11). The most common contact allergies in patients with hand eczema are metals, the Fragrance Mix, *Myroxylon pereirae*, and colophonium (12).

Fragrance allergy may be a relevant problem in patients with hand eczema; perfumes are present in consumer products to which their hands are exposed (13). A significant relationship between hand eczema and fragrance contact allergy has been found in some studies based on patients investigated for contact allergy (14). However, hand eczema is a multi-factorial disease and the clinical significance of fragrance contact allergy in (severe) chronic hand eczema may not be clear. A review on the subject has been published (15).

Axillae

Bilateral axillary dermatitis may be caused by perfume in deodorants and, if the reaction is severe, it may spread down the arms and to other areas of the body (8) [pp 158–170]. In individuals who consulted a dermatologist, a history of such first-time symptoms was significantly related to the later diagnosis of perfume allergy (9).

Face

Facial eczema is an important manifestation of fragrance allergy from the use of cosmetic products (16). In men, aftershave products can cause an eczematous eruption of the beard area and the adjacent part of the neck (8) [pp 158–170], and men using wet shaving as opposed to dry have been shown to have an increased risk of 2.9 of being fragrance allergic (17).

4.1.2. Irritant reactions (including contact urticaria)

Irritant effects of some individual fragrance ingredients, e.g. citral (18, 19), are known. Irritant contact dermatitis from perfumes is believed to be common, but there are no existing investigations to substantiate this (7). Many more people complain about intolerance or rashes to perfumes/perfumed products than are shown to be allergic by testing (9). This may be due to irritant effects or inadequate diagnostic procedures.

Fragrances may cause a dose-related contact urticaria of the non-immunological type (irritant contact urticaria). Cinnamal, cinnamic alcohol, and *Myroxylon pereirae* are well recognised causes of contact urticaria, but others, including menthol, vanillin and benzaldehyde have also been reported (20). The reactions to *Myroxylon pereirae* may be due to cinnamates (21).

A relationship to delayed contact hypersensitivity was suggested (22), but no significant difference was found between a fragrance-allergic group and a control group in the frequency of immediate reactions to fragrance ingredients (20), in keeping with a non-immunological basis for the reactions seen.

4.1.3. Pigmentary anomalies

The term “pigmented cosmetic dermatitis” was introduced in 1973 for what had previously been known as melanosis faciei feminae when the mechanism (type IV allergy) and causative allergens were clarified (23). It refers to increased pigmentation, usually on the face/neck, often following sub-clinical contact dermatitis. Many cosmetic ingredients were patch tested at non-irritant concentrations and statistical evaluation showed that a number of fragrance ingredients were associated: jasmine absolute, ylang-ylang oil, cananga oil, benzyl salicylate, hydroxycitronellal, sandalwood oil, geraniol, geranium oil (24).

4.1.4. Photo-reactions

Musk ambrette produced a considerable number of allergic photocontact reactions (in which UV-light is required) in the 1970s (25) and was later banned from use in the EU. Nowadays, photoallergic contact dermatitis is uncommon (26). Furocoumarins (psoralens) in some plant-derived fragrance ingredients caused phototoxic reactions with erythema followed by hyperpigmentation resulting in Berloque dermatitis (8) [pp 417–432]. There are now limits for the amount of furocoumarins in fragrance products. Phototoxic reactions still occur but are rare (27).

4.1.5. General/respiratory

Fragrances are volatile and therefore, in addition to skin exposure, a perfume also exposes the eyes and naso-respiratory tract. It is estimated that 2–4% of the adult population is affected by respiratory or eye symptoms by such an exposure (28). It is known that exposure to fragrances may exacerbate pre-existing asthma (29). Asthma-like symptoms can be provoked by sensory mechanisms (30). In an epidemiological investigation, a significant association was found between respiratory complaints related to fragrances and contact allergy to fragrance ingredients, in addition to hand eczema, which were independent risk factors in a multivariate analysis (31).

4.2. Patch testing

The diagnosis of contact sensitisation (or contact allergy – regarded here as synonymous) as the immunological alteration underlying allergic contact dermatitis is made by patch testing. This diagnostic tool involves the standardised application of small doses of a set of potential or individually suspected allergens for a period of 1 day or, mostly, 2 days. In the following days, exposed skin sites are checked for the occurrence of allergic reactions, which morphologically mimic allergic contact dermatitis occurring elsewhere, after exposure to culprit products. International guidelines for the application, reading and interpretation of the patch test exist (32). The present brief section does not intend to reiterate all technical and scientific aspects, but to outline some aspects of diagnostic patch testing which are often misunderstood (for a recent comment see also (33)).

- The patch test identifies whether the patient has contact allergy to a substance, but cannot contribute information on the clinical relevance of that contact allergy for the eczema that led to consultation and to patch testing (see 4.4.1).
- Exposure conditions of the patch test (one-time, prolonged occlusive application, usually in petrolatum or water, of a single substance) have been optimised to achieve above diagnostic aim, and thereby have nothing in common with exposures which lead to sensitisation and elicitation of allergic contact dermatitis. These are normally repetitive, often over weeks, months or years, non-occlusive, and to much lower concentrations and doses/area, respectively, but possibly on damaged or inflamed skin. In fact, the repeated open application test (ROAT), which is sometimes used after a positive patch test of uncertain validity to verify that contact allergy indeed exists mimicks these day-to-day exposure conditions, and typically involves single dosings which are a small fraction of the one-time patch test dose (see 11).
- It is self-evident that such (repeated, low-level) exposures must have occurred and have culminated in an adaptive immune response – therefore it is axiomatic that the substance involved is a skin sensitizer in humans (33).
- Repeated patch testing, which is a relatively rare event, does not contribute significantly to contact allergy (to fragrance allergens).
- Most allergen test preparations, and certainly those that are included in international baseline series, have evolved from studies critically (re-) appraising their diagnostic validity, i.e., sensitivity and specificity. Notwithstanding this, false-positive and false-negative reactions do occur (as with any diagnostic tool). While in the individual case such diagnostic misclassification may have unfortunate consequences, it will hardly impair epidemiological estimates of contact allergy frequency – at least as long as a reasonable balance between false-positive and false-negative reactions is achieved.

4.3. Epidemiology of fragrance allergy

4.3.1. Substances used for screening of contact allergy to fragrance ingredients

A fragrance formula may consist of ten to 300 or more different ingredients. The CosIng database lists 2587 ingredients used for perfuming¹, as well as several other materials classified as odour “masking” agents, which is equivalent with regard to allergy. A mixture of seven fragrance chemicals and one natural extract, which have been identified as major fragrance allergens in the past (34), are used for diagnosing contact allergy to fragrance

¹ <http://ec.europa.eu/enterprise/cosmetics/cosing/index.cfm?fuseaction=search.results&function=66&search>, last accessed 2009-10-14.

ingredients (Table 4-1). This mixture is called the Fragrance Mix (FM I) and is included in the standard patch test tray containing the most common allergens in Europe.

Table 4-1: Ingredients of Fragrance Mix I (FM I; 8% allergens in petrolatum).

Single constituent: INCI name (common name)	Conc. (%)
Amyl cinnamal (alpha-amyl cinnamal)	1
Cinnamyl alcohol (cinnamic alcohol)	1
Cinnamal (cinnamic aldehyde)	1
Eugenol	1
Geraniol	1
Hydroxycitronellal	1
Isoeugenol	1
Oak moss absolute (a natural extract; INCI: <i>Evernia prunastri</i>)	1
Sorbitan sesquioleate (added as an emulsifier)	5

Note: All single allergens of the above, when used for breakdown testing, are also in petrolatum.

However, due to the introduction of new fragrance ingredients (with allergenic potential), the above Fragrance Mix I was deemed not to be sufficient for the diagnosis of fragrance allergy. Thus, Fragrance Mix II was devised to supplement Fragrance Mix I in a European multicentre study (35, 36). Since then, FM II has been included in the European baseline series. Table 4-2 lists the ingredients of FM II. In addition to being tested in FM II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) is also tested separately at 5% test concentration in the baseline series (37).

Table 4-2: Ingredients of Fragrance Mix II (FM II; 14% allergens in petrolatum).

Single constituent: INCI name (common name)	Conc. (%)
Citronellol	0.5
Citral	1
Coumarin	2.5
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)	2.5
Farnesol	2.5
Alpha-hexyl-cinnamal	5

Note: All single allergens of the above, when used for breakdown testing, are also in petrolatum.

Patch test results in patients and in population samples with these two screening mixes, and single allergens, will be presented and discussed in the following two sections.

4.3.2. Clinical epidemiology

For a number of reasons the bulk of the evidence regarding the frequency of contact allergy to fragrance ingredients relies on clinical data, i.e. the history, clinical presentation and test results of patients patch tested for suspected allergic contact dermatitis – in general, and not specifically due to fragrance ingredients. The frequency of contact allergy to fragrance ingredients (or other contact allergies, for that matter) cannot be related to the population

directly, as it is derived from a subgroup (of patients) selected for specific morbidity. Nevertheless, these data can be examined epidemiologically assuming a largely similar selection process: (i) across time in a given department; and (ii) between departments at any point of time. If the notion of similarity, and thus direct comparability, does not appear valid, adjustment or standardisation techniques can be employed to account for differences, e.g. the average age of patients in a time series on a (fragrance) allergen with age-associated risk of sensitisation. In this situation, changes in the age composition of the patients tested may confound a time trend. A distinction must be made between patch testing “consecutive” patients, i.e. all patients who are patch tested for suspected contact sensitisation, and “aimed” patch testing, i.e. application of allergens only in the subset of patients in whom exposure to the particular allergens of the applied “special series” is suspected. For any given allergen, the latter “aimed” approach will usually yield higher sensitisation prevalences than the testing of not-further-selected “consecutive” patients. Thus, information on the inclusion of an allergen either in a baseline series (tested in virtually all patients) or in a special series (applied in an aimed fashion) must be considered and is given in the following tables, where available in the cited references.

Notwithstanding the potential pitfalls of clinical data, they have proven useful in identifying emerging trends or persisting problems, and also in evaluating the effect of preventive action – either regarding the entire population, or subgroups thereof, such as certain occupations. Regarding the fragrance mixes (FM I and FM II) mentioned above, evidence regarding sensitisation frequencies published since 1999 will be outlined below, thus supplementing the data presented in the SCCNFP opinion on Fragrance Allergy in 1999 (1).

Fragrance Mix I ("Larsen Mix")

Table 4-3: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in Europe: Fragrance Mix "I" (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Sweden (38)	Consecutive patients	2000	3790	6.9
Hungary (39)		1998-1999	3604	8.2 (7.3–9.1) [§]
Czech Republic (40)		1997-2001	12058	5.8 (5.4–6.2) [§]
Ljubljana, Slovenia (41)	Consecutive patients	1989-1998	6129	5.9 (5.3–6.5) [§]
Germany (42)	Consecutive IVDK patients	1996-2002	59298	11.3 (11.0–11.5) [§]
Germany (43)	Consecutive IVDK patients	2005-2008	36961	7.3 (7.0–7.6) [§]
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	9.1 (8.1–10.3) [§]
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	295	5.8 (3.4–9.1) [§]
The Netherlands (45)	Consecutive patients	09/1998-04/1999	1825	10.6 (9.2–12.1)
The Netherlands (46)	Patients (cosmetic allergy suspected)	1994-1998	757	14.8 (12.3–17.5) [§]
Leuven, Belgium (47)	Consecutive patients	1990-2005	10128	9.1 (8.6–9.7) [§]
Coimbra, Portugal (48)	Consecutive patients	07/1989-06/1999	2600	10.9 (9.7–12.2) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	4.5 (3.4–5.8) [§]
Sheffield, UK (50)	Consecutive patients	1994-1995	744	11.4 (9.2–13.9) [§]
St. John's, London, UK (51)	Consecutive patients	1980-2004	34072	7.7 (7.4–8.0) [§]
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	7.2 (6.8–7.6) [§]
ESSCA (53)	Consecutive patients	2002-2003	9663	7.1 (6.6–7.6) [§]
ESSCA (54)	Consecutive patients	2004	9941	7.6 (7.1–8.2) [§]
ESSCA (55)	Consecutive patients	2005-2006	18542	7.0 (6.6–7.4) [§]

Table 4-4: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in non-European countries: Fragrance Mix “I” (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
South Korea (56)	Consecutive patients	04/2002–06/2003	422	9.7 (7.1–13.0) [§]
Lahore, Pakistan (57)	Dermatitis patients	2 years prior to 2002	350	7.7 (5.2–11.0) [§]
Manipal, India (58)	Dermatitis patients	1989-1998	1780	3.1 (2.3–4.0) [§]
Tel Aviv, Israel [§] (59)	Consecutive patients	1999-2000	943	8.5 (6.8–10.5) [§]
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	7.1 (6.1–8.3) [§]
Tehran, Iran (61)	Consecutive patients	2002-2004	250	4.0 (1.9–7.2) [§]
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) [§]
Beijing, China (63)	Consecutive patients	2000-2003	378	15.9 (12.3–20.0) [§]
USA (Canada) (64)	Probably consecutive patients	2003	1603	5.9
NACDG 2009 (US and Canada) (65)	Consecutive patients	2005-2006	4439	11.5

Note: § Possibly included in (60).

Beyond the studies discussed above, regarding a time trend of sensitisation to FM I, a significant increase of positive results to FM I until 1998, and a significant drop thereafter has been noted in the IVDK study covering 1996 to 2002 (42). A similar drop from 1999 to 2007 has been observed in female, but not male patients from Copenhagen (52). In accordance with these findings, the prevalence of positive reactions to FM I doubled, or thereabouts, from 1989-1993 to 1994-1998 in Ljubljana, Slovenia (41).

Within Europe, a comparison between different countries and clinical departments is possible. An EECDRG study covering 1996-2000 found 9.7% positives to FM I (range: 5.0–12.6% in ten departments from seven European countries (66). A different European study, covering 10/1997-10/1998, found 11.3% (95% CI: 9.9–12.9%) positive reactions to FM I in 1,855 patients; the variation between centres was marked: Gentofte 8.2% vs. Leuven 23.0% as extremes (67). In the first study of the European Surveillance System on Contact Allergies (ESSCA), covering 2002 and 2003, 9663 patients were patch tested with FM I, overall yielding 7.1% positive reactions with marked variation between participating departments. In Dortmund, Germany, the minimum frequency of 3.7% was noted, while in Lahti, Finland, the highest prevalence, namely 10.4%, was found (53). Subsequently, in the year 2004, the overall prevalence was 7.6%, i.e. largely unchanged (54). In the most recent study by ESSCA, based on 2005/2006 PT data across Europe, significant differences were again noted, this time on the aggregated level of European regions, with FM I sensitisation being the least frequent in the Southern countries (4.8% [95% CI: 3.9–5.5%] age- and sex-standardised prevalence) vs. 7.7% (95% CI: 7.0–8.4%) in the central European departments, with the Finnish, Polish and Lithuanian departments (5.7% [95%

CI: 4.6 – 6.8%]) and the UK network (6.8% [95% CI: 6.3 – 7.3%]) in an intermediate position (55).

Fragrance Mix II

Table 4-5: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis: Fragrance Mix “II” (see Table 4-2). The FM II was only conceived in 2005, so results are still sparse). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ^(§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
EU (35)	Six clinical depts.	10/2002-06/2003	1701	2.9 (2.2–3.9) [§]
IVDK, Germany (68)	Consecutive patients	01/2005-12/2008	35633	4.9 (4.7–5.1) [§]
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	227	9.3 (5.8–13.8) [§]
Leuven, Belgium (47)	Consecutive patients	2005 only	335	2.1 (0.8–4.3) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	0.6 (0.2–1.1) [§]
Denmark (69) on behalf of the DCDG, 2010	Consecutive patients	2005-2008	12302	4.5 (4.1–4.9) [§]

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see section 7.1).

HICC was recognised as an allergen in 1995 (70) and later included in the new perfume mixture, Fragrance Mix II (71), which is routinely used for the diagnosis of perfume allergy, see above. Furthermore, it is recommended to test separately with HICC, because it is a very frequent allergen (37) and detects relevant fragrance sensitisation which would otherwise have been missed (49). In the studies performed in European dermatology clinics, 0.5-2.7% of eczema patients have been found to be allergic to HICC with the highest frequency in central Europe (55). For further details see Table 4-6.

Table 4-6: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **HICC** (5% pet. if not stated otherwise). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ^(§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Lithuania (72)	Consecutive patients	04/2006-10/2008	816	0.9 (0.3–1.8) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	852	0.8 (0.3–1.7) [§]
Germany (CH, AT) (73)	Consecutive patients	03/2000-02/2001	3245	1.9 (1.5–2.4) [§]

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Germany (CH, AT) (74)	Consecutive patients	01/2003-12/2004	21325	2.4 (2.2–2.6) [§]
Germany (CH, AT) (68)	Consecutive patients	01/2005-12/2008	35582	2.3 (2.2–2.5) [§]
Belgium (47)	Consecutive patients	2002-2005	2901	2.1 (1.6–2.7) [§]
Denmark (69)	Consecutive patients	2005-2008	12302	2.4 (2.1–2.7) [§]
South Korea (56)	Consecutive patients	04/2002–06/2003	422	1.7 (0.6–3.4) [§]
USA, Canada (64)	Probably consecutive patients	2003	1603	0.4 (0.2–0.9) [§]

***Myroxylon pereirae* (Balsam of Peru)**

Myroxylon pereirae is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75). This natural mixture has been employed as screening agent in the baseline series for many decades. Hence, a wealth of data is available; Table 4-7 summarises results of the past 10 years.

Table 4-7: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: ***Myroxylon pereirae* resin** (Balsam of Peru) (25% pet.). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Tel Aviv, Israel (59) #	Consecutive patients	1999-2000	943	6.6 (5.1–8.4) [§]
South Korea (56)	Consecutive patients	04/2002 – 06/2003	422	7.3 (5.1–10.3) [§]
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	3.6 (2.9–4.5) [§]
Manipal, India (58)	Dermatitis patients	1989-1998	1780	1.0 (0.5 – 1.5) [§]
Tehran, Iran (61)	Consecutive patients	2002-2004	250	2.4 (0.9–5.2) [§]
Sevilla, Spain (76)	Consecutive patients	2002-2004	863	5.8 (4.3–7.6) [§]
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) [§]
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	5.4 (4.6–6.3) [§]
Czech Republic (40)	Consecutive patients	1997-2001	12058	7.3 (6.8–7.8) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	6.4 (5.1–7.9) [§]

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	3.9 (3.6–4.2) [§]
Sweden (38)	Consecutive patients	2000	3790	6.5
Nine European countries (53)	Consecutive patients	2002-2003	9672	6.1
Germany, three Swiss and one Austrian Dept. (43)	Consecutive patients	2005-2008	36919	8.0 (7.7–8.3)
Ten depts. From seven EU countries (66)	Consecutive patients	1996-2000	26210	6.0
USA (Canada) (64)	Probably consecutive patients	2003	1603	6.6
NACDG 2009 (65)	Consecutive patients	2005-2006	4449	11.9

Oil of turpentine

This natural extract is not tested in all baseline series. It is considered as a minor screening allergen for fragrance contact allergy. Moreover, oil of turpentine is used as a raw material in perfumery (see Annex I). Table 4-8 summarises results of the past 10 years with patch testing of consecutive patients.

Table 4-8: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Oil of turpentine** (10% pet.) patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Lisbon, Portugal (77); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 (1.9–2.8) [§]
Birmingham, UK (78)	Potters with occup. hand dermatitis	6 months; prior to 1996	24	14/4 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (79)	Consecutive patients	1992-1995	27658	0.47 (0.39–0.55) [§]
Austria/Germany (IVDK) (42)	Consecutive patients	1996-2002	59478	Annual prevalence 1.6 to 4.4%
Augsburg, Germany (80)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (53)	Consecutive patients	2002/03	3767	1.6%
Austria/Germany/Switzerland (IVDK) (43)	Consecutive patients	2005-2008	37163	1.8%

An “overall burden” of fragrance contact allergy, in terms of the prevalence of contact allergy to at least one of the up-to-five screening allergens present in the baseline series (FM I, FM II, HICC, *Myroxylon pereirae*, oil of turpentine) has not been given in the published studies. A re-analysis of data from the two published studies of the IVDK (43, 68), covering central Europe from 2005 to 2008 (Germany, Austria and Switzerland), yielded an estimate of such overall prevalence of 16.2% (95% CI: 15.8-16.6%) (IVDK technical report, 2011-11-18).

4.3.3. Population-based epidemiology

In principle, the examination of a representative sample of the population is the most valid approach for estimating disease frequency, as there is no systematic selection process. However, in practice, participation of much less than 70% of those approached introduces the possibility of self-selection and thus of biased morbidity (or risk) estimates. Moreover, the resources needed prohibit regular, e.g. yearly, patch test studies in a sample of several thousand persons. For these reasons few studies exist (see Table 4-9).

A Swedish study of hand eczema in an industrial city showed that among 1,087 individuals recruited from the general population with symptoms of present or previous hand eczema, 5.8% were positive to the Fragrance Mix (81). In Denmark, Fragrance Mix sensitivity was found in 1.1% (0.3-2.1%) of 567 persons drawn as a sample from the general Danish population; only nickel sensitivity was more prevalent (82). In Italy, female patients with hand eczema caused by contact with detergents were patch tested. Of 1100 women, 3.1% reacted to Fragrance Mix I (83). A control group of 619 female patients with no eczema disease were also patch tested; 1.3% were positive to the Fragrance Mix (83). On the other hand, in a sample of 593 healthy Italian recruits, only three positive reactions (0.50%) to FM I were observed (84). Among Danish school children, 14-15 years of age, fragrance contact allergy was detected in 1.8% by patch testing with Fragrance Mix I (85). A study of 85 American student nurses showed that 15 (17.6%) had a positive reaction to Fragrance Mix I; 12 of the individuals also had a positive history of contact dermatitis (86). In this study the concentration of Fragrance Mix I was 16% as opposed to the currently recommended concentration of 8% and the study included only young females. Both of these factors may have contributed to the high prevalence of fragrance sensitivity found.

In 1990, 1998 and 2006, samples of the Danish adult population living in the Copenhagen area were patch tested with the European baseline series. In total 4299 individuals aged 18-69 years (18-41 years only in 1998) completed a pre-mailed questionnaire and were patch tested with FM I and *Myroxylon pereirae* (82, 87, 88). In 1990, 1.1% were found positive to FM I and in 2006, 1.6% were positive, which means no general change. However, when the age group of 18-41 years was analysed, the prevalence of FM I sensitisation followed an inverted V-pattern among women, i.e. an increase from 0.7% in 1990 to 3.9% in 1998, followed by a decrease to 2.3% in 2006. The participation rate varied in the three samples from 71.5% in 1990 to 52.4% in 1998, and to 43.7% in 2006 (82, 87, 88).

Contact sensitisation to FM I is strongly age related, with the relative risk more than doubling in the older age groups, compared to younger PT patients. This has been found in both bivariate (89) and adjusted multifactorial analyses (90). Hence, in older samples of the population, the prevalence of contact allergy to fragrance ingredients in general, and to FM I in particular, can be expected to be higher than in younger samples. From this background, the strikingly high prevalence observed in the MONICA/KORA allergy study in Augsburg, Germany (see Table 4-9) (80), may be explained, together with some residual confounding from the rather complex sampling process.

Table 4-9: Results from patch testing with Fragrance Mix I in different population based groups.

Country (Ref.)	Population	Year(s)	No. tested	% positive (95% CI)
Italy (83)	Females without eczema	Not given	619	1.3
Italy (84)	Male recruits	Not given	593	0.50
Denmark (82)	Population sample adults, 15-69 years	1990-91	567	1.1
Denmark (85)	School children 12-16 years old	1995/96	717	1.8
Denmark (82, 87)	Population sample adults, 18-41 years	Jan-Nov 1998	414	2.7
Denmark (88)	Population sample adults, 18-69 years	June 2006–May 2008	3460	1.6
Norway (91)	Population sample adults, 18-69 years. (Results reported in 2007)	1994 (92)	1236	1.8 (1.1–2.7)
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	1141	11.4
USA (86)	Student nurses, females	1980	85	17.6*
Sweden (81)	Population sample adults, age 20-65 years reporting hand eczema	1983-84	1087	5.8*

Note: * Testing performed with Fragrance Mix I, containing 16% allergens; the currently used Fragrance Mix I contains 8% allergens (see above).

Table 4-10: Results from patch testing with other fragrance allergens in different population based groups. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ^(§).

Country (Ref.)	Population	Year(s)	Fragrance allergen	No. tested	% positive (95% CI) [§]
Thailand (93)	Convenience sample (via advertisement), age 18-55	Not given	Isoeugenol, <i>Evernia prunastri</i> , <i>Myroxylon pereirae</i> *	2545	Positive to at least one of three allergens: 2.5 (1.9–3.2) [§]
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	<i>Myroxylon pereirae</i>	1141	2.4
Denmark (88)	Population sample, age 18-69	1990 2006	<i>Myroxylon pereirae</i>	567 3460	1.1 0.1

Note: * *Myroxylon pereirae* is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75).

4.4. Consumer products as a cause of fragrance contact sensitisation and allergic contact dermatitis

4.4.1. Clinical relevance

Clinical relevance is a concept used to describe the significance of a positive (allergic) patch test reaction for an individual patient: a reaction is deemed relevant if contact allergy to the substance is associated with previous or current episodes of allergic contact dermatitis. Thereby, the evaluation of clinical relevance links past exposure to morbidity. For the evaluation of relevance, past or recent exposure(s) to the allergen need to be identified in the patient's history. The success of this process generally depends on:

- The patient's understanding and awareness;
- The dermatologist's knowledge concerning exposures;
- Ingredient labelling; and
- Information about the actual chemical composition of the implicated product.

As these requirements may be met to a varying extent, the validity of relevance information as reported in clinical studies may also be variable. However, information on clinical relevance is important, in principle, because the proportion of currently relevant sensitisations reflects the amount of current exposure and resulting disease state, which may increase or decrease with time. In this way, current relevance also reflects the direct burden of a fragrance contact allergy to the individual and indirectly to society. Further important aspects of the evaluation of clinical relevance as a final step of patch testing have been discussed (32, 94-96).

Generally, clinical relevance is categorised as “current”, “previous” or “unknown”. Further differentiation has been introduced by adding information on:

- Occupational versus non-occupational causation; and
- The level of certainty of the relevance statement, e.g. as “certain”, “probable”, “possible”.

In some cases, clinical relevance may not be established due to:

- Immunological cross-reactivity with an individual allergen, diagnosed or not;
- Active sensitisation by the patch testing;
- Contact sensitisation not caused by the substance, but by a contaminating constituent; or
- Failure to test with a true hapten (e.g. haptens formed from prehaptens on exposure to air, see chapter 5).

It should be noted that this statement on clinical relevance refers to the past history of a patient. This implies that a lack of, or unknown, clinical relevance does not make future allergen avoidance unnecessary.

In the context of contact allergy to fragrance ingredients, a number of alternative concepts of relevance have been used, for example:

- A history of intolerance to perfume or to perfumed products;
- A history of intolerance to perfume actually containing the allergen diagnosed;
- Detection of the culprit allergen in a perfume previously used.

4.4.2. Elicitation with clinical symptoms/signs, current and past

In case reports or small series, the clinical relevance of positive patch test reactions is usually well established and presented in detail. Moreover, a few large-scale clinical studies on contact allergy to fragrance ingredients have reported results on clinical relevance, which will be presented and discussed in this section. The studies can be subdivided into those which focus on medical history, patch testing with consumer products or detection of specific allergens in consumer products used by patients.

Medical history

A series of studies conducted in the 1990s showed that most individuals with contact allergy to fragrance ingredients were aware that they could not tolerate fragranced products on their skin and were able to specifically name product categories that initiated their disease (9). In this context, colognes, deodorants and lotions were named significantly more often by fragrance allergic dermatitis patients than by patients without fragrance contact allergy (3). These studies are described in the SCCNFP opinion on fragrance allergy of 1999 (1). Newer studies are outlined below.

NACDG 2009 study (65)

The definition of “present” clinical relevance in this North American network study was strict, requiring:

- A positive use or patch test with the suspected item(s) for “definite” relevance; and
- Verification of the presence of the allergen in known skin contactants, and consistent clinical presentation for “probable”.

If these conditions were not met, but skin contact to items generally containing the item was likely, “possible” was used.

Regarding fragrance allergens, the proportions were as described in Table 4-11.

Table 4-11: Extract from ((65) Table 3) regarding the proportion of patients with “present clinical relevance” (see text) and “past clinical relevance” (criteria not given).

Fragrance allergen	n (tested)	% (pos.)	Current relevance (%)			Past relevance (%)
			Definite	Probable	Possible	
<i>Myroxylon pereirae</i>	4449	11.9	1.3	33	53	2.7
FM I	4439	11.5	2.0	29.4	54.3	4.3
Cinnamal	4435	3.1	1.5	33.8	50	2.9
Ylang-Ylang oil	4434	1.5	4.6	10.8	73.8	1.5
Jasmine absolute	4447	1.1	0	24.5	67.3	6.1

Frosch 2002 (a) study (67)

In this study, 1,855 consecutive patients were patch tested with FM I and a series of a further 14 fragrance chemicals. Prior to the test, the history of adverse reactions to fragrances was classified as “certain” (6.6%), “probable” (8.0%), “questionable” (9.2%) or “none” (76.1%) (see (71)).

Frosch 2002 (b) study (97)

A series of 18 essential oils or components thereof, together with FM I, was assessed in 1,606 consecutive patients. Similar to the above study, the proportions of patients with a “certain” or “probable” history (or otherwise) and positive reactions to either FM I or the

special series, or both, were cross-tabulated. Of note, 53.7% of patients with positive reactions to FM I only, had no history. Similarly 54.2% of patients with positive reactions only to one of the essential oils had no history. However, in cases of reactivity to both FM I and one of the essential oils, the proportion of patients with no history was only 36.5%.

Frosch 2005 study (35)

The diagnostic properties of FM I and the new FM II were evaluated in 1,701 consecutive patients patch tested in six European centres. Contrasting a “certain” (found in 8.7% of patients) with “no history” (75.3% of patients), the sensitivity of FM I was 25.2%, and the positive predictive value (PPV) 45.1%. In comparison, the sensitivity of FM II at 14% concentration was 13.5% and the PPV was 55.6%. The combination of the two mixes was important, as more patients with a “certain” history, but also independently from history, reacted to just one of the mixes rather than to both.

Danish Contact Dermatitis Group 2005-2008 (69)

In 12302 consecutive patients patch tested in seven dermatology clinics and three university hospitals, 10.6% were positive to one or more of the fragrance allergy markers (FM I, FM II, *Myroxylon pereirae* or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)). Clinical relevance covered current and/or past relevance based on: 1) medical history; 2) results of patch and/or use tests; 3) ingredient labelling; or 4) chemical analysis. Clinical relevance was found in 71.0% of cases positive to FM I, 72.2% of those positive to FM II and 76.7% of those positive to HICC. These proportions were higher than the average for other cosmetic allergens such as preservatives and hair dyes, which gave relevant reactions in about 50% of those positive, as did *Myroxylon pereirae*. *Myroxylon pereirae* itself is not used in cosmetics as it is banned, but sensitisation may be caused by exposures to related substances and thus relevance may be difficult to determine.

Cosmetic products

Fragrance formulae from cosmetic products

Popular fine fragrances (5), as well as toilet soaps, shampoos, lotions, deodorants, and aftershaves have been shown to provoke allergic contact dermatitis in patients when used for patch testing (5, 6, 98, 99). Moreover, commercially available fragrance formulae and dilutions of individual fragrance allergens were potent elicitors of allergic contact dermatitis under simulated use conditions (10, 100, 101).

More recently, deodorants spiked with the fragrance allergens cinnamal, hydroxycitronellal and HICC, respectively, in realistic in-use concentrations were shown to elicit allergic contact dermatitis in 89-100% of the fragrance allergic individuals tested (102-104). In 87.5% of HICC sensitised individuals the use of a cream (and in 82.8% the use of an ethanol solution) spiked with HICC provoked dermatitis (105). These studies are discussed in more detail in chapter 11 on quantitative aspects. Other new studies are mentioned below:

IVDK “own perfumes” study (106)

A different perspective on clinical relevance is provided by assessing the proportion of positive reactions to the FM I or single fragrance allergens in patients who had not tolerated certain perfumed products, such as deodorants and aftershaves and who were patch test positive to these cosmetics. The following two tables are taken from this publication.

Table 4-12: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own deodorant.

Fragrance allergen	Conc. (%)	Deodorant positive (n=66)		Deodorant negative (n=855)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	61	38.0 (24.1-51.9)	805	15.0 (12.5-17.5)
<i>Myroxylon pereirae</i>	25	60	22.9 (12.7-33.1)	806	9.1 (7.2-11.0)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
Isoeugenol	1	33	6.5 (0.7-12.3)	204	7.2 (3.6-10.8)
Cinnamal	1	29	11.3 (0-24.1)	133	1.1 (0-2.7)
Geraniol	1	29	8.3 (0-20.4)	141	0 (0-2.1)

Of the 66 patients with a positive patch test reaction to their own deodorant, most had positive reactions to one or more fragrance allergens. This was much more prevalent than those patients in whom no positive reaction to their deodorant was observed. This observation supports the notion that the respective fragrance allergens are important in contact allergy to fragrance ingredients caused by deodorants, supporting data regarding exposure (chapter 10.1).

Table 4-13: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own aftershave, eau de toilette or perfume.

Fragrance allergen	Conc. (%)	Product positive (n=63)		Product negative (n=819)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	56	57.1 (46.2-68.1)	764	13.9 (11.4-16.4)
<i>Myroxylon pereirae</i>	25	56	13.9 (7.3-20.4)	766	8.8 (6.8-10.7)
HICC	5	20	58.3 (37.5-79.0)	310	1.3 (0-2.7)
<i>Evernia prunastri</i>	1	28	22.1 (7.0-37.2)	153	8.8 (4.2-13.4)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
<i>Cananga odorata</i> (ylang-ylang oil)	10	7	16.3 (2.0-30.5)	43	5.0 (0-11.3)

Similar results were obtained from the subgroup of patients with a positive reaction to their eau de toilette, aftershave (hydroalcohol solutions) or perfumes (Table 4-13). However, notable differences were: (i) the greater relative importance of *Evernia prunastri* (Oak moss absolute); and (ii) generally an extremely high proportion of positive reactions to various other fragrance ingredients.

4.4.3. Elicitation in diagnostic patch tests without clinical history

In a variable proportion of patients, a positive patch test reaction does not correlate with recent or past episodes of presumptive allergic contact dermatitis. Apart from particular circumstances, such as cross-reactivity or reactivity to contaminants outlined above, there are several possible explanations for this:

- The patch test reaction was a false-positive (irritant).
- There was erroneous recall/interpretation of the patient's history (false-negative).
- Lack of knowledge concerning exposures.

- If the patient is weakly sensitised (e.g. by a low induction dose), the occlusive exposure during patch testing may have been the only exposure above the individual elicitation threshold capable of eliciting an unequivocal allergic contact reaction. In this situation, clinical relevance would be classified as “unknown”. Nevertheless, there is an alteration of the immune status of the individual.

Sometimes, a repeated open application or provocative use test is employed to mimic “normal” exposure to the allergen. A positive reaction to such a use-related test confirms actual sensitisation. Moreover, the positive result supports the necessity of future allergen avoidance. Apart from the risk of developing allergic contact dermatitis in the future, sensitisation means an alteration of the immune status of the individual.

4.5. Socio-economic impact of contact allergy

4.5.1. Health related quality of life

Skin diseases in general are known to affect quality of life significantly (107); this also applies to eczema, where most studies concern atopic dermatitis and hand eczema patients (108, 109). Hand eczema has a poor prognosis and may affect the self-image, limit social activities and lead to occupational restrictions (109, 110). The quality of life in hand eczema patients with fragrance contact allergy is affected in a similar degree as patients with other contact allergies (111).

In a questionnaire study of 117 patients recently diagnosed with contact allergy to fragrance ingredients, most presented with hand or facial eczema. In response to the question if and how fragrance allergy had affected their life situation, 67.5% replied that they often had to take special precautions, 47.0% replied that they were often bothered by eczema and itch, 17.1% said that they had had to take sick leave due to their fragrance contact allergy and 45.3% felt that fragrance contact allergy had significantly influenced their daily living (112).

4.5.2. Occupational restrictions

Contact allergy is known to influence severity and prognosis of hand eczema (113, 114) including risk of sick leave (111). Fragrance contact allergy is mostly of a non-occupational origin (90) related to the personal use of scented cosmetics, but may have secondary occupational consequences. This may be due to exposure to fragrance ingredients also in the work place or because hand eczema has developed. Hand eczema itself may make it impossible to remain in the trade even if protective equipment is used. In young people, fragrance allergy may limit the choice of occupations, as it will be difficult to work as a hairdresser, cosmetologist or in other occupations with a significant skin exposure to fragranced products.

4.5.3. Costs to health care/health economics

In a population based study of 3,460 individuals, contact allergy to FM I was found in 1.6%; logistic regression analyses showed that medical consultation due to cosmetic dermatitis (OR 3.37, 95% CI 1.83-6.20) and cosmetic dermatitis within the past 12 months (OR 3.53, CI 2.02-6.17) were significantly associated with sensitisation to FM I (88). Further, as mentioned above, fragrance allergy may lead to sick leave (112). No specific cost estimates for fragrance allergy exist, but the yearly total costs of contact dermatitis in Western Europe was estimated to be 5.2 billion Euro in 1997. Prices were based on the Allergy White Paper (1997) and on results of investigations and extrapolations of known data for Western Europe (115). Fragrance allergy is the second most frequent cause of contact allergy after nickel allergy and is seen in every 10th patient investigated for contact allergy. Even a modest reduction in nickel allergy has been estimated to have the value of 12 million Euro/year/million people in Denmark (Environmental Project Nr. 929, 2004; <http://www2.mst.dk/Udgiv/publications/2004/87-7614-295-7/pdf/87-7614-296-5.pdf>, last

accessed 2011-11-13). The costs are likely to differ in other countries, some with higher expenses and some with lower costs. These estimates show that the cost of contact allergy in the population may be considerable.

4.6. Allergen avoidance

Generally, “allergen avoidance” can be regarded as having two aspects: (i) primary prevention of the acquisition of contact allergy achieved by avoiding or limiting exposure of the general population, or certain parts of it, to allergens; and (ii) secondary prevention in terms of avoiding (re-)elicitation of allergic contact dermatitis in sensitised individuals.

4.6.1. Primary prevention: limiting or eliminating exposure to allergens in the population

The main aim of public health is the primary prevention of disease in populations. Allergic contact dermatitis (to fragrances) has the potential to have a significant impact on quality of life, including effects on fitness for work (chapter 4.5). Moreover, it is a common phenomenon and therefore a reduction of exposure to (fragrance) allergens must be an objective of effective Public Health measures.

Means of limiting or eliminating exposure to fragrance allergens include the following:

- *Prohibition* by regulatory measures or other means.
- *Restriction* by regulatory measures or other means of the maximum permissible concentration of a substance, or a critical component of natural mixtures, possibly according to different uses and product types, respectively.
- *Substitution* with suitable, but less or non-allergenic compounds. Substitution by a component which is chemically different, but effectively not different in terms of allergenicity or cross-reactivity, is not adequate (e.g. an ester) (chapter 5).
- *Formulating the fragrance* with the aim of limiting or eliminating those substances for which a sensitising potential has been shown. One difficulty with this approach is that sometimes no sensitisation data exist for those components of a fragrance formula which are used to replace a “known sensitiser”.
- *Deliberate avoidance* of the use of fragrances where they are not essential to the function of a finished product, but used merely to add to its appeal. Examples could include most cosmetics, topical medicaments, detergents etc., but obviously not perfumes, eau de toilette and other products used for their scent.
- *Information, e.g. labelling* so that the consumer may make an informed choice to avoid exposure to a particular ingredient.

4.6.2. Secondary prevention: avoiding re-exposure to (a) specific sensitiser(s) in clinically diagnosed individuals

In clinical dermatology, avoidance of re-exposure to an allergen is central to the care of sensitised patients. Contact sensitisation, as a latent condition, persists life-long, and therefore allergen avoidance is the only means of avoiding potentially severe and/or handicapping disease, which affects quality of life and may affect fitness for work, i.e. allergic contact dermatitis.

In this context, the valid diagnosis of sensitisation, by patch testing (32) with standardised materials, is a prerequisite of successful allergen avoidance.

In the case of fragrances, a history clearly indicative of “fragrance dermatitis” but in which patch testing with commercially available test preparations is negative, most probably reflects a shortcoming of the patch test procedure, namely, a false-negative investigation. An important cause is inadequate information on the presence of fragrance substances

present in cosmetic products (and consumer products in general). This means that patients cannot be tested for relevant substances.

A false-negative investigation can also be due to a number of other reasons: (i) non-adherence to scientific recommendations (32) or guidelines (e.g. (116)); (ii) sub-optimal patch test concentration; or (iii) use of non-oxidised material if oxidised material is the true allergen.

In an “ideal” case, from the point of view of successful patient management, the test procedure identifies all the allergen(s) to which the patient has developed contact allergy, according to the information on the culprit product(s) brought in by the patient. Such contact sensitisation is termed “clinically relevant” (65), and the need for allergen avoidance in the future is unequivocally evident in these cases. However, not infrequently, clinical relevance of an allergic patch test reaction cannot be ascertained for various reasons, which may be beyond control by the clinician (see chapter 4.4). Nevertheless, future elicitation of allergic contact dermatitis by sufficient contact with the identified “non-relevant” allergen may be expected. Hence, the patient will need to avoid the respective substance(s).

In a less “ideal” case, only part of the fragrance allergens having caused allergic contact dermatitis are identified (and can subsequently be avoided), while another part remains unidentified, for instance because it is: (i) not labelled on the product; and/or (ii) not available for routine diagnostic patch testing (special investigations such as chemical analysis of the culprit product, and break-down patch testing of its individual components, are performed rarely). Such “residual” undetermined sensitisation will hamper the success of secondary prevention of allergic contact dermatitis due to fragrances.

The above consideration raises the question for the patient of how to identify fragrance chemicals in cosmetics and other products coming into contact with the skin, such as detergents and household products, topical medicaments, products used professionally (e.g. by hairdressers, beauticians, masseurs, aromatherapists), and in other industrially used categories of products (7) (see also chapter 9). In this regard, the labelling with “perfume” or “contains fragrances” does not provide sufficient information. Moreover, such general labelling has two main disadvantages:

- It does not aid the identification of past exposure to specific agents when planning a patch test and later, when interpreting possible positive patch test results regarding clinical relevance.
- The diagnosis of allergic contact sensitisation to unidentified fragrance allergens will lead to unnecessary avoidance of other fragrance substances to which the patient is not sensitised, which are, however, included under the label “perfume”.

Furthermore, the attribute “fragrance-free” may be misleading, as it merely states that no substance was added to the product to give it a scent, assuming it is used correctly at all. Nevertheless, fragrance substances used for other purposes, e.g. as preservatives, may expose the “fragrance allergic” patient to the allergen even in a “fragrance free” product (117). However, in terms of cosmetic ingredient labelling, such other uses are less problematic, as each ingredient not used as a fragrance component must be labelled. Also the use of natural products (essential oils) as preservatives must be considered in this context.

Ingredient labelling of 26 individual fragrance ingredients, identified as allergens in humans, was introduced for cosmetics in 2005. The intention was to provide a tool for clinicians for optimizing the investigation of patients with suspected fragrance allergy, as well as for fragrance allergic patients for avoiding products containing substances they have been shown to be allergic to. Both these aims are objectives of secondary prevention and seem to have been well accepted. In a study of fragrance allergic patients and their utilisation of ingredient labelling (112), most responded that they used the ingredient labelling (86.3%) and of those who used it, the majority (65.3%) found it helpful (112). Most allergic patients used the ingredient labelling (83.2%) to find out if the product was scented, while 35.6%

also looked for specific ingredients. Many (84.9%) found that a clearer labelling, e.g. easier names and a larger font size, would increase their benefit.

4.7. Conclusions

Contact allergy to fragrances is relatively common, affecting 1 to 3% of the general population, based on limited testing with eight common fragrance allergens and about 16 % of patients patch tested for suspected allergic contact dermatitis. Fragrance contact allergy is mostly non-occupational and related to the personal use of cosmetic products.

Allergic contact dermatitis can be severe and widespread, with a significant impairment of quality of life and potential consequences for fitness for work. Thus, prevention of contact sensitisation to fragrances, both in terms of primary prevention (avoiding sensitisation) and secondary prevention (avoiding relapses of allergic contact dermatitis in those already sensitised), is an important objective of public health risk management measures.

5. Activation of weak or non-sensitising substances into sensitisers - prehapten and prohapten

Fragrance allergens act as haptens, i.e. low molecular weight chemicals that are immunogenic only when attached to a carrier protein. However, not all sensitising fragrance chemicals are directly reactive, but require previous activation.

A prehapten is a chemical that itself is non- or low-sensitising, but that is transformed into a hapten outside the skin by simple chemical transformation (air oxidation, photoactivation) and without the requirement of specific enzymatic systems.

A prohapten is a chemical that itself is non- or low-sensitising but that is transformed into a hapten in the skin (bioactivation) usually via enzyme catalysis.

It is not always possible to know whether a particular allergen that is not directly reactive acts as a prehapten or as a prohapten, or both, because air oxidation and bioactivation can often give the same product (geraniol is an example).

Some chemicals might act by all three pathways. One example is geranial (an isomer of citral) which is a hapten itself with a moderate sensitisation potency, but can be activated to more potent sensitisers via air oxidation (autooxidation) thus acting as a prehapten and also via bioactivation (metabolic activation) thus acting as a prohapten (118).

Increased understanding of the importance of activation through interaction with the environment that turns non-sensitising compounds into sensitisers has made it important to distinguish between prehapten and prohapten. This distinction facilitates discussions by emphasizing the differences in activation mechanisms between the two types of compounds requiring activation to become haptens. It is important to note that prehapten activation, in contrast to bioactivation, can be prevented to a certain extent by avoidance of air exposure during the handling and storage of the chemicals. This concerns the most prominent haptens formed by autooxidation i.e. the hydroperoxides. In bioactivation, hydroperoxides have not been identified as metabolites, but other allergenic oxidation products (in particular aldehydes and epoxides) have been identified as being formed by both activation routes depending on the structure of the compound. One thoroughly studied example is geraniol which forms the aldehyde geranial, epoxy-geraniol, and also epoxy-geranial via both pathways of activation (autooxidation and metabolic oxidation) (119, 120). When haptens are formed by both pathways, the impact on the sensitisation potency depends on the degree of autooxidation in relation to the amount of metabolic oxidation.

Human data on established prehapten are presented in Table 5-1 and Table 5-2. In Table 5-1 the results from patch testing with air exposed samples of the prehapten are given. Table 5-2 shows the results from testing with the prehapten themselves without intended air exposure. In addition to the data given in this chapter, animal data (LLNA) on the pure prehapten or after controlled air exposure are given in Table 8-2. Possible pro- and prehapten are identified by SAR analyses in chapter 9.

5.1. Prehapten

Autooxidation is a free radical chain reaction in which hydrogen atom abstraction in combination with addition of oxygen forms peroxy radicals. The reaction shows selectivity for positions where stable radicals can be formed. So far, all fragrance substances that have been investigated with regard to the influence of autooxidation on the allergenic potential, including identification of formed oxidation products, have oxidisable allylic positions that are able to form hydroperoxides and/or hydrogen peroxide as primary oxidation products upon air exposure. Once the hydroperoxides have been formed outside the skin they form specific antigens and act as skin sensitisers (121). Secondary oxidation products such as aldehydes and epoxides can also be allergenic, thus further increasing the sensitisation potency of the autooxidation mixture (122). The process of photoactivation may also play a role, but further research is required to establish whether this activation route is currently underestimated in importance due to insufficient knowledge of the true haptens in this context.

Most terpenes with oxidisable allylic positions can be expected to autoxidise on air exposure due to their inherent properties. Depending on the stability of the oxidation products that are formed, a difference in the sensitisation potency of the oxidised terpenes can be seen. Oxidation products of commonly used fragrance terpenes (limonene, linalool, geraniol, linalyl acetate) have been identified as potent sensitisers in predictive animal tests (119, 123-128) (see chapter 8). This is also demonstrated for alpha-terpinene (129) and citronellol (AT Karlberg, personal communication 2012). The oxidised fragrance terpenes limonene, linalool and linalyl acetate have been tested in consecutive dermatitis patients and give frequent allergic contact reactions (130-135). Not all oxidised fragrance substances are strong sensitisers, e.g. caryophyllene is readily oxidised but has a low sensitisation potency after autoxidation (136). This is supported by clinical studies showing oxidised caryophyllene to be a less frequent allergen compared to oxidised limonene and oxidised linalool (133). Details are given in Table 5-1. The non-oxidised compounds rarely cause allergic reactions (43-45, 67, 70, 74, 97, 137-139), for details see Table 5-2. As oxidised and non-oxidised fragrance terpenes were not patch tested simultaneously in the same patients, the results are presented in two separate tables (Table 5-1 and Table 5-2).

Oxidised fragrance terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available.

It should be noted that activation of substances via air oxidation results in various haptens that might be the same or cross-reacting with other haptens (allergens). The main allergens after air oxidation of linalool and linalyl acetate are the hydroperoxides. If linalyl acetate is chemically hydrolysed outside the skin it can thereafter be oxidised to the same haptens as seen for linalool. A corresponding example is citronellol and citronellyl acetate. In clinical studies, concomitant reactions to oxidised linalool and oxidised linalyl acetate have been observed (140, 141). Whether these reactions depend on cross-reactivity or are due to exposure to both fragrance substances cannot be elucidated as both have an allergenic effect themselves.

Linalool and linalyl acetate are the main components of lavender oil. They autoxidise on air exposure also when present in the essential oil, and form the same oxidation products found in previous studies of the pure synthetic terpenes. Experimental sensitisation studies showed that air exposure of lavender oil increased the sensitisation potency. Patch test results in dermatitis patients showed a connection between positive reactions to oxidised linalool, linalyl acetate and lavender oil (140).

Air oxidation of prehaptens can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autoxidation rate depends not only on the compound itself, but also on its purity. The prevention of autoxidation using antioxidants needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation, which is the case for alpha-terpinene from tea tree oil (129). Alpha-Terpinene together with its analogue gamma-terpinene has been suggested as an agent for maintaining the oxidative stability of different matrices, such as food, cosmetics and medicaments (142-144). As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

Due to the complexity of scented products, which are mixtures of many different fragrance substances, there are at present no published data identifying the presence of individual hydroperoxides in cosmetic products containing the above fragrance terpenes. However, clinical studies show a clear connection between contact allergy to oxidised limonene and oxidised linalool, and contact allergy to other markers of fragrance contact allergy (130-135); see Table 5-3.

Table 5-1: Contact allergic reactions to the autoxidised fragrance substances limonene, linalool, caryophyllene, myrcene and linalyl acetate in consecutive dermatitis patients.

INCI name	CAS no	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
D-Limonene (ox.)	5989-27-5	5	18/703 (2.6%)	§ (130)
		3	28/1172 (1.6%)	
		2	3/362 (0.83%)	
D-Limonene (ox.)	5989-27-5	3	63/2273 (2.8%) variation between centres: 0.3-6.5%	§ (131)
D-Limonene (ox.)	5989-27-5, 5989-54-8, 138-86-3	3	49/1812 (2.3%)	§ (134)
L-Limonene (ox.)			36/1812 (2.0%)	
D – and/or L- Limonene (ox.)			63/2411 (2.6%)	
Linalool (ox.)	78-70-6	2	20/1511 (1.3%) variation between centres: 0.4-2.7%	§ (133)
Caryophyllene (ox.)	88-44-5	3.9	2/1511 (0.1%)	
Myrcene (ox.)	123-35-3	3	1/1511 (0.1%)	
Linalool (ox.)	78-70-6	2	14/1693 (0.83%)	§ (135)
		4	67/2075 (3.2%)	
		6	91/1725 (5.3%)	
		11	72/1004 (7.2%)	
Linalool (ox.)	78-70-6	3	11/483 (2.3%)	(145)
Linalyl acetate (ox.)	115-95-7	6	13/1217 (1.1%)	(141)

Notes: § Bicentric or multicentre studies.
(ox.) Oxidised.

Table 5-2: Contact allergic reactions to limonene, linalool, linalyl acetate and caryophyllene in consecutive dermatitis patient. Please observe that several studies have been performed using the test substances without reporting the autoxidation status but it has been intended to be low. For precise information see the original references.

INCI name	CAS number	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
Limonene	138-86-3	2	0/1200	(137)
Limonene			3/2396 (0.1%)	§ (74)
DL-Limonene			11/1241 (0.88%)	§ (43)
Limonene			0/320	(44)
DL-Limonene			3/2396 (0.1%)	§ (74)
Linalool	78-70-6	30	0/179	(139)
		20	3/1825 (0.2%)	§ (45)
		10	2/320 (0.6%)	(44)
		10	4/792 (0.5%)	(138)
		5 and 1	0/100	(70)
		10	7/2401 (0.3%)	§ (74)
Linalool, "stabilised" *	78-70-6	10	2/985 (0.2%)	§ (43)
		10	2/985 (0.2%)	§ (43)
Linalyl acetate	115-95-7	1, 5	0/100	(70)
		10	4/1855 (0.2%)	§ (67)
beta-Caryophyllene	87-44-5	5	10/1606 (0.6%)	§ (97)

Notes: § Bicentric or multicentre studies.

(ox.) Oxidised.

* Stabilised: according to the manufacturer contained additional substances aimed at limiting oxidation.

Table 5-3: Concomitant reactions to fragrance markers: Fragrance Mix I and II (FM I, FM II), *Myroxylon pereire* (MP) and to colophonium (coloph.) in the baseline series in patients with positive or negative patch test reactions to oxidised fragrance substances.

	Total number of pos. and/or neg. reactions	Pos. to FM I		Pos. to MP		Pos. to coloph.		Ref.
		n	%	n	%	n	%	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction	Pos.: 49	20	41	12	24	12	24	(130) *
	Neg.: 2751	223	8.1	142	5.2	131	4.8	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction ^a	Pos.: 60	22	37	11	18	13	22	(132) *
	Neg.: 729	141	19	71	9.7	58	8	

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Reactions to ox. D- limonene and/or ox. L- limonene ^a	Pos. to ox. D- limonene: 41	14	34	11	27	11	27	(134)*		
	Neg. to ox. D- limonene: 1771	113	6.4	91	5.1	62	3.5			
	Pos. to ox. L- limonene: 36	11	31	12	33	9	25			
	Neg. to ox. L- limonene: 1776	116	6.5	80	4.5	64	3.6			
Reactions to any of ox. linalool, myrcene, caryophyllene	Pos. to any of the tested ox. subst.: 31	12	39	6	31	12	39	(133)*		
	Neg. to any of the tested ox. subst: 1480	93	6	63	4	46	3			
		Pos. to FM I		Pos. to FM II		Pos. to MP		Pos. to coloph.		
		n	%	n	%	n	%	n	%	
Reactions to ox. linalool	Pos. at test conc. 4%: 30	8	26.7	5	16.7	10	33.3	5	16.7	(135)*
	Pos. at test conc. 6%: 55	12	21.8	8	14.5	11	20	8	14.5	
	Pos. at test conc. 11%: 72	14	19.4	9	12.5	14	19.4	9	12.5	
	Total pos. at any test conc: 75/1004	n.g.		n.g.		n.g		n.g.		
	Total neg. at any test conc: 929/1004	56	6.0	29	3.1	45	4.8	24	2.6	

Notes: * Bicentric or multicentre studies.

n.g. Not given.

(ox.) Oxidised.

5.2. Prohaptens

Compounds that are bioactivated in the skin and thereby form haptens are referred to as prohaptens. The human skin expresses enzyme systems that are able to metabolise xenobiotics (146), modifying their chemical structure to increase hydrophilicity and allow elimination from the body. Xenobiotic metabolism can be divided into two phases: phase I and phase II. Phase I transformations are known as activation or functionalisation reactions, which normally introduce or unmask hydrophilic functional groups. If the metabolites are sufficiently polar at this point they will be eliminated. However, many phase I products have to undergo subsequent phase II transformations, i.e. conjugation to make them sufficiently water soluble to be eliminated. Although the purpose of xenobiotic metabolism is detoxification, it can also convert relatively harmless compounds into reactive species. Cutaneous enzymes that catalyse phase I transformations include the cytochrome P450 mixed-function oxidase system, alcohol and aldehyde dehydrogenases, monoamine oxidases, flavin-containing monooxygenases and hydrolytic enzymes. Acyltransferases, glutathione S-transferases, UDP-glucuronosyltransferases and sulfotransferases are

examples of phase II enzymes that have been shown to be present in human skin (146). These enzymes are known to catalyse both activating and deactivating biotransformations (147), but the influence of the reactions on the allergenic activity of skin sensitisers has not been studied in detail.

Skin sensitising prohaptens can be recognised and grouped into chemical classes based on knowledge of xenobiotic bioactivation reactions, clinical observations and/or *in vivo* and *in vitro* studies of sensitisation potential and chemical reactivity. Few mechanistic investigations of prohaptens have so far been published. Investigations that are important for the bioactivation of fragrance substances are studies on alkenes, e.g. alpha- terpinene (148-150), the allylic primary alcohols geraniol (120) cinnamyl alcohol (151-155), eugenol and isoeugenol (156).

In order to be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests where intrinsic bioactivating systems are lacking. So far, no such predictive non-animal methods have been developed that take account of this.

When bioactivation occurs, the risk of cross-reactivity also needs to be considered. Cross-reactivity between certain aldehydes and their corresponding alcohols, e.g. cinnamal - cinnamyl alcohol and geranial - geraniol, due to the metabolic oxidation of the alcohols to the aldehydes in the skin is demonstrated (120, 151-155).

When using derivatives of a fragrance substance, it must be taken into account that the derivative could be metabolically transformed in the skin into the parent or cross-reacting compounds. A prominent example of such bioactivation is the hydrolysis of esters by esterases to the corresponding original alcohols. The metabolic product obtained can act as a hapten or a prohaptens in exactly the same way as the non-esterified parent compound.

Isoeugenol and its derivatives are an important example for this mechanism from which general conclusions may be drawn. As only the use of isoeugenol in fragranced products needs to be indicated on the ingredients list, the additional exposure to isoeugenol through its derivatives should also be taken into account. In a study it was shown that several EDP/EDT/aftershave lotions contained high levels of isoeugenyl acetate and isoeugenol methyl ether (Table 5-4) (157). Isoeugenyl acetate will be hydrolysed by esterases in the skin to generate isoeugenol. The situation may be similar for eugenyl acetate and geranyl acetate, which might be used in fragrance formulations instead of eugenol and geraniol, respectively. Moreover, such derivatives will contribute to exceeding any established 'acceptable dose/area level' of the parent compound, i.e., yield unduly high concentrations on the skin.

Table 5-4: Mean and median content of isoeugenol and its derivatives in the 29 perfume products.

Fragrance compound INCI Name	Products containing the fragrance		Content (ppm)			
	No.	%	Range	Mean	SD	Median
Isoeugenol	16	55	27-203	71	54	45
Isoeugenyl acetate	10	34	20-4689	985	1570	166
Isoeugenyl methyl ether	13	45	65-1755	360	442.3	222

5.3. Conclusions

- Many fragrance substances can act as prehaptenes or prohaptenes, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation.
- Fragrance substances of clinical importance known to be prehaptenes and to form sensitising compounds by air oxidation include limonene, linalool, and linalyl acetate.
- Fragrance substances of clinical importance known to be prohaptenes and to form sensitising compounds by metabolic transformation include cinnamyl alcohol, eugenol, isoeugenol and isoeugenol acetate.
- Fragrance substances of clinical importance with published data known to be both prehaptenes and prohaptenes and to form sensitising compounds by air oxidation (prehaptenes) and by metabolic transformation include geraniol and alpha-terpinene.
- A fragrance substance that sensitises without activation, but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is, as one example, geranial (one isomer of citral).
- In the case of prehaptenes, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product, and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

It should be noted that the possibility to reduce the sensitisation potency by preventing air oxidation is also important for a direct acting hapten or prohapten, if a further activation by air oxidation to more allergenic compounds has been shown.

- In the case of prohaptenes, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geranial (citral) and between cinnamyl alcohol and cinnamal.

Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenol acetate, eugenyl acetate and geranyl acetate all of which are known to be used as fragrance ingredients.

- Further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is clearly needed to increase the safety for the consumer. Compounds suspected to act as prehaptenes and/or prohaptenes should be considered as allergens, unless it could be demonstrated that they do not become activated by one of the described pathways.

6. Retrieval of evidence and classification of fragrance substances

For a systematic review, a structured approach of identifying, grading and aggregating available information should be used. Regarding the classification of substances as allergens, a number of approaches have been suggested (158-160). The categorisation of skin sensitisers according to sensitising potency has also been proposed (161, 162). For this opinion, these discussions were extended to reconcile different perspectives and to arrive at a strategy that is both consistent and applicable in practice.

By default, positive human evidence (clinical data) overrides negative results obtained in animals. This implies that the observation of a sufficient number of positive clinical cases is more important than potency information derived from animal experiments (LLNA).

Cosmetovigilance information based on consumer complaints only is of limited value in the evaluation of sensitisation risk associated with cosmetic allergens, including fragrances, as it does not identify specific causative substances, and likely to severely under-estimate the frequency of contact dermatitis. An exception is the combination with qualified diagnostic work-up, as in the French REVIDAL/GERDA system (299); however, such data are generally published, thus publicly available, and considered in the present opinion.

6.1. Retrieval of evidence

A systematic search strategy was employed for the retrieval of clinical data, as outlined below. Experimental data are often not published hence the exact definition of the scope considered for the review is necessary and is given below. Additional LLNA data were reviewed, if identified by the search strategy, e.g. in chapter 8.1.2 and, as “additional information”, in Annex I of this opinion. This supplemental evidence was, however, not considered for the final categorisation in Table 13-2.

6.1.1. Search strategy for clinical data

Method of literature search:

1. Manual search of the issues of the journal “Contact Dermatitis” (for the 26 “annex substances”, which were re-evaluated in the present opinion, starting 1999) up to October 2010, identifying all studies with fragrance substances.
2. PubMed search of CAS numbers identified in the previous opinion, reviews and already identified clinical studies, respectively, and manual screening of identified publications (narrowed for the last 10 years for the 26 “annex substances”), if necessary narrowing the search results by adding “dermatitis” or “allergy”. For example, for citral: 5392-40-5 AND (dermatitis or allergy), translated into
 "5392-40-5"[EC/RN Number] AND
 (
 ("dermatitis"[MeSH Terms] OR "dermatitis"[All Fields])
 OR
 ("hypersensitivity"[MeSH Terms] OR "hypersensitivity"[All Fields] OR "allergy"[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields])
)
3. Manual search of all RIFM reviews published in supplement issues of “Food and Chemical Toxicology²” in the past 20 years. In case of the least evidence on human sensitisation the substances were preliminarily selected and further research initiated.

² Food and Chemical Toxicology, Elsevier Ltd. <http://www.sciencedirect.com/science/journal/02786915>.

4. Consideration of the most important (“top 100”) fragrance compounds in terms of volumes used (disregarding functional additives such as solvents) as supplied by the International Fragrance Association IFRA (personal communication 2010).
5. Consideration of fragrance compounds ranking 101 to 200 on the list of use volumes, if they were self-classified by manufacturers as skin sensitisers (R 43).

For the present systematic overview of available clinical data, only original studies were considered, as only these provide direct evidence, while other reviews, partly being based on the same original reports, only served to identify additional literature. In contrast, selected reviews, guidelines and similar publications were used as basis for methodological approaches (e.g., in section 11).

6.1.2. Collection of experimental (LLNA) data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, industry submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report. A summary is given in chapter 8 and full data are given in Annex II. EC3 values on some additional fragrance substances in two published reviews (165, 166) have also been considered. Additional EC3 values may be available in the scientific literature and there may also be other unpublished data.

6.2. Grading of evidence

Assembled evidence has to be graded in two steps: (i) the quality of each single study, and (ii) the strength of evidence underlying the eventual classification as an allergen. Generally, studies (published or not) which are eligible for consideration will contribute to the final overall judgement to different degrees.

- Positive human data, if sufficiently demonstrated (point (i) below), will always over rule experimental (animal), *in vitro* or *in silico* data of similar internal validity, as they provide direct evidence on allergenicity in humans.
- Small study groups will contribute less precise information than larger studies of otherwise similar quality. As a minimum requirement, the size of the study groups and the numbers of events must be stated in the reports.

The following subsections will address special aspects of clinical and experimental studies, respectively.

6.2.1. Quality of a clinical study

Two major types of clinical studies must be distinguished because they provide a different scope of information:

- (i) Case reports or small case series, focusing on patients with positive (test) reactions to the target substance, sometimes including a set of non-exposed, possibly non-diseased “control patients”; these should present a concise summary of all relevant aspects of the patient's history, diagnostic procedures and possibly further outcomes.
- (ii) Clinical series in which results of a group of patients patch tested with the target substance, often combined with other substances, are presented. In the latter type of report, usually only a minority of patients tested show a positive reaction to the test substance. This implies that the majority of patients can be used to illustrate the proportion of irritant, doubtful and negative reactions. The degree of detail on the patients' histories is usually limited in such studies, compared to case reports.

Some of the basic quality criteria in clinical patch testing which should be considered are:

- Adherence to international patch test guidelines (32, 96).
- Material(s) tested should be characterised.
- Total number of patients tested must be given.
- Patient selection should be described.
- Relevance may be demonstrated either on a case-by-case basis, following pertinent guidelines, or in terms of a significant epidemiological association between sensitisation and exposure or valid markers of exposure.

Concerning relevance, it must be noted that while clinical relevance can provide important information (see 4.4.1), it is ideally based on comprehensive knowledge of prior exposures. Since the implementation of labelling 26 fragrances, previous exposure to these can often be ascertained in the assessment of relevance of a positive patch test reaction (44). However, exposure to substances not listed on a product ingredient label is obscure, except in very rare cases where elaborate diagnostics and chemical analyses are feasible (e.g. (167)). Thus, a lack of information on relevance (reported in studies) does not invalidate the impact of diagnosed contact sensitisation.

6.2.2. Quality of an experimental study

International guidelines such as the pertinent OECD guidelines for testing sensitisation have been developed and adopted. Experimental studies following these guidelines are considered as valid. However, a vast number of non-guideline studies are available and should be assessed on a case-by-case basis.

6.2.3. Quality of “other” evidence

Supporting evidence besides human and animal (experimental) data comprises *in vitro* test systems, *in chemico* experiments and structure activity relationships (SARs).

SAR analysis has at present no formal regulatory validation for skin sensitisation, nevertheless it may provide useful indicative information on sensitising potential when no or limited clinical or animal data are available.

SAR studies must consider a possible formation of haptens (allergens) from compounds able to act as prehapten by, e.g. autoxidation outside the body as well as metabolic activation in the skin of compounds able to act as prohapten (122, 168).

6.3. Aggregating evidence for a final conclusion

The criteria listed below are followed as a flow chart to arrive at a conclusion. This implies that if classification into one category is achieved, subsequent categories need not be considered. Based on the above criteria, fragrance substances were selected to be included in the present opinion if classified in one of the categories defined below.

6.3.1. Established contact allergen in humans

To qualify as an *established contact allergen*, the SCCS considers that *at least one* of the following two criteria must be met:

- At least two clinical series fulfilling the quality criteria from two different centres with cases of sensitisation, or at least three separate clinical series from different centres if a study, or studies, do not meet all quality criteria. (→ *sufficient human evidence present*)
or
- Case reports from at least two independent centres describing more than two

patients altogether in whom clinically relevant contact sensitisation had unequivocally been proven (→ *sufficient human evidence present*)
or

- At least one clinical series fulfilling the quality criteria, together with at least one case report of clinically relevant contact sensitisation (→ *sufficient human evidence present*);
or
- Experimentally induced sensitisation (e.g. unequivocally positive human maximisation tests/repeated insult patch test)³ (→ *sufficient human evidence present*).

6.3.2. Established contact allergen in animals

To qualify as an *established contact allergen*, the following criterion must be met:

- At least one positive animal study carried out according to accepted guidelines, providing evidence of a sensitisation potential (→ *sufficient animal evidence present*).

6.3.3. Likely contact allergen, if human, animal and other evidence is considered

To qualify as an *likely contact allergen*, if classification as “established ...” is not applicable, *at least two* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

6.3.4. Possible contact allergen, if human, animal and other evidence is considered

To qualify as a *possible contact allergen*, if classification as “established ...” or as “likely ...” contact allergen is not applicable, *at least one* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

³ It should be noted that the SCCS considers such tests unethical (169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:).

6.4. Conclusions

The present opinion includes (i) a well-defined search strategy for retrieving pertinent evidence; (ii) a definition of criteria used to evaluate available evidence; and, finally (iii) a set of rules to categorise the substances with regard to the relevant toxicological endpoint, i.e. sensitisation in man, based on the evidence.

7. Reported fragrance allergens from the clinical perspective

In this chapter, clinical evidence regarding sensitisation to individual fragrance chemicals and to natural extracts (essential oils) is tabulated. In this report “single chemicals” refers to chemicals of natural or synthetic origin whose chemical identity is fully known. The term “natural extracts” refers to plant or animal derived mixtures of natural chemicals, for example lavender oil, whose composition may be variable and may or may not have been fully or partly established. Full information, including possible synonyms, structural formulas (in the case of single chemicals only), a short summary of available evidence and further information, e.g. on regulatory status, is presented in Annex I.

7.1. Tabular summary of evaluated individual fragrance chemicals

Regarding nomenclature, INCI names are used wherever possible. If an INCI name is not available, the perfuming name as listed by CosIng is used. Detailed information on the publications identified and considered for this report can be found in Annex I. Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I, these substances have not been further evaluated and are thus not included in this chapter.

In this section, a tabular overview on the classification of substances considered is presented in four tables listing:

1. Established contact allergens in humans (→ *sufficient human evidence present*).
2. Substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans” (→ *limited human evidence present*).
3. Substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.
4. Substances eligible for inclusion (see beginning of chapter 6) for which no human data are available.

A critical point in understanding this scheme is that there is publication bias in reporting allergens. This is due to the fact that once a substance has been reported and accepted as a contact allergen in humans, further reports are less likely to be published unless they are part of an epidemiological survey or when there is a novel source of exposure. Moreover, the number of patients displaying positive test reactions obviously not only depends on the underlying prevalence of sensitisation, but also on how often a substance is patch tested. This implies that inclusion of an allergen or allergen mixture in the baseline patch test series (as for Fragrance Mix I and II, *Myroxylon pereirae* and HICC, and partly also other substances/mixtures) will yield the maximum possible number of cases. In contrast, patch testing in “special” series, e.g. as a break-down of single constituents of the respective mix in case of a positive reaction to the latter, or with application only in the case of strongly suspected fragrance intolerance, will mostly result in higher relative numbers than testing the same compound consecutively, but also in lower absolute numbers.

In Table 7-1, the single substances are listed with a semi-quantification of their impact which were categorised as established contact allergens in humans according to the criteria given in chapter 6.3.

Established contact allergens in humans, according to the criteria outlined in chapter 6.3.1, were categorised according to the number of patients reacting positively and to the number of patients tested, based on the publications considered (see annex I for references). The following categories were used:

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+	Up to 10 positive test reactions reported
++	11 to 100
+++	101 to 1000
++++	> 1000

If a test allergen has been tested in less than 1,000 patients, “r.t.” (rarely tested) is added in the following tables. For this categorisation, absolute numbers of cases of sensitisation, and not the relative frequency of positive patch tests, were used, because relative frequencies depend heavily on the selection of patients for patch testing. Thereby, an important allergen tested routinely, in the baseline series, may yield 1 to 2% positive reactions (usually in several thousand patients), while an allergen tested in a selective fashion (in much fewer patients) may yield an even higher relative frequency. Moreover, case reports/series cannot be interpreted in terms of relative frequencies. The calculation of absolute numbers was based on all available literature, as detailed in the annex I to this opinion, i.e., regarding the 26 substances already listed in Annex III to the Cosmetics Directive includes data already evaluated in the previous opinion.

Table 7-1: Established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ACETYLCEBRENE	32388-55-9	+
AMYL CINNAMAL	122-40-7	++
AMYL CINNAMYL ALCOHOL	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISYL ALCOHOL	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL	100-51-6	++
BENZYL BENZOATE	120-51-4	++
BENZYL CINNAMATE	103-41-3	++
BENZYL SALICYLATE	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL (Lilial®)	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL	104-55-2	+++
CINNAMYL ALCOHOL	104-54-1	+++
CITRAL	5392-40-5	+++
CITRONELLOL	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN	91-64-5	+++
(DAMASCENONE)	23696-85-7	+ (r.t.)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ROSE KETONE-4		
alpha-DAMASCONE (TMCHB) [#]	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE [#]	23726-92-3	+
delta-DAMASCONE [#]	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL	97-53-0	+++
FARNESOL	4602-84-0	+++
GERANIOL	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE (HICC)	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL	107-75-5	+++
ISOEUGENOL	97-54-1	+++
alpha-ISOMETHYL IONONE	127-51-5	++
(DL)-LIMONENE	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN [#]	92-48-8	++ (photo-allergy)
METHYL 2-OCTYNOATE	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	++
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

Those substances which were categorised as +++ or more, i.e. those with the most reported cases, were also the top ranking substances in large series of patients tested with the 26 labelled fragrance ingredients ((44, 74) and additionally (170)). Geraniol is an exception, as it was all negative in the Danish study (170), but was still among the top ten in the Dutch and German studies (44, 74), with prevalences of 0.5%-0.6% positives. Geraniol has, in addition, caused many cases of contact allergy in other areas of Europe (49).

The use of absolute numbers allows the pooling of studies with different selection criteria. Limonene and linalool were not tested in their oxidized forms in the three studies (44, 74, 170) and would not have been identified, if only these publications had been the basis of assessment.

It should be noted that oxidised fragrance terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available (see also chapter 5).

Table 7-2 lists those substances which gave rise to a few reported cases of contact sensitisation only, or where results have been reported from just one clinical department. Thus, the level of evidence concerning human data must be regarded as *limited*, according to the definitions given in chapter 6.3.

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Table 7-2: Fragrance substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
AMBRETTOLIDE	7779-50-2	3.4% positive reactions in 178 patients	(171)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (172))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(139)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(173)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(171)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(174)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(175).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(171)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(173)
ETHYL VANILLIN	121-32-4	1 occupational case	(176)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(97)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(173)
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, possibly “excited back syndrome” 0 / 95 in another study with <= 1/10 of above test conc.	(139) (70)
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(171)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(177)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(173)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(178)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(179)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(97) (70)
METHYLIONANTHEME	55599-63-8	1 case	(180)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(97)
METHYL OCTINE CARBONATE	111-80-8	1 case	(181)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(133)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(182)
NEROL	106-25-2	6.0% positive	(173)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(183)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly "excited back syndrome"	(139)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(139) (70)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(178) (184)
PHENYLPROPANOL	122-97-4	2 / 218	(173)
PHYTOL	150-86-7	1 case in human max. test	(185)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(186)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(187)

For a number of substances negative patch tests results were obtained, usually in rather small patient samples (max. 313 patients). For some of these substances exposure is substantial, according to data submitted from IFRA. It should be noted that a negative result does not rule out a notable sensitisation prevalence, as the study size has to be larger than, e.g. n=298 to yield a 95% CI which excludes a prevalence of 1% and larger than n=597 to exclude a prevalence of 0.5%.

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Table 7-3: Fragrance substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Results / Comment	Ref.
6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	21145-77-7	0 / 313 consecutive patients in 2 centres	(70)
AMYL CYCLOPENTANONE	4819-67-4	0 / 178	(171)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(70)
2-TERT-BUTYL CYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(70)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(70)
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(171)
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(70)
HEXYL SALICYLATE	6259-76-3	0 / 218 "top 100" substance and classified as R43	(173)
HIBISCOLIDE	6707-60-4	0 / 178	(171)
alpha-IONONE	127-41-3	0 / 205	(70)
beta-IONONE	79-77-6	0 / 205 "top 100" substance	(70)
ISOBORNYL ACETATE	125-12-2	0 / 107 "top 100" substance	(70)
METHYL ANTHRANILATE	134-20-3	0 / 91 "top 100" substance	(188)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 "top 100" substance	(70)
OXALIDE	1725-01-5	0 / 178	(171)
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 "top 100" substance	(70)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(139)
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(171)

For yet another subset of substances, no human data were publicly available. However, exposure to these substances is important as they are used in high volumes (this being the sole criterion for inclusion in this list) and, therefore their hazard with regard to contact sensitisation should be examined.

Table 7-4: Fragrance substances lacking human data and used in high volumes according to industry information.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
ANISALDEHYDE	123-11-5
BENZYL ACETONE	2550-26-7
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0
CITRONELLYL NITRILE	51566-62-2
CYCLAMEN ALDEHYDE	103-95-7
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0
DECANAL	112-31-2
DIHYDROMYRCENOL	18479-58-8
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	68039-49-6
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6
DIPHENYL ETHER	101-84-8
ETHYL 2-METHYLBUTYRATE	7452-79-1
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6
ETHYLENE BRASSYLATE	105-95-3
EUCALYPTOL	470-82-6
GERANYL ACETATE	105-87-3
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0
HEXYL ACETATE	142-92-7
IONONE isomeric mixture	8013-90-9
ISOAMYL ACETATE	123-92-2
ISOBERGAMATE [#]	68683-20-5
Longifolene	475-20-7
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0
METHYLBENZYL ACETATE	93-92-5
METHYL DECENOL	81782-77-6
METHYL beta-NAPHTHYL ETHER	93-04-9
METHYLUNDECANAL	110-41-8
OXACYCLOHEXADECENONE	34902-57-3
PENTADECALACTONE	106-02-5
PHENETHYL ACETATE	103-45-7
PHENOXYETHYL ISOBUTYRATE	103-60-6
PHENYLISOHEXANOL	55066-48-3
Tetrahydrolinalool	78-69-3
TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	63500-71-0

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
TRICHLOROMETHYL PHENYL CARBINYL ACETATE	90-17-5
TRICYCLODECENYL PROPIONATE	17511-60-3
TRIMETHYLHEXYL ACETATE	58430-94-7
gamma-UNDECALACTONE	104-67-6
VERDYL ACETATE	2500-83-6/ 5413-60-5

7.2. Tabular summary of evaluated natural extracts/essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic the demand for perfumes based on natural materials is considerable (189).

The three main methods used to concentrate plant fragrance substances (190); distillation, mechanical separation ("pressing"), and solvent extraction, yield very different extracts. Essential oils are obtained by water steam, water, ethanol, or water/ethanol distillation. Essence oils are essential oils that separate from the aqueous phase in the distillation receiver during the distillative concentration of fruit, usually citrus, juices. Citrus peel oils, apart from distilled lime oil, are prepared in a special way by pressing the peel to release mostly volatile substances from the pericarp in small oil glands, mostly highly volatile terpene hydrocarbons. However, they also contain small amounts of non-volatile compounds such as dyes, waxes and furocoumarins. The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concretes, an extract of fresh plant material with nonpolar solvents, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolutes, which are prepared by taking up concretes in ethanol; compounds that precipitate on cooling are removed by filtration, yielding a wax-free residue called absolute. Resinoids, used for their fixative properties, are prepared by extracting plant exudates with alcohols or nonpolar solvents. The products are usually highly viscous and thus sometimes diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are concentrates prepared from spices by solvent extraction (189).

An ISO norm exists regarding the nomenclature of aromatic natural raw materials (ISO/DIS 9235 Aromatic raw materials - vocabulary; International Standardisation Organisation, Geneva, Switzerland). This nomenclature has been considered in Annex I, whereas in the present opinion, nomenclature is according to the CosIng database. Concerning extraction processes for many essential oils, ISO standards exist; for detailed information see Annex I to this opinion.

Regarding clinical data in terms of contact allergy to essential oils and natural extracts, the main focus is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g. masseurs, physiotherapists (191, 192), aromatherapists (193-197), beauticians performing massages (198). For further details, e.g. PT results with various essential oils, see Annex I.

In this section, a tabular overview on the classification of substances considered is presented in three tables listing:

1. Extracts identified as *established contact allergens* in humans(→ *sufficient human evidence present*).

2. Extracts with positive human data, which are, however, not sufficient to categorise as *established contact allergen* in humans (→ *limited human evidence present*).
3. Extracts with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

In Table 7-5, essential oils with sufficient human evidence to categorise these as *established contact allergens* in humans are presented.

Table 7-5: Natural extracts classified as established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion, including variants of botanical nomenclature.

INCI name (or, if none exists, ^{\$} perfuming name according to CosIng ⁴) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment: see text
CANANGA ODORATA and <i>Ylang-ylang oil</i>	83863-30-3; 8006-81-3	+++
CEDRUS ATLANTICA BARK OIL	92201-55-3; 8000-27-9	++
CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
CITRUS AURANTIUM AMARA FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
CITRUS BERGAMIA PEEL OIL EXPRESSED ^{\$}	89957-91-5	+ (r.t.)
CITRUS LIMONUM PEEL OIL EXPRESSED [#]	84929-31-7	++
CITRUS SINENSIS (syn.: <i>AURANTIUM DULCIS</i>) PEEL OIL EXPRESSED ^{\$}	97766-30-8; 8028-48-6	++
CYMOPOGON CITRATUS / SCHOENANTHUS OILS	89998-14-1; 8007-02-1; 89998-16-3	++
EUCALYPTUS SPP. LEAF OIL ^{\$}	92502-70-0; 8000-48-4	++
EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL	8000-34-8	+++
EVERNIA FURFURACEA EXTRACT ⁵ (Tree moss)	90028-67-4	+++
EVERNIA PRUNASTRI EXTRACT (Oak moss) [#]	90028-68-5	+++
JASMINUM GRANDIFLORUM / OFFICINALE	84776-64-7; 90045-94-6; 8022-96-6	+++
JUNIPERUS VIRGINIANA	8000-27-9; 85085-41-2	++
LAURUS NOBILIS	8002-41-3; 8007-48-5; 84603-73-6	++
LAVANDULA HYBRIDA	91722-69-9	+ (r.t.)
LAVANDULA OFFICINALIS ^{\$}	84776-65-8	++
MENTHA PIPERITA	8006-90-4; 84082-70-2	++
MENTHA SPICATA	84696-51-5	++
MYROXYLON PEREIRAE (Balsam of Peru) [#]	8007-00-9	++++

⁴ <http://ec.europa.eu/consumers/cosmetics/cosing/>

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INCI name (or, if none exists, ^{\$} perfuming name according to CosIng ⁴) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment: see text
NARCISSUS SPP.	<i>diverse</i>	++
PELARGONIUM GRAVEOLENS	90082-51-2; 8000-46-2	++
PINUS MUGO/ PUMILA [#]	90082-72-7 / 97676-05-6	++
POGOSTEMON CABLIN	8014-09-3; 84238-39-1	++
ROSE FLOWER OIL (ROSA SPP.)	<i>Diverse</i>	++
SANTALUM ALBUM	84787-70-2; 8006-87-9	+++
TURPENTINE (oil) [#]	8006-64-2; 9005-90-7; 8052-14-0	++++
VERBENA absolute [#]	8024-12-2	++

Notes: r.t. Rarely tested.

Table 7-6 lists a number of essential oils, mostly tested in just one clinical department, and thus, or for other reasons, not satisfying the criteria for being categorised as *established contact allergen* in humans (i.e. *limited human evidence present*).

Table 7-6: Natural extracts with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment	Ref.
ACORUS CALAMUS ROOT OIL	84775-39-3	n=7 pos. reactions to “calamus”	(199)
CEDRUS DEODARA WOOD OIL	91771-47-0	Rudzki 1976/1986 found 3 / 3 positive reactions	(199, 200).
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Several cases in 2 series from 1 centre	(199, 200)
CITRUS TANGERINA ...	223748-44-5	1 case	(201)
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Several cases in 2 series from 1 centre	(199, 200)
ILLICIUM VERUM FRUIT OIL	84650-59-9	Cases of active sensitisation; 34% consecutive patients pos. to 1%	(202)

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INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment	Ref.
LAVANDULA SPICA	97722-12-8	Several cases in 2 series from 1 centre	(199, 200)
LITSEA CUBEBA	90063-59-5	Several cases in 2 series from 1 centre	(199, 200)
PELARGONIUM ROSEUM	90082-55-6	2.1% pos. of 1483 patients	(203)
ROSMARINUS OFFICINALIS	84604-14-8	3 cases in 2 series from 1 centre	(199, 200)
SALVIA spp.	Diverse	Several cases in 2 series from 1 centre	(199, 200)
TAGETES PATULA	91722-29-1	1 case (aromatherapist)	(193)
THYMUS spp.	84929-51-1	4 / 84 pos	(199)
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	1 / 200 and 9 / 86 pos.	(199, 200)

The final table is an indicative list of natural extracts which lack published human data, but which are of interest: (i) as high-volume exposure; (ii) due to published positive animal experiments; or (iii) because they contain well-known (established) contact allergens.

Table 7-7: Indicative list illustrating natural extracts containing established human allergens or having R43-label or positive LLNA, lacking published human data.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment
CITRUS PARADISI PEEL OIL	8016-20-4	high volume substance, classified as R43
CYMBOPOGON MARTINI HERB EXTRACT	84649-81-0	Pos. LLNA study by RIFM: EC3 value 9.6% (204).
MENTHA ARVENSIS	68917-18-0	high volume, classified as R43
OCIMUM BASILICUM	84775-71-3	Pos. LLNA study by RIFM: EC3 value < 2.5% (204).
PIMENTA RACEMOSA	85085-61-6	Contains, among other substances, the established contact allergen eugenol (42-56%)
SANTALUM SPICATA	8024-35-9	Contains, among other substances, the established contact allergens santalols (75%) and farnesol (10%)

7.3. Conclusions

- According to the criteria described in chapter 6.3 a total of 54 individual chemicals and 28 natural extracts (essential oils) can be categorised as *established contact allergens* in humans, including all currently regulated substances.
- Of the 54 individual chemicals which are established contact allergens in humans, 12 are considered to be of special concern due to the high number of reported cases, (> 100, i.e. category +++ or ++++ in Table 7-1). These are further considered in chapter 5 (limonene and linalool) and the remainder in chapter 11. In particular one ingredient stands out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1,500 reported cases since the 1999 opinion (see also chapter 4.3.1, chapter 11.3 and Annex I).
- For an additional 33 individual chemicals (Table 7-2) and 14 natural extracts (Table 7-6), positive patch test results have been reported. However, they do not qualify for the above category, i.e. only *limited human evidence* is present.
- For a number of fragrance substances (n=18, Table 7-3) patch testing did not yield positive results. However, numbers of patients tested are generally too small to rule out the existence of clinical contact sensitisation with sufficient confidence. No clinical evidence has been identified for 39 individual chemicals that have been reported to be frequently used (Table 7-4).
- For the substances (and, if possible, also for the main constituents of the natural mixtures) with limited or no human evidence, additional animal data and/or SAR considerations are taken into account. Aggregated data for these substances are presented in chapter 13.

8. Animal data

8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test⁶ and the local lymph node assay (LLNA)⁷. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH⁸. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling⁹, substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol “Xi” and the risk phrase “R43: May cause sensitisation by skin contact”; or, according to the recent regulation on classification, labelling and packaging (CLP¹⁰) “H317: May cause an allergic skin reaction”.

As yet, there is no officially validated *in vitro* test method for skin sensitisation. Therefore, for cosmetic ingredients the LLNA, the GPMT and the Buehler test have also been used in risk assessment for regulatory purposes.

Positive results from the OECD guideline animal tests mentioned above which are sufficient to classify a substance as a skin sensitizer (R43) are:

- GPMT; at least 30% of the animals have a positive response.
- Buehler test; at least 15% of the animals have a positive response.
- LLNA; at least a 3-fold increase in lymph node cell proliferative activity is induced, compared to vehicle-treated controls (stimulation index $SI \geq 3$). For positive LLNAs, an EC3 value is calculated which gives the estimated concentration of a chemical necessary to give a 3-fold increase in proliferative activity compared to vehicle-treated controls.

Further categorisation of substances classified with R43 into three groups according to allergen potency (extreme, strong and moderate) has been proposed by a European Commission expert group on skin sensitisation (161, 205), and proposed also in the ECHA guidance document on application of the CLP criteria (162). Such categorisation is based on EC3 values in the LLNA, on intradermal induction concentration in the GPMT, and topical induction concentration in the Buehler test. The potency categories and their default concentration values based on EC3 values in the LLNA as defined in (161): extreme sensitizer (EC3 value ≤ 0.2); strong sensitizer (EC3 $> 0.2 - \leq 2$); and moderate sensitizer (EC3 value > 2). When LLNA EC3 values are available from more than one study, the lowest value should normally be used. Where multiple animal data sets lead to different categorisation of the same substance, the higher potency category should apply (161, 205).

The potency categorisation of substances based on the LLNA is applied by the SCCP in risk assessment of cosmetic ingredients, particularly hair dye substances (206).

⁶ OECD Guideline for testing of chemicals. Guideline 406: Skin Sensitisation. OECD, Adopted 12 May 1981, updated 17th July 1992.

⁷ OECD Guideline for testing of chemicals. Guideline 429: Skin Sensitisation: Local Lymph Node Assay. OECD, Adopted 22 July 2010.

⁸ Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

⁹ Directives 67/548/EEC and 1999/45/EC.

¹⁰ Regulation No. 1272/2008.

8.1.1. LLNA data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, IFRA submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report.

Table 8-1 displays the EC3 values for fragrance substances in the report submitted by industry (164). EC3 values for some additional fragrance substances in two published reviews (165, 166) have also been included in Table 8-1. Table 8-2 presents LLNA results for oxidised substances. Full data are given in Annex II. Table 8-3 summarises the distribution of fragrance substances, by potency category, according to EC3 values.

Additional EC3 values may be available in the scientific literature. Many more animal experiments may have been performed, but have not been published.

Table 8-1: Summary of local lymph node assay (LLNA) data on 66 fragrance substances, based on a report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009 (164)) and in published reviews by Gerberick et al. 2005 (165) and Kern et al. 2010 (166), respectively. EC3 values (% and M) are given. The order of substances is by decreasing sensitisation potency as assessed by LLNA EC3 values (lowest EC3 value indicating highest potency).

Substance	CAS no.	EC3 value		Reference
		%	M	
Hexyl salicylate	6259-76-3	0.18	0.008	(164, 166)
Cinnamal	104-55-2	0.2	0.015	(164)
Methyl 2-octynoate	111-12-6	<0.5	<0.032	(164, 166)
Isoeugenol	97-54-1	0.54	0.033	(164)
Citral	5392-40-5	1.2	0.079	(164)
2-Hexylidene cyclopentanone	17373-89-6	2.4	0.14	(164)
Methyl octine carbonate	111-80-8	2.5	0.15	(164)
Peru balsam absolute	8007-00-9	2.5	n/a	(164)
trans-2-Hexenal	6728-26-3	2.6	0.26	(164)
Benzyl Salicylate	118-58-1	2.9	0.23	(164, 166)
Butylphenyl methylpropional (BMHCA)	80-54-6	2.9	0.14	(164)
Phenylacetaldehyde	122-78-1	3	0.25	(164, 165)
Allyl phenoxyacetate	7493-74-5	3.1	0.16	(164)
Benzylideneacetone	122-57-6	3.7	0.25	(165)
3-Propylidenephthalide	17369-59-4	3.7	0.21	(164, 165)
<i>Evernia prunastri</i> extract oak moss	90028-68-5	3.9	n/a	(164)
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	4	n/a	(164)
Farnesol	4602-84-0	4.1	0.18	(164)
p-t-Butyl-dihydrocinnamaldehyde	18127-01-0	4.3	0.23	(164)
α-Methyl cinnamic aldehyde	101-39-3	4.5	0.31	(164, 165)

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Substance	CAS no.	EC3 value		Reference
		%	M	
Eugenol	97-53-0	5.3	0.32	(164)
Hexyl cinnamal	101-86-0	5.3	0.25	(164)
Dihydrocoumarin	119-84-6	5.6	0.38	(165)
Geraniol	106-24-1	5.6	0.36	(164)
Carvone	6485-40-1	5.7	0.38	(164)
Diethyl maleate	141-05-9	5.8	0.34	(165)
2-Methoxy-4-methylphenol	93-51-6	5.8	0.42	(164, 165)
Anise alcohol	105-13-5	5.9	0.43	(164, 166)
Jasmine absolute (<i>Grandiflorum</i>)	8022-96-6	5.9	N/a	(164)
Dibenzyl ether	103-50-4	6.3	0.32	(164)
<i>Cananga odorata</i> leaf/flower oil ylang ylang "extra"	8006-81-3	6.8	N/a	(164)
Isocyclocitral	1335-66-6	7.3	0.48	(164)
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	7.5	0.50	(165)
Amyl cinnamal	122-40-7	7.6	0.38	(164)
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	8.1	0.54	(164, 165)
p-Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	9.5	0.46	(164)
d-Limonene*	5989-27-5	<10	<0.73	(164)
Methylundecanal	110-41-8	10	0.54	(165)
Acetylcedrene	32388-55-9	13.9	0.57	(166)
Methylenedioxyphenyl methylpropanal	1205-17-0	16.4	0.85	(164, 166)
Benzyl benzoate	120-51-4	17	0.80	(165)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	17.1	0.81	(164, 165)
Benzyl cinnamate	103-41-3	18.4	0.77	(164, 166)
Hydroxycitronellal	107-75-5	19.3	1.12	(164)
Cinnamyl alcohol	104-54-1	21	1.57	(165)
α -iso-Methylionone	127-51-5	21.8	1.06	(164, 166)
Cyklamen aldehyde	103-95-7	22	1.64	(165)
4-Methoxy- α -methyl benzenpropanal	5462-06-6	23.6	1.32	(164)
Amyl cinnamyl alcohol	101-85-9	~25	~1.22	(164, 166)
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	25.1	1.07	(164)
Ethyl acrylate	140-88-5	28	2.8	(165)
Linalool*	78-70-6	30	1.94	(165)
Trimethylbenzenepropanol Majantol	103694-68-4	30	~1.68	(164)
Jasminum Sambac Flower CERA/Extract/Water	91770-14-8	35.4	N/a	(164)

Substance	CAS no.	EC3 value		Reference
		%	M	
Citronellol	106-22-9	43.5	2.78	(164, 166)
No EC3 value was established; higher concentrations should also have been tested				
6-Methyl-3,5-heptadien-2-one	1604-28-0	>5	>0.40	(164)
<i>Camellia sinensis</i> leaf tea leaf absolute	84650-60-2	>5	N/a	(164)
Cinnamyl nitrile	1885-38-7	>10	>0.77	(164)
Menthadiene-7-methyl formate	68683-20-5	>10	>0.51	(164)
<i>Evernia furfuracea</i> extract tree moss absolute	90028-67-4	>20	N/a	(164)
Isocyclogeraniol	68527-77-5	>25	>1.62	(164)
1-Octen-3-yl acetate	2442-10-6	>30	>1.76	(164)
Benzyl alcohol	100-51-6	>50	>4.62	(164)
Coumarin	91-64-5	>50	>3.42	(164)
Vanillin	121-33-5	>50	>3.3	(164)
No EC3 value calculated				
Benzaldehyde	100-52-7	-		(165)

Notes: * Material with low levels of oxidation according to (164)

n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

8.1.2. LLNA data on oxidised fragrance substances

For fragrance substances that can autoxidise upon air exposure, it is also important to investigate the sensitisation potency after air exposure. The oxidised compounds are clinically relevant as they represent what the consumers could come in contact with from perfumes and fragranced products. In Table 8-2 the LLNA data for some of the most commonly used fragrance substances, pure and after autoxidation, are presented. The EC3 values obtained for the pure substances are 5-10 times higher compared to those obtained for the same substances after air exposure. The experimental air exposure simulated air exposure that can take place during normal handling and storage. In the production process, some perfumes are “matured” aerobically, stirring included. During this process, some fragrance substances may be oxidised. It should be noted that, although only a few substances capable of oxidation have so far been investigated, structural alerts indicating possible autoxidation are common among the fragrance substances listed in this document (see chapter 9). It is important to further investigate this issue for increased understanding of the associated risk.

Table 8-2: Local lymph node assay (LLNA) data on four fragrance substances and one essential oil before and after air exposure, comparing the sensitisation potency of the pure (not oxidised) substance with the potency of the oxidised.

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
D-Limonene (ox. 10 w)	5989-27-5	1, 5, 25	3.0	(207)
D-Limonene (pure)	5989-27-5	25, 50, 100	30	

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
Linalool (ox. 10 w)	78-70-6	5, 10, 25	9.4	(127)
Linalool (ox. 45 w)	78-70-6	2.5, 10, 25	4.8	
Linalool (pure)	78-70-6	25, 50, 100	46.2	
Linalyl acetate (ox. 10 w)	115-95-7	0.5, 10, 40	3.6	(128)
Linalyl acetate (pure)	115-95-7	10, 30, 100	25	
Geraniol (ox. 10 w)	106-24-1	1, 3, 6, 10, 20	4.4	(119)
Geraniol (ox. 45 w)	106-24-1	0.5, 1, 3, 6, 10	5.8	
Geraniol (pure)	106-24-1	5, 10, 15, 20, 30	22.4	
Lavender oil (ox. 10 w)		1, 5, 10, 20, 50	11	(140)
Lavender oil (ox. 45 w)		1, 5, 10, 20, 50	4.4	
Lavender oil (not ox.)		5, 25, 100	36	

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.

Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.

Ox. x w: Oxidised by air exposure during x weeks.

* Acetone:olive oil.

8.2. Methodological considerations

EC3 mean values

In the submitted poster (163) and the report by IFRA (164), the LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) are presented. The SCCS considers it is misleading to present EC3 values as mean values from tests performed with different vehicles. It is generally agreed that the lowest EC3 value should be used if there is more than one study fulfilling the OECD guideline requirements (161, 205), and these have been introduced into Table 8-1. The EC3 values in the reviews by Gerberick et al. and Kern et al. (165, 166) were based on single representative experiments with a vehicle described in the OECD guideline 429 (see above), and preferably with acetone:olive oil. EC3 mean values, as in the submission by IFRA, were not presented in these two reviews.

Vehicle

The most frequently used *vehicle* in the submission by IFRA (164) was ethanol:diethyl phthalate (1:3), followed by acetone:olive oil (4:1). In some experiments, antioxidants were mixed with ethanol:diethyl phthalate. The vehicle was not reported in some of the references, and no rationale for using vehicles other than those recommended was given in the report (164). According to the OECD guideline 429 (see above), the recommended vehicles are acetone:olive oil (4:1), N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulphoxide, but others may be used if sufficient scientific rationale is provided. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus as an *additional control*, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.

Number of doses and animals

According to the OECD guideline 429 (see above), a minimum of three concentrations should be tested. The number of consecutive doses used in the reported data, was generally five, sometimes three and in few experiments two. The SCCS considers that too few concentrations were tested in four studies in which only two concentrations were used. Lower concentrations than those tested should have been used in experiments with five fragrance substances, in which the EC3 value could not be determined. Higher concentrations than those tested should also have been used in experiments with 12 substances, in which the EC3 value could not be determined.

The *number of animals* per dose group was generally four plus a non-exposed control group, sometimes five, and in few experiments six; the minimum according to the OECD guideline being four.

Units for concentrations

In the submission by IFRA (164) the EC3 values are given in weight per area unit ($\mu\text{g}/\text{cm}^2$). The SCCS considers that the EC3 values (%) are the values of primary interest in communicating risk assessment, as EU legislation, OECD guideline 429 and scientific literature refer to EC3 values (%). However, the SCCS recommends that molar (M) EC3 values should be considered, as they give the concentration based on the molecular weight of substances. They have thus been calculated and introduced into Table 8-1.

EC3 values (%) overestimate the intrinsic molecular sensitisation potency for low molecular weight compounds while compounds with a high molecular weight are underestimated. Regarding the differences in molecular weight between the studied fragrance substances, a variation is seen if the ranking list of the sensitisation potency is based on EC3 (%) or EC3 (M) since some substances have a molecular weight twice as high as others.

From comparisons in Table 8-1, we notice that, e.g. hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has an EC3 value of 17.1 %, or 0.81 M when the calculation includes its molecular weight, while for trans-2-hexenal the corresponding values are 2.6% and 0.26 M. The example shows that comparing the sensitisation potency between these two substances using the EC3 values in % exaggerates the sensitisation potency of trans-2-hexenal compared to that of HICC. When using the EC3 values in molar concentrations the difference is not so pronounced.

8.3. Summary of animal data by LLNA

The distribution of sensitising potency of fragrance substances compared to other substances, (e.g. biocides, dyes, plastic materials) taken from three references (164-166) as assessed by EC3 values in the LLNA, is shown in Figure 8-1 and Table 8-3.

For 10 substances, no EC3-value could be established. These should have been tested at higher concentrations – some of these would most probably have generated an EC3 value. However, we reported here “No EC3 value established”. 5 substances should have been tested also at lower concentration and in these cases the EC3 value could have been lowered, meaning a more severe potency category could have been achieved. In all, approx 150 experiments were reported in (164), listed in Annex II.

The median EC3 value of evaluable fragrance substances (5.9%) is similar to other substances tested (5.5%). However, very few fragrance substances have low EC3 values (≤ 2).

Substances with an EC3 value ≤ 2 may be categorised as strong or extreme sensitisers. Such potent sensitisers are comparatively rare among fragrance substances assessed in the LLNA. Nevertheless, fragrances are important allergens in humans, which points to repeated skin exposure to less potent sensitisers as a factor strongly determining sensitisation risk.

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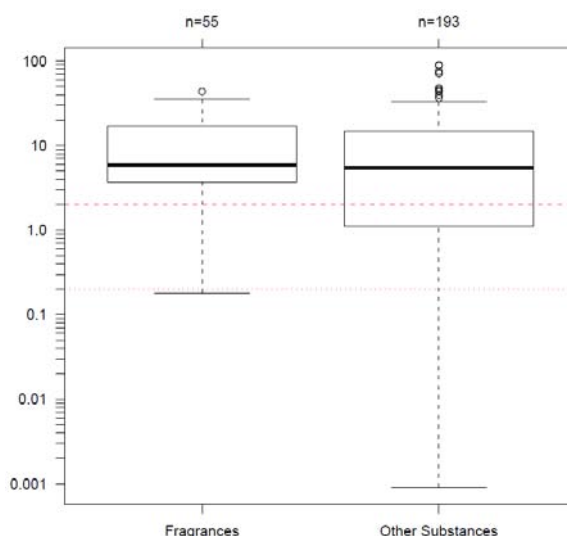


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in the box the median, and the top of the box the 3rd quartile (75% percentile). Outliers, i.e. below the 25% and above the 75% percentiles, are shown as whiskers. Beyond the 1.5-fold interquartile range, single values are shown as circles instead of whiskers. The difference in distribution is not significant (Wilcoxon test: $p=0.061$).

Note: EC3 values for the five oxidised fragrances additionally examined (Table 8-2) range from 3.0 to 4.8 (median 4.4) and are lower by a factor of around 7 than EC3 values of the respective non-oxidised material.

Table 8-3: Summary of EC3 values for fragrance substances in Table 8-1 and for other substances, all taken from the three references (164-166). The EC3 value intervals for potency categorisation (161, 205) were used for comparison of fragrances substances vs other substances.

EC3 value interval	Fragrance substances		Other substances	
	n	%	n	%
≤ 0.2	2	3%	28	11%
$> 0.2 - \leq 2$	3	4%	38	15%
> 2	50	71%	127	49%
No EC3 value established *	10	14%	0	0%
No EC3 value calculated (NC)	5	7%	69	26%
All substances	70		262	

Note: * Substances should have been tested also at higher concentrations.

8.4. Conclusions

- In the event that human data are lacking, the LLNA provides important information on skin sensitising potential and potency.
- Animal data on fragrance substances submitted by IFRA (164) and assessed in this opinion were generated exclusively by LLNA. Other guideline methods are, however, also available.
- The vast majority of the submitted (164) and additional (165, 166) fragrance substances tested by the LLNA are skin sensitisers.
- Several studies in the IFRA report (164) were of insufficient quality, not following the OECD guideline.

- Fragrance substances that can be predicted to autoxidise upon air exposure should also be tested after air exposure, as oxidation may significantly increase their sensitising potency.
- It can be concluded that the skin sensitising potency, as assessed by the LLNA, is only one of several factors that are of importance for sensitisation to fragrance substances. This is illustrated by the fact that only a small fraction of sensitising fragrance substances can be categorised as an extreme allergen based on LLNA test results. Therefore, doses from repeated deposition onto skin must be considered a driving force of sensitisation risk.

9. Structure activity relationships (SAR): grouping of substances based on expert judgement

Whether or not a particular chemical will be a sensitiser, and how potent it will be if it is a sensitiser, depends on its ability, either directly or after activation, to react with appropriate proteins in the skin. This fundamental concept was initially demonstrated by Landsteiner and Jacobs in 1936 (208) and subsequently validated by numerous studies with various types of chemicals (some key references: (209-213)). The ability to predict sensitisation potency, or lack of it, depends on being able to predict reactivity to skin proteins. This is the basis of SAR analysis for skin sensitisation. The prediction can often be made based on the chemical structure, recognising structural features (referred to as **structural alerts**) that are associated with reactivity.

The relationships between molecular structure and reactivity that form the basis for structural alerts are based on well established principles of mechanistic organic chemistry (214). Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and α,β -unsaturated carbonyl groups, C=C-CO- (alerting to the possibility of sensitisation via Michael addition of protein thiol groups). Major mechanistic reactivity domains have been discussed in detail by Aptula and Roberts (215). Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) is more complex compared to that of compounds that act as direct haptens without any activation. The autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers linalool and geraniol results in different major haptens/allergens. Moreover, the complexity of the prediction increases further for those compounds that can act both as pre- and prohaptens. In such cases, the impact on the sensitisation potency depends on the degree of abiotic activation (e.g. autoxidation) in relation to the metabolic activation.

These structural alerts can be applied by computerized expert systems, i.e. *in silico* or by estimations made by organic chemists (*in cerebro*) using their experience. When an organic chemist looks at a chemical structure, they recognise parts of the structure that they can associate with reactivity, the type of reactivity (i.e. assign the reaction mechanistic domain), and other features of the molecular structure that will affect the reactivity positively or negatively. Human experts should be aware of the complexities, and how structural modification can alter the reactivity associated with structural alerts, etc. Importantly, they can also recognise where there are unfamiliar structural features whose effects they cannot confidently predict. In such cases they can call for experimental chemistry work (*in chemico*) to be done to ascertain the presence or nature of, and degree of reactivity. *In chemico* methods include organic chemistry experimentation to identify chemical reaction products from oxidation and/or reaction with model nucleophiles, identification of mechanisms of reaction. In so called *in chemico* reactivity methods, the ability of a specific chemical to react with selected peptides is determined so as to predict the sensitisation potential of the chemical under investigation (216, 217). To make *in chemico* reactivity methods able to predict the activity of prohaptens, the addition of horseradish peroxidase and hydrogen peroxide oxidation system has been tested to model the enzymatic oxidation in the skin (218, 219).

Although computerized expert systems are derived from input by human experts, they are less well able to capture the subtleties of structure reactivity relationships, and they sometimes fail to detect aspects of chemistry that are obvious to organic chemists. Human experts should be aware of the complexities, as well as how structural modification can alter the reactivity associated with structural alerts, etc.

The SAR evaluation made in the section below is based on *in cerebro* alerts applied by organic chemists.

Depending on the type of reactivity (the **reaction mechanistic domain**), it is sometimes possible to make a quantitative prediction of potency in the LLNA, which can be used to predict potency in humans relative to related known human sensitisers. These predictions use quantitative mechanistic models (**QMMs**) based on reactivity expressed quantitatively

by model parameters, and sometimes in combination with hydrophobicity. For example, potency of aliphatic aldehydes and ketones (the Schiff base domain) in the LLNA is modelled by a combination of reactivity and hydrophobicity (220), whereas the LLNA potency of DNCB analogues (the S_NAr domain) is well modelled by reactivity alone (221).

QMMs aiming not only to predict the potential to be a sensitiser but also to predict the potency, promise to be a useful tool in non-animal based risk assessment for skin sensitisation. However, in the field of fragrance substances there are major gaps in our present ability to apply QSAR/QMM. This is largely because many of the fragrance substances of interest have the potential to act via abiotic or metabolic activation (pre- and/or prohaptens, i.e. they themselves are only weak or non-sensitisers, but have the potential to be activated to form more potent sensitisers. Resulting sensitisation potency will depend on the extent of activation and the nature of the resulting products. It is possible to apply SAR analysis to identify these plausible possibilities, but QSAR modelling for these cases is not yet developed. However, much progress has been made in identifying structural alerts for the various activation mechanisms that have been recognised. This is reviewed by Karlberg et al. (122).

Chemicals with no structural alerts for direct reactivity, or for known activation mechanisms, and no unfamiliar structural features that might be associated with as yet unidentified activation mechanisms, can be predicted to be non-sensitising. Chemicals that do have alerts for reactivity (direct or via activation) are not necessarily sensitisers – they may be insufficiently reactive and/or insufficiently hydrophobic.

Substances meeting the inclusion criteria (see chapter 6), for which, however, no categorisation as established contact allergen in humans or established contact allergen in animals was possible, have been assessed for structural alerts. The results are presented in four tables based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitiser; structural alerts:
Compounds containing structural alerts comprising direct reactive compounds and for compounds that after specific abiotic or metabolic activation (prohaptens and prehaptens) can be predicted to be sensitisers by structural comparison to known allergens.
- Possible sensitiser; structural alerts:
Compounds containing structural alerts that by comparison to known allergens with similar structures were expected to be less reactive and hence less likely to be sensitising. Also compounds with structural alerts indicating a possible abiotic or metabolic activation (possible prehaptens or prohaptens) but with no structural data available for comparison, were included in this group. Consequently, a possible sensitiser may turn out to be a non sensitiser when tested in vivo.
- Predicted non-sensitiser (NS); no obvious structural alerts
- Not predictable due to insufficient/conflicting data

Table 9-1: Predicted sensitisers.

Substance (INCI) name	CAS number	Structural alerts
p-tert.-Butyldihydrocinnamaldehyde ^s	18127-01-0	Schiff base
Citronellal	106-23-0	Schiff base and possible prehapten
Citronellyl nitrile	51566-62-2	Possible prehapten
Decanal	112-31-2	Schiff base
3,7-Dimethyl-1,6-nonadien-3-ol	10339-55-6	Prehapten
Geranyl acetate	105-87-3	Prehapten and prohapten

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Isoamyl salicylate	87-20-7	Acyltransfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehaptent
Nerol	106-25-2	Prehaptent and prohaptent
Nerolidol (isomer not specified)	7212-44-4	Possible prehaptent
Oxacyclohexadecanone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehaptent

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

Substance (INCI) name	CAS number	Structural alerts
Ambrettolide	7779-50-2	Possible prehaptent
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohaptent via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehaptent
Cuminaldehyde	122-03-2	Schiff base and possible prehaptent
alpha-Cyclohexylidene benzeneacetone	10461-98-0	Possible Michael acceptor
Cyclopentadecanone	502-72-7	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
trans-beta-Damascone	23726-91-2	Possible Michael acceptor
trans-trans-delta-Damascone	71048-82-3	Possible Michael acceptor and possible prehaptent
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehaptent
Dihydromyrcenol	18479-58-8	Possible prehaptent
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehaptent and possible prohaptent
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehaptent
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehaptent
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehaptent
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehaptent
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohaptent
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl	54830-99-8	Possible prehaptent

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Substance (INCI) name	CAS number	Structural alerts
acetate		
Hexahydro-methanoindenyl propionate	68912-13-0	Possible prehapten
Ionone isomeric mixture	8013-90-9	Possible Michael acceptor and possible prehapten
alpha-Ionone	127-41-3	Possible Michael acceptor and possible prehapten
beta-Ionone	79-77-6	Possible Michael acceptor
Isobergamate	68683-20-5	Possible prehapten
Isolongifoleneketone	33407-62-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Longifolene [§]	475-20-7	Possible prehapten
Methoxycitronellal	3613-30-7	Schiff base
Methyl decenol	81782-77-6	Possible prehapten
Methyl ionone (mixture of isomers)	1335-46-2	Possible Michael acceptor and possible prehapten
Methylionantheme	55599-63-8	Possible Michael acceptor and possible prehapten
5-Methyl-alpha-ionone	79-69-6	Possible Michael acceptor and possible prehapten
Myrtenol	515-00-4	Possible prehapten
Nopyl acetate	128-51-8	Possible prehapten
Phytol	150-86-7	Possible prehapten and/or prohaptent
Rhodinol	6812-78-8	Possible prehapten
Terpineol acetate (isomer mixture)	8007-35-0	Possible prehapten
alpha-Terpinyl acetate	80-26-2	Possible prehapten
Tricyclodecenyl propionate	17511-60-3	Possible prehapten
Verdyl acetate	2500-83-6/ 5413-60-5	Possible prehapten

Note: [§] Classified as R43.

Table 9-3: Predicted non-sensitisers with no obvious structural alerts.

Substance (INCI) name	CAS number	Structural alerts
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	
Benzyl acetone	2550-26-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
2-tert.-Butylcyclohexyl acetate	88-41-5	
4-tert.-Butylcyclohexyl acetate	32210-23-4	
Cyclohexyl acetate	622-45-7	
Diphenyl ether	101-84-8	

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Substance (INCI) name	CAS number	Structural alerts
Ethyl 2-methylbutyrate	7452-79-1	
Ethylene dodecanioate	54982-83-1	
Ethylene brassylate	105-95-3	
Eucalyptol	470-82-6	
Hexyl acetate	142-92-7	
Hibiscolide	6707-60-4	
Hydroxycitronellol	107-74-4	However, dehydration followed by autoxidation could give sensitising impurities
Isoamyl acetate	123-92-2	
Isobornyl acetate	125-12-2	
Methoxytrimethylheptanol	41890-92-0	
Methyl p-anisate	121-98-2	
Methyl anthranilate	134-20-3	
Methylbenzyl acetate	93-92-5	
Methyl dihydrojasmonate	24851-98-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
Oxalide	1725-01-5	
Pentadecalactone	106-02-5	
Phenethyl acetate	103-45-7	
Phenethyl alcohol	60-12-8	
Phenoxyethyl isobutyrate	103-60-6	
Phenylisohexanol	55066-48-3	
Phenylpropanol	122-97-4	
Tetrahydrolinalool	78-69-3	
Tetrahydro-methyl-methylpropyl)-pyran-4-ol	63500-71-0	
Trimethylhexyl acetate	58430-94-7	
Trimethyl-propylcyclohexanepropanol (tmch)	70788-30-6	
gamma-Undecalactone	104-67-6	

Table 9-4: Not predictable.

Substance (INCI) name	CAS number	Structural alerts
Anisaldehyde	123-11-5	Due to insufficient /conflicting data; structural similarities to benzaldehyde suggest certain activity in man
Trichloromethyl phenyl carbonyl acetate	90-17-5	Due to insufficient /conflicting data
Methyl beta-naphthyl ether	93-04-9	Due to insufficient /conflicting data

9.1. General results

From this work with the included SAR predictions, the following observations can be made.

- For substances for which sufficient experimental/clinical evidence is missing, SAR analyses have been performed to predict a probable or possible risk of allergenic (sensitising) effect. These predictions are based on chemical reactivity and the recognition of structural features in a substance that are in common with the structural features that have been shown to cause sensitisation from other substances. In cases where the SAR analysis indicates a sensitisation potential, the substance should be investigated further to confirm or reject the conclusion drawn from the SAR analysis.
- Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) becomes more complex compared to that of compounds that act as direct haptens without any activation.
- The complexity of the prediction increases further for those compounds that can act both as prehaptens and prohaptens.
- Prediction of the sensitisation potential of compounds that can act as prehaptens is further complicated by the fact that the autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers of linalool and geraniol results in different major haptens/allergens.

9.2. Conclusions

The SAR evaluation made in this section is based on *in cerebro* alerts applied by organic chemists.

- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods.
- SAR, as performed here, is only one consideration in the overall weight of evidence.

10. Exposure

Exposure to fragrance chemicals and other potential allergens is most commonly by direct skin contact. Exposures to fragrance chemicals occur from:

- Personal cosmetic use;
- Detergents and other household products;
- Medicaments;
- Occupation, i.e. personal hygiene, manufacturing ingredient(s), product in work process, plant materials;
- Secondary exposure from another individual (e.g. spouse, child);
- Toys;
- Oral intake;
- Airborne exposure.

Factors that are important for both the induction and elicitation of contact allergy are:

- Dose per unit area;
- Vehicle effects including penetration enhancers;
- Presence of skin irritants;
- Presence of other allergens (combination effects);
- Duration of skin exposure;
- Frequency of applications;
- Anatomical sites of exposure;
- Condition of the skin (barrier function impairment, pre-existing inflammation);
- Occlusion (e.g. in flexures, under clothing and personal protective equipment).

Fragrance mix ingredients are commonly present in cosmetic formulations (71, 222-224). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (225). The clinical significance of exposure to natural extracts is difficult to determine as there is often “hidden and variable” exposure to important and potent allergens in natural products.

10.1. Concentrations and quantities used

Consumers are exposed in daily life to fragrance chemicals from a large variety of products, such as cosmetics, toys, detergents and other cleaning products, etc. The fragrance exposure may be via dermal and/or inhalation route. With respect to “Terms of Reference” to the SCCS, only dermal exposure from cosmetics is addressed in this opinion. As cosmetics are the perfumed products most commonly used in daily life, potential fragrance allergens identified by the use of cosmetics also represent the exposures of these chemicals from other product categories. In recent years, it has become a trend to add fragrance chemicals to many other types of consumer products, such as children’s toys, toilet paper and nappies, which may contribute significantly to the fragrance exposure of the consumer by the dermal route.

Factors for the fragrance exposure assessment by the dermal route require knowledge on:

- Product types (categorisation of scented products) used by the consumer.
- Market survey (impression of the qualitative and quantitative contents of different allergens in consumer products).

- Hydrolysis, metabolism or oxidation of a fragrance material, which may generate a potential skin allergen.
- Chemicals in the product matrix, which may significantly enhance or reduce dermal absorption of a fragrance material.

Fragrance materials, both defined chemical substances and natural mixtures of chemicals (essential oils), are used in all types of cosmetic products: perfumes, eau de cologne, eau de perfume (EDP), and eau de toilette (EDT), aftershave lotion, deodorants, skin care products, skin cleansers, make-up cosmetics, hair care products, and oral care products, etc. However, some unscented cosmetic products have also reached the market in the last decade. Products containing the highest concentration of fragrance chemicals are perfumes, followed by eau de cologne, eau de perfume (EDP) and eau de toilette (EDT). Concentrations of fragrance chemicals in deodorant products are lower than those in EDT/EDP products, but still significant. Aftershave products also contain relatively high amounts of fragrance chemicals. Other cosmetic products contain relatively low amounts, 0.1-1% of fragrance compound, compared to up to 30% fragrance compound in EDT/EDP (226). The fragrance compound are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (226). For the exposure assessment, levels of fragrance chemicals in cosmetics containing significant amounts of fragrance materials (i.e. EDP/EDT/aftershave/deodorant) should be selected. It may not be possible to detect/measure the amounts of all fragrance chemicals when present in highly diluted form in a cosmetic product such as skin care products, make-up cosmetics etc. On the other hand, if a fragrance is evaluated safe for use when present in significant amounts in a product, it will also be safe for use in other products. Also the analysis of trend of the use of individual fragrance materials should be based on monitoring their contents in fine perfumes and deodorants.

Ninety of the 100 fragrance materials used in annual volumes > 175 tons in perfume formulations are fragrances and the remaining ten are used for other functions such as solvents or antioxidants (IFRA, personal communication 2010).

Among the 26 fragrances currently requiring individual labelling, amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone, and linalool are used in volumes greater than 175 ton. α -Amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri*), tree moss (*Evernia furfuracea*) are used in volumes less than 175 ton.

According to the information from the fragrance industry, 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products.

Since the implementation of the regulation of labelling of 26 fragrance substances in cosmetic products, qualitative information on fragrance exposure from cosmetics is provided in some market surveys performed on cosmetics (Table 10-1, (227)) and (Table 10-2, (228)) and on consumer products including cosmetics (Table 10-3, (229); Table 10-4, (115); and Figure 10-1, (105)). Thus, the implementation of the regulation of fragrance allergens in detergents (Directive 648/2004/EC), similar to that for cosmetics, has also added to the knowledge of fragrance exposure to the consumer. These market surveys revealed that fragrance ingredients which are potent allergens and frequently cause allergies in consumers are used as ingredients in consumer products including cosmetics. The results of these surveys further revealed that limonene and linalool were the most commonly used fragrance chemicals in cosmetics, while anisyl alcohol, cinnamal, α -amylcinnamyl alcohol, oak moss and tree moss were the least used fragrance ingredients in cosmetics and other consumer products. In general, the most potent allergens were also the most infrequently used ingredients. Prior to the regulation of the 26 allergens, analysis of 21 selected fragrance chemicals in deodorants also revealed additional 66 potential allergens in these products on the basis of structure activity relationship (230).

Table 10-1: Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (227).

Fragrance substance		% Products labelled to contain the fragrance substance
INCI name	CAS number	
Amyl cinnamal	122-40-7	8.2
alpha-Amylcinnamyl alcohol	101-85-9	2.9
Anise alcohol	105-13-5	0
Benzyl alcohol	100-51-6	9.6
Benzyl benzoate	120-51-4	9.1
Benzyl cinnamate	103-41-3	2.9
Benzyl salicylate	118-58-1	9.6
Butyl phenyl methyl propional	80-54-6	7.7
Cinnamal	104-55-2	1
Cinnamyl alcohol	104-54-1	6.7
Citral	5392-40-5	8.2
Citronellol	106-22-9	10.5
Coumarin	91-64-5	4.8
Eugenol	97-53-0	7.2
Farnesol	4602-84-0	2.9
Geraniol	106-24-1	12
Hexyl cinnamal	101-86-0	10.1
Hydroxycitronellal	107-75-5	6.3
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	31906-04-4	5.8
Isoeugenol	97-54-1	0.5
Alpha-isomethyl ionone	127-51-5	5.8
<i>d</i> -Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
<i>Evernia prunastri</i> /oak moss	90028-68-5	0
<i>Evernia furfuracea</i> /tree moss	90028-67-4	0

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Table 10-2: Usage trends in deodorants of fragrance chemicals that are required to be labelled in cosmetics.

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
INCI name	CAS number	% Products labelled to contain the fragrance	Content in 23 selected products		Content in all 70 products	
			% Products found to contain the fragrance	Range(ppm)	% Products found to contain the fragrance	Range (ppm)
Amyl cinnamal [■]	122-40-7	10.2	17	2.3-165	31	1-617
alpha-amyl cinnamyl alcohol	101-85-9	-	-	-	n.a.	n.a.
Anise alcohol	105-13-5	2.3	9	1, 51	n.a.	n.a.
Benzyl alcohol	100-51-6	17.1	26	32-166	76	1-629*
Benzyl benzoate	120-51-4	25.0	48	3-4054	71	1-1075
Benzyl cinnamate	103-41-3	3.4	9	74, 143	n.a.	n.a.
Benzyl salicylate	118-58-1	39.8	48	136-5279	49	1-18758
Butyl phenyl methyl propional	80-54-6	48.9	70	1-5455	51	1-3732
Cinnamal [■]	104-55-2	1.1	4	5	17	1-424
Cinnamyl alcohol [■]	104-54-1	12.5	48	2-503	39	6-1169
Citral [□]	5392-40-5	26.1	44	39-554	n.a.	n.a.
Citronellol [□]	106-22-9	65.9	91	1-5848	81	1-5585
Coumarin [□]	91-64-5	33.0	52	3.8-1255	57	1-1411
Eugenol [■]	97-53-0	27.3	30	1-514	57	1-2355
Farnesol [□]	4602-84-0	14.8	39	9-1791	n.a.	n.a.
Geraniol [■]	106-24-1	48.9	87	1-399	76	1-1178

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Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
Hexyl cinnamal [□]	101-86-0	33.0	48	1-4434	71	2-1684
Hydroxycitronellal [■]	107-75-5	27.3	70	1-1746	50	1-1023
HICC [□]	31906-04-4	33.0	74	1-4431	53	1-1874
Isoeugenol [■]	97-54-1	9.1	35	1-138	29	1-458
Alpha-isomethyl ionone	127-51-5	46.6	65	6-2588	61	1-2765
D-Limonene [°]	5989-27-5	53.4	70	1022-11386	n.a.	n.a.
Linalool [°]	78-70-6	53.4	96	8-3447	97	9-1927
Methyl-2-octynoat [°]	111-12-6	1.1	-	-	n.a.	n.a.
<i>Evernia prunastri</i> [■] /oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
<i>Evernia furfuracea</i> [■] /tree moss	90028-67-4	2.3	n.a.	n.a.	n.a.	n.a.

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.

- Fragrance not detected in any product.

n.a. Not analysed.

* Benzyl alcohol could not be determined in 49% of the products due to interference.

The most common fragrance allergens are contained in the two mixtures, which are used for diagnosing fragrance allergy, called Fragrance Mix I (■) and Fragrance Mix II (°), besides the oxidation product of terpens (°), and tree moss extract are common allergens. Methyl-2-octynoate is an extreme, but rare allergen.

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Table 10-3: Frequency of occurrence in consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (229).

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyr TM	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evermia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evermia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0.3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

INCI, International Nomenclature of Cosmetic Ingredients; PCP, personal care products; MP, men's products; HP, household products; WP, women's perfumes; Cos, cosmetics; Deo, deodorants; Dent, dental products; BPMP, butyl phenyl methyl propional; LyrTM, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

Table 10-4: Frequency in 516 consumer products of the 26 fragrance substances that are required to be labelled in cosmetics* (115).

Fragrance substance INCI name	% Product containing the chemical
D-Limonene	48.3
Linalool	35.8
Butyl phenyl methyl propional	24.8
Geraniol	22.1
Alpha-isomethyl ionone	21.7
Hexyl cinnamal	21.3
Citronellol	21.1
Benzyl salicylate	18.6
Coumarin	17.0
Eugenol	15.7
Benzyl alcohol	15.3
Benzyl benzoate	14.7
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	12.8

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Fragrance substance INCI name	% Product containing the chemical
Citral	11.6
Hydroxycitronellal	10.8
Amyl Cinnamal	7.9
Anise alcohol	7.0
Cinnamyl alcohol	6.4
Farnesol	3.9
Isoeugenol	3.1
Cinnamal	2.5
Benzyl cinnamate	2.3
Amylcinnamyl alcohol	1.9
Methyl-2-octynoate	1.0
<i>Evernia prunastri</i> -oak moss	0.8
<i>Evernia furfuracea</i> -tree moss	0.4

Note: * Consumer Products: Cosmetics and household products with labelling of the 26 fragrance allergens. The content of these fragrances was confirmed by chemical analysis.

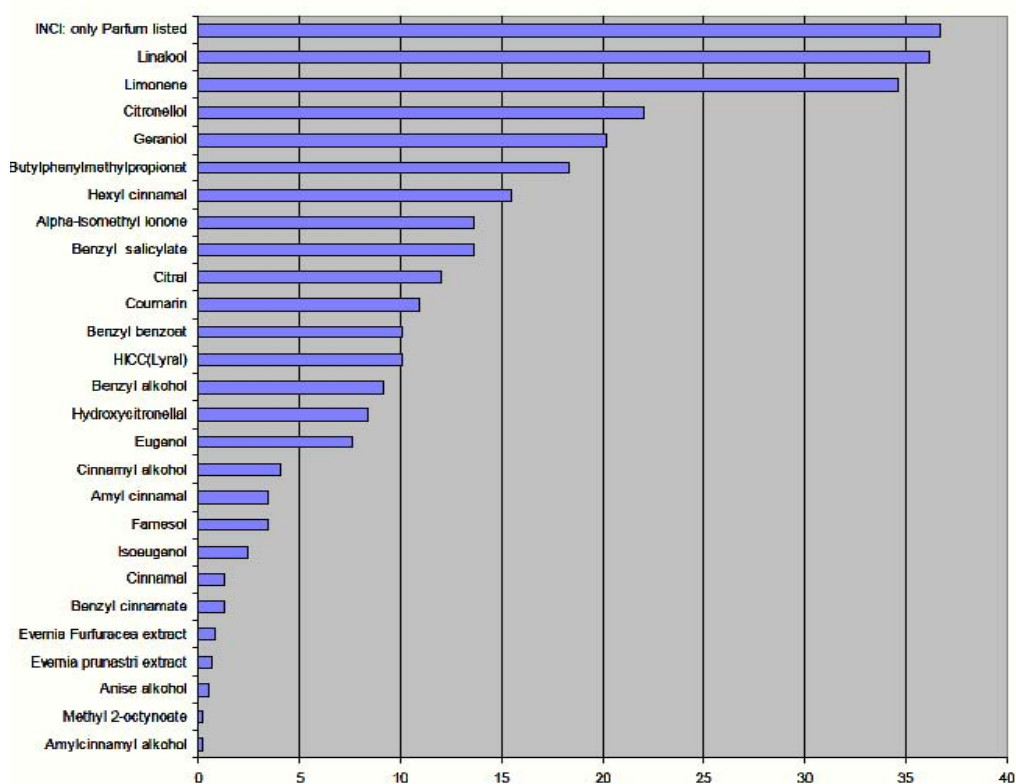


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

Contents of fragrance substances determined in cosmetic products have been described in several studies, both before and after the regulation of the 26 fragrance allergens. The studies prior to the regulation of the 26 fragrance allergens included many, but not all of these 26 allergens. On the other hand, these studies included some other possible fragrance allergens. The quantitative analysis of fragrance substances has been performed in prestige perfumes (5, 157, 232-234), deodorants (228, 231), children's cosmetics and cosmetic toys (115, 227, 235), products marketed as natural cosmetics (225) and in cosmetics used by patients with contact allergy to fragranced products (35, 71). Quantitative analyses have revealed that the consumer is exposed to most, but not all of the 26 fragrance allergens from the use of cosmetics. However, when fragrance exposure from other consumer products, for example detergents and other household products is also taken into consideration (Table 10-3, Table 10-4, Figure 10-1), (105, 115, 229, 236), exposure to all of the 26 allergens is foreseeable in daily life. Although from the data available, the exposure to α -amylcinnamyl alcohol, cinnamal, methyl-2-octynoate, *Evernia prunastri* (oak moss) and tree moss may appear to be low, these are very strong allergens.

The changes in the use of fragrance chemicals in cosmetic formulations, during last 12 years, i.e. before and after the regulation of the 26 fragrance allergens, is reflected in the studies concerning contents of fragrances substances in popular perfumes (5, 232). As described in Table 10-5, the content of FM I allergens in prestige perfumes was significantly reduced from 1996 to 2003. Whether this is also the case for the perfumes sold as natural cosmetics (Table 10-6) has not yet been investigated.

Table 10-5: Concentration of Fragrance Mix I ingredients in five prestige perfumes before and after the regulation of the 26 fragrance allergens.

Fragrance INCI name	Concentration in the perfumes before regulation (5)			Concentration in the perfumes after regulation (232)		
	In no. of perfumes	Range % (w/w)	Mean % (w/w)	In no. of perfumes	Range % (w/w)	Mean % (w/w)
Geraniol*	5	0.072- 0.432	0.340	5	0.090- 0.236	0.156
Cinnamal	2	0.002- 0.002	0.002	0	-	-
Hydroxy- citronellal	5	0.222- 0.979	0.615	5	0.015- 0.478	0.169
Cinnamyl alcohol	4	0.068- 0.232	0.147	0	-	-
Eugenol	5	0.032- 0.738	0.337	2	0.001, 0.001	0.001
Isoeugenol	3	0.026- 0.249	0.119	2	0.001, 0.004	0.003
Amyl cinnamal	1	0.019	0.019	0	-	-

Note: * Due to interference by linalyl acetate, concentration of geraniol+linalyl acetate is reported.

Table 10-6: Concentrations of Fragrance Mix I ingredients, hexyl cinnamal and coumarin in 22 perfumes marketed as natural cosmetics investigated in 1996.

Fragrance	In no. of perfumes	Concentration % (w/w)
Geraniol	14	1.191*
Cinnamal	3	0.089, 0.109, 2.101
Hydroxycitronellal	5	0.135-6.044
Cinnamyl alcohol	8	0.035-2.289
Eugenol	2	0.027, 0.139
Isoeugenol	8	0.194-3.039
Amyl cinnamal	9	0.105-7.706
Coumarin	11	0.046-6.043

Note: * Quantification was performed in one sample only, due to interference by a very large amount of linalyl acetate in other samples.

The trend in the use of most of the fragrance allergens in deodorants before and after their regulation is reflected by the two studies performed by Rastogi et al. (228, 231). The results of these studies cannot be directly compared, because the study from 1998 included randomly selected deodorants, while selection of the deodorants for the 2007 study was based on the labelling of the presence of known strong fragrance allergens in these products. The number of products analysed in the 1998 study were three times more than those analysed in 2007, but not all of the 26 fragrance allergens were analysed in the 1997 study. However, an indication of the change in the use of the fragrance allergens during 1998-2007 may be obtained by reviewing the results of these two studies. Among the 17 common fragrance substances studied in the two studies, the frequency of use of 16 of these substances in deodorants was reduced in 2007 compared to that in 1998 (Table 10-2). The frequency of use of butyl phenyl methyl propional in deodorants appeared to be unchanged. The contents of benzyl alcohol, benzyl salicylate, cinnamal, cinnamyl alcohol, eugenol, geraniol, isoeugenol and linalool were found to be lower in the deodorants analysed in 2007 compared to those in 1998. Citronellol, coumarin and alpha-isomethylionone contents in the deodorants were similar in both studies, but concentrations of benzyl benzoate, butyl phenyl methyl propional, hexyl cinnamal, hydroxyisohexyl-3-cyclohexene carboxyaldehyde and linalool were much higher in deodorants in 2007 compared to those in 1998. This analysis of trend of use of fragrance allergens in cosmetic products indicates that the regulated fragrance allergens are used less frequently, but exposures from some of the regulated fragrance allergens may be much higher compared to those before regulation.

Table 10-7: Atranol and chloroatranol content in eau de toilette/eau de perfume, investigated in 2004 and in 2007.

	2007 Study	2004 Study
No. of samples	22	17
Atranol present in no. of samples	15 (68%)	12 (70%)
Atranol content	ppb (ng/ml)	ppb (ng/ml)
Range	n.d.-880	n.d.-791
Mean±SD	157±249	97±224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Atranol content	ppb (ng/ml)	Ppb (ng/ml)
Range	0.9-208	1-175
Mean±SD	63±73	36±51
Median	22	10

Notes: n.d. Not detected.

* $P < 0.05$ (chi-square test).

SD: Standard deviation.

Atranol (CAS no. 526-37-4) and chloroatranol (CAS no. 57074-21-2), constituents of oak moss and tree moss have been shown to be very potent fragrance allergens (237, 238). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (239). Two other commonly used fragrance chemicals, isoeugenol (240) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (71), have also been shown to be important contact allergens. The contents of atranol, chloroatranol, isoeugenol and hydroxyisohexyl-3-cyclohexene carboxyaldehyde in fine fragrances was determined for the exposure assessment of these fragrances (233). The results revealed that isoeugenol was present in 56%, HICC in 72%, atranol in 59%, and chloroatranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below 0.02% which is the maximum concentration recommended by the fragrance industry. HICC reached a maximum concentration of 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the EC Scientific Committee (241). The concentrations of atranol and chloroatranol in the products investigated in 2007 were comparable to those found in similar products in 2004 (Table 10-7, (233, 234). A significant decrease in the frequency of the presence of chloroatranol in the products was found in 2007 (Table 10-7).

10.2. Global exposure (household and occupational exposures)

Fragrances are used in cosmetics that the consumer applies to themselves, as described in the previous section. In addition, exposure to fragrance substances is possible by a number of other exposure routes briefly outlined in this section.

Topical pharmaceutical products

In a study from Belgium, 370 of the 3,280 topical products marketed in Belgium have been found to contain one or more of 66 fragrance substances (242). This publication also contains a description of causative fragrance allergens in 127 patients reacting to 48 specific topical products. In a broader sense, exposure of the patient by extracts used in aromatherapy falls in this category as well.

Childrens products and toys

Children's products may contain fragrance allergens and high levels may be present (235). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (243).

Clothing

Washed fabrics have been reported to contain fragrances (244). Odour-neutralising agents are sometimes used for shoe insoles. In one case, an insole containing cinnamon, has been reported to lead to plantar vesicular contact dermatitis due to contact sensitisation to FM I and, in the breakdown, to cinnamal and cinnamyl alcohol (245).

Cleaning agents and other household products

Contact dermatitis from geraniol in washing-up liquid has been reported (246). Terpenes are used as solvents and cleansing agents (e.g. limonene) (247) and have been reported as cause of hand dermatitis (248, 249). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (250). In a review of 301 cosmetic and detergent consumer products in Sweden, in half of the cosmetics and one-third of the detergents, one or more of the 26 fragrances requiring labelling were identified (251). In the UK, a review of 300 consumer products showed that linalool and limonene were present in 63% of products. Dental products contained on average 1.1 fragrance substances that are presently required to be labelled and women's perfumes contained 12 of these fragrance substances (Table 4-1 and Table 4-3) (229).

Candles

The dermal hand transfer of three fragrance materials (cinnamic aldehyde, d-limonene and eugenol) from scented candles was determined in ten subjects (i.e. 20 hands) after grasping scented candles for five consecutive 20 second exposures/grasps. The total mean residues of cinnamal and eugenol transferred per grasp from the candles to the hands were 0.255 µg/cm(2) and 0.279 µg/cm(2), respectively (252).

Food

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (253)) or lichen planus-like lesions (e.g. due to cinnamal (254)) or contact gingivitis (e.g. due to eugenol (255)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (256) may cause allergic contact dermatitis by handling this food.

Occupational exposure

In a number of occupations, contact allergy to fragrances is more common than in others, including geriatric nurses, masseurs and physiotherapists, metal furnace operators and potters/glass makers, according to a multifactorial analysis (90). Moreover, hairdressers, beauty therapists and aroma therapists are examples of occupations where there is occupational exposure to fragrance-containing cosmetic and other products. Cleaners are exposed to fragrance-containing household products (e.g. detergents). Cooks and bakers are exposed to flavour chemicals and spices. Healthcare workers are also at risk of acquiring fragrance contact allergy. "Odour maskers" may contain important fragrance allergens (89, 90, 257-259). Occupational exposure and

occupational ACD to fragrances have been described in perfume bottlers (260). Industrial use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (259).

A number of fragrance chemicals are also used as biocides (see Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, published 11.12.2007 EN Official Journal of the European Union L 325/3 –L325/65), see Table 10-8 below.

Table 10-8: Parts of Annex I to (EC) No 1451/2007 (see above): "Active substances identified as existing", if use is 'perfuming' or 'masking' according to CosIng.

Biocide	EINECS	CAS number	Biocidal product group
Linalool	201-134-4	78-70-6	19
Geraniol	203-377-1	106-24-1	18, 19
Benzyl benzoate	204-402-9	120-51-4	2, 18
Eugenol	202-589-1	97-53-0	Not given
Farnesol	225-004-1	4602-84-0	Not given
(R)-p-mentha-1,8-diene	227-813-5	5989-27-5	12
Citriodiol/mixture of cis- and trans-p-menthane-3,8 diol	255-953-7	42822-86-6	1, 2, 19
Citral	226-394-6	5392-40-5	Not given
Pine ext.	304-455-9	94266-48-5	10
TANACETUM CINERARIIFOLIUM FLOWER EXTRACT	289-699-3	89997-63-7	18
Citrus oils (main component: limonene)	several	various	
Clove oil (main component: eugenol (83.8 %), caryophyllene (12.4 %))	/	8000-34-8	

Product groups(According to Biocide Directive 98/8/EC)

- 1 Human hygiene biocidal products
- 2 Private area and public health area disinfectants and other biocidal products
- 3 Veterinary hygiene biocidal products
- 10 Masonry preservatives
- 12 Slimicides
- 18 Insecticides, acaricides and products to control other arthropods
- 19 Repellents and attractants

The above illustrates that the consumer is exposed to fragrance substances from a wide variety of cosmetic products, other consumer products, pharmaceuticals and occupational exposures.

All these exposures are of importance in the context of contact allergy as it is not the source of exposure that is critical for both induction and elicitation, but the cumulative dose per unit area.

10.3. Exposures related to particular anatomical sites

Contact allergy to fragrances most often causes dermatitis of the hands, face and axillae. Axillary involvement has been shown to be statistically related to fragrance allergy (9). It is recognised that the axillary skin is a problematic area as it is moist, occluded and is easily irritated. Moreover, facial eczema is a common manifestation of fragrance allergy (3, 47). There is an association between fragrance allergy and hand eczema or aggravation of hand eczema (13-15). Vehicles may influence elicitation capacity of an allergen and the presence of detergents (surfactants) as in hand cleaning products may increase the clinical response by a factor of 4-6 (261). Men using wet shaving as opposed to electric razors have an increased risk of being fragrance allergic (17), most likely due to microtraumata and to the presence of surface active substances in shaving foam.

In use tests, the upper arm has been shown to be more sensitive than the forehead and lower arm (262). The axillae, neck and face are more sensitive than the upper arms (10). The threshold of elicitation may vary depending on the volatility of the substance (263). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (264).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (74). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (265). Following this, eczema may appear, or be worsened by, the use of a variety of product types including other cosmetics, household products, industrial products and flavours.

The association between contact allergy to fragrance ingredients and certain anatomical sites, which mirrors exposure to fragrance-containing products on these anatomical sites, has been described in several publications (266, 267), see above. However, due to the potential confounding effect of other factors, at least on some anatomical sites, an adjusted analysis will provide a more valid impression of the association between certain anatomical sites and contact allergy to fragrance ingredients. As an adjusted, multifactorial analysis relies on: (i) a substantial number of observations (patients tested); and (ii) an outcome prevalence not too close to 0%, such an approach has, hitherto, been limited to FM I.

In a paper published 2001, data from the IVDK in terms of patch test reactions to FM I and relevant clinical and demographic information of the patients tested (n=57,779) was studied by Poisson regression analysis (90). Risk was quantified by the prevalence ratio, which can be interpreted as an estimate of relative risk, i.e. the factor by which the risk of being sensitised to FM I (in this example) is to be multiplied (RR > 1: elevated risk; or RR < 1: reduced risk) if a certain "risk factor" is present, compared to those patients in whom this risk factor is not present (the reference category) (general aspects of such analyses are discussed in (268)). In the analysis, potential risk factors and confounders, respectively, including occupation, year of patch testing (to address a possible time trend), sex, age, past or current atopic dermatitis, in addition to anatomical site. The relevant part of Table 3 of (90) is reproduced below.

Table 10-9: Result of a Poisson regression analysis of patients tested with the Fragrance Mix between January 1992 and December 1998, considering two alternative outcomes – part I: non-occupational factors

Attribute	Prevalence (%)	At least + (11.5%)		At least ++ (4.0%)	
		PR	95% CI	PR	95% CI
Age:					
≤30	26.7	1.00	Reference	1.00	Reference
>30–44	23.8	1.42	1.31 to 1.53	1.61	1.40 to 1.84
>44–58	25.6	1.67	1.55 to 1.80	1.90	1.66 to 2.16
>58	23.9	1.93	1.77 to 2.10	2.07	1.79 to 2.39
Sex (female)	64.5	1.29	1.21 to 1.37	1.18	1.07 to 1.31
Main site:*					
Trunk	2.9	1.00	Reference	1.00	Reference
Hands	29.9	1.24	1.07 to 1.46	1.28	0.98 to 1.67
Arm	3.8	1.23	1.01 to 1.49	1.19	0.86 to 1.65
Face	15.2	1.20	1.03 to 1.42	1.13	0.86 to 1.48
Neck	1.4	1.39	1.10 to 1.75	1.31	0.88 to 1.94
Feet	2.8	1.26	1.02 to 1.55	1.19	0.84 to 1.68
Leg	8.7	1.59	1.36 to 1.89	1.50	1.14 to 1.99
Axilla	0.9	2.77	2.20 to 3.46	2.73	1.87 to 4.00
Other site	8.9	0.66	0.55 to 0.80	0.48	0.35 to 0.67

*Additionally controlled for several more sites—none of these associated with a significantly increased or decreased risk.

Compared to the trunk, which was arbitrarily chosen as the reference category, all other anatomical sites are associated with an increased risk of being sensitised to FM I (significantly if the lower limit of 95% CI is > 1). Most evidently, dermatitis of the axilla(e) is strongly associated with contact allergy to FM I, presumably due to the application of deodorants. Furthermore, the part of the table shown above illustrates a strong, positive age gradient, i.e. the older patients are, the more likely they are to be sensitised to FM I, the risk being almost double when comparing the oldest with the youngest age group. This observation is in concordance with a bivariate (unadjusted) association between age and contact allergy to FM I found in another study (89). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (269): (i) an even stronger age gradient; and (ii) no particular association to axillary dermatitis (included in the “other” category) was found (Table 10-10).

Table 10-10: Association between selected risk factors and positive patch test to *Myroxylon pereirae* resin. For full model see (269). Risk quantified with the prevalence ratio (PR) with accompanying 95% confidence interval (CI).

Factor	PR	95% CI
Atopic dermatitis, past or present	1.02	(0.95-1.10)
Female sex	1.13	(1.06-1.20)
Site		
Trunk	1.00	(reference)
Hand or Arm	1.03	(0.94-1.12)
Foot or Leg	1.76	(1.61-1.92)
Head or Neck	0.94	(0.86-1.03)
“Other” site	0.72	(0.64-0.81)
Missing site	1.07	(0.97-1.19)
Age		
30 years and younger	1.00	(reference)
31 to 44	1.92	(1.73-2.12)
45 to 58	2.87	(2.61-3.16)
58 or older	3.85	(3.49-4.25)

10.4. Conclusion

There are various modes of exposure to fragrances, including not only products used for their scent, such as perfumes and eau de toilette, after shaves, and deodorants, but also types of products where scent is an added feature, such as other cosmetic categories (including wipes), topical pharmaceuticals, household products, and products encountered in the occupational setting.

Consumer exposure can change over time, both qualitatively and quantitatively.

Different routes of exposure are reflected by certain anatomical sites affected: deodorants are associated with axillary dermatitis, the axillary skin being particularly vulnerable to sensitisation due to occlusion, maceration and irritation. However, while sensitisation and initial disease may follow a distinct pattern, later less specific exposures, e.g. via hand creams, cleaning lotions etc. may be sufficient to cause allergic contact dermatitis.

11. Dose-response relationships and thresholds

The dose-response relationship between exposure to contact allergens and induction of allergy, i.e. sensitisation, is well established in animal models and by experiments in healthy volunteers (270). It seems that not only the dose per unit area of allergen (271), but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (272). The induction of contact allergy is an immunological process (type IV-allergy), which is without any clinical symptoms. In the case of continued exposure or re-exposure with a sufficient dose of allergen, elicitation will occur. Elicitation is an inflammatory response (eczema) with clinical symptoms of erythema, induration and in some cases vesicles. Studies of the elicitation response are normally done in patients with an allergy to the substance in question. Different provocation models exist (see chapter 11.2.1). Elicitation experiments in healthy human volunteers following the induction have only rarely been performed (273) and may be considered a less valid model than patient studies. The reason is that following experimental induction, the level of sensitivity may not be at the same level as in a real life situation and that individuals who have actually acquired the disease are a more relevant endpoint to study.

Knowledge of the dose-response relationship provides an opportunity to establish levels of exposure which are safe for the majority of individuals. In the following chapter, the use of different data and models for the establishment of such safe levels in relation to fragrance ingredients are explored. The focus will be on those chemicals, which have been identified in chapter 7.1 as established contact allergens in humans and which have already given rise to a significant number of published cases (category 3 or more): cinnamal, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, isoeugenol. Limonene and linalool are considered in chapter 5 as their ability to cause sensitisation depends on air oxidation, and hydroxyisohexyl 3-cyclohexene carboxaldehyde is considered in chapter 4.3.2 and 11.4.

11.1. Induction

A model for dermal sensitisation quantitative risk assessment (QRA) has been developed and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments, mainly the local lymph node assay (LLNA) (see chapter 8.1). A set of safety factors are applied for inter-individual differences, for vehicle effects and for use considerations, stated to give rise to a safety margin from 10 to 1000 (274). In this way, a so-called “acceptable exposure level” is derived. The exposure to an allergen in different types of products should be below this level. The restrictions, which have been introduced by the fragrance industry based on the QRA model, are given in Table 11-1 for some important product categories.

The IFRA guidelines give concentration limits for 11 product categories (http://www.ifraorg.org/en-us/standards_1, last accessed 2011-11-02), three of which are mentioned in Table 11-1. These three products have the lowest concentrations except for lip products, which give a slightly lower concentration limit.

Table 11-1: Current IFRA restrictions based on induction experiments.

Fragrance chemicals	IFRA guideline ¹		
	Deodorant (%)	Hand cream (%)	Perfume (%)
Cinnamal	0.02	0.05	0.05
Cinnamyl alcohol	0.1	0.4	0.4
Citral	0.05	0.3	0.6
Coumarin	0.13	0.8	1.6
Eugenol	0.2	0.5	0.5
Farnesol	0.11	0.6	1.2
Geraniol	0.4	2.8	5.3
Hydroxycitronellal ²	0.2	1.0	1.0
Isoeugenol ²	0.01	0.02	0.02

Notes: 1) Exposure per mg/cm²/day is based on 8.5 mg/cm²/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.
 2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

The SCCP evaluated this methodology (275) as well as its application to three model fragrance substances.

It was, among other things, concluded that:

“The data provided show that the application of the dermal sensitisation QRA approach would allow increased exposures to allergens already known to cause allergic contact dermatitis in consumers. The model has not been validated and no strategy of validation has been suggested. There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer.”

and that:

“Identification of safe levels of exposure to existing substances known to cause allergic contact dermatitis in the consumer should be based on clinical data and/or elicitation low-effect levels. Currently, these are the only methods which have proven efficient in reducing/preventing existing problems of sensitisation/allergic contact dermatitis in the consumer.”

11.2. Elicitation

11.2.1. General considerations

A response in terms of elicitation of allergic contact dermatitis by application of the (suspected) allergen under standardised conditions is the outcome of interest of the routine diagnostic procedure for suspected contact allergy, the patch test. While the patch test procedure is largely standardised, it is optimised as a diagnostic tool for contact allergy. Thus exposure conditions are not comparable to actual exposures occurring in the daily life or working environment of the patient, which often involve long-term, repeated and low-dose contact with the allergen. Here, procedures such as the repeated open application test (ROAT) or provocative use test are often used, because they reflect actual exposure much better and can be used, for instance, to validate the current clinical relevance of a positive PT reaction.

Generally, exposure of a sensitised patient to a set of graded doses (quantity/area) of the suspected allergen, i.e. threshold testing, will allow not only quantitative diagnosis of the presence or absence of specific contact sensitisation but will additionally provide evidence on the intensity (degree) of sensitisation. This may have important individual consequences in terms of everyday or occupational exposures being capable (or not) of eliciting allergic contact dermatitis. However, beyond the individual perspective, clinical dose-response data collected from sensitised individuals provide a valuable estimate of the usual doses/unit area resulting in a positive, allergic response in a certain proportion of sensitised persons, e.g. 10, 50 or 90%. Maximum concentration levels can be derived, which are safe in terms of eliciting allergic reactions in only a defined low percentage of sensitised persons. As such data will always be based on small samples, the precision of the estimate should be considered, and therefore results are preferably given with confidence intervals.

A statistically significant relationship between threshold concentrations in the ROAT and patch test has been found, on analysing results from different allergens (see Table 11-2) (276), but the dose of allergen per unit area per application needed to elicit a reaction in the two study methods is not the same. A translation factor between the two methods has been suggested for non-volatile substances: $ED_{xx}(ROAT) = 0.0296 \cdot ED_{xx}(\text{patch test})$ based on testing nickel and methyl dibromo glutaronitrile (276). Based on this the eliciting dose per application in an open test is 33 times lower than in the patch test. In practice it means that the cumulative dose in a ROAT (in $\mu\text{g}/\text{cm}^2$) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in $\mu\text{g}/\text{cm}^2$) for a given number of responders (see Figure 11-1). For a given cut-off point the elicitation dose determined by patch testing will be higher than determined by ROATs.

Table 11-2: Spearman's rank correlation between the threshold concentration in the patch test and the repeated open application test for three allergens.

Allergen	Number of patients	Correlation coefficient	P-value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011

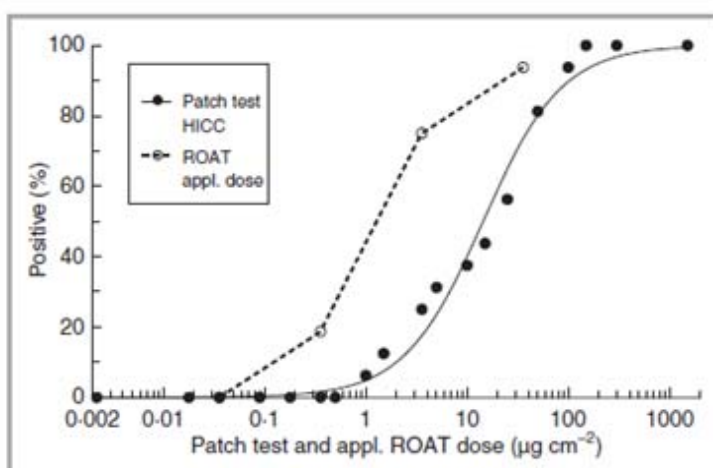


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded application as in the patch test.

In the translation between methods, evaporation needs to be taken into consideration for volatile substances. The experience, based on a study of the fragrance ingredient HICC and using the results from the literature on isoeugenol, is that if the same equation is used as for non-volatile substances, the response in the ROAT will be overestimated by a factor 3 to 4. Thus, the translation factor would be 0.1060 instead of 0.0296, but this needs to be confirmed by other fragrance allergens. This implies that for the fragrance ingredients tested, the eliciting dose per application in a ROAT was 9.4 times lower than the patch test compared to a 33 times lower dose for non-volatile substances (276). This needs to be confirmed by studying other fragrance allergens. Thus, according to these experiments, the dose ($\mu\text{g}/\text{cm}^2$) eliciting a response in threshold patch testing will be at most 33 times higher than established in the ROAT if an identical vehicle is used.

Volatility effects in skin sensitisation

The potency of volatile skin sensitisers can be underestimated, to an extent depending on how rapidly it evaporates, by assays such as the LLNA in which the test substance is applied topically to exposed healthy skin without occlusion. Such sensitisers present a greater sensitisation risk to consumers when the skin is occluded by clothing and/or compromised, than when healthy non-occluded skin is exposed.

Volatility at physiological temperature, say 40°C , is represented by the vapour pressure p_{40} at that temperature. This is related to the boiling point T_B by the Clapeyron-Clausius equation, which can be written (277):

$$\text{Log}(p_{40}) = - (T_B - 40)\text{Tr}/2.303RT$$

Where p is in atmospheres, T_B is in $^\circ\text{C}$, R is the gas constant, Tr is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to $22 \text{ cal}\cdot\text{deg}^{-1}$ for many organic compounds) and T is physiological temperature in degrees absolute ($= 313$ for 40°C).

It has been shown, in experiments where evaporation from a glass slide is measured under simulated LLNA conditions, that 2-hexenal ($T_B = 146\text{-}149^\circ\text{C}$, $p_{40} = 17 \text{ mmHg}$) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal ($T_B = 248^\circ\text{C}$, $p_{40} = 0.5 \text{ mmHg}$), more than 90% remains after 1 hour (278). In agreement with these findings, cinnamal fits a QSAR relating LLNA EC3 to reactivity, whereas the EC3 for 2-hexenal is higher (lower potency) than predicted from its reactivity.

The above is only a partial rationalisation, since different solubilities in different vehicles will influence the tendency to evaporate, according to Henry's law.

11.2.2. Studies on specific fragrance ingredients

Studies concerning chloroatranol/atranol, cinnamal, hydroxycitronellal, hydroxyisohexyl 3-cyclohexenecarboxaldehyde and isoeugenol have been identified. These are summarised in Annex III.

Overview of results

In four studies dummy deodorants spiked with a single fragrance allergen in realistic use concentrations have been used to study elicitation responses, unscented deodorants were used as control products in paired designs. The deodorants were used by patients sensitised to the fragrance allergen in question as well as a healthy control group

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(without fragrance allergy) (102,103,104,279). Between 76 and 100% of the sensitised individuals reacted to the deodorants spiked with allergen, isoeugenol, cinnamal, hydroxycitronellal and hydroxyisohexyl 3-cyclohexene carboxaldehyde, and none of the controls (Table 11-4).

Table 11-3: Overview of results of deodorant provocation investigations with different allergens. Frequency in % of test groups, which reacted at different doses of allergen applied in a roll-on deodorant in the axilla, is given in the table.

Dose in ppm in deodorant	Isoeugenol	Cinnamal (1)	Cinnamal (2)	Hydroxycitronellal	HICC
0	0	0	0	0	0
63	23				
100			11		
200	69				64
320		25	55	57	
600					85
630	76				
1000		75	88	71	
1800					100
3200		100		100	
No. test persons	13	8	9	7	14
No. of control persons	10	20		7	10
% control persons, who reacted	0	0		0	0
Exposure according to study should be:	< 63 ppm	<100 ppm		<320 ppm	< 200 ppm
Reference	(279)	(103)		(104)	(102)

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Eleven studies concerning dose-response results of the five allergens listed above were identified, including the above mentioned studies of deodorants. An overview of the results of the studies concerning thresholds is given in Table 11-4. In Annex III the details of each study are given.

Table 11-4: Overview of threshold results from clinical studies.

“Observed” means that the proportion was actually observed in the study while “estimated” means that the value is derived from a fitted curve, i.e. is interpolated.

Chloroatranol			
ROAT			Ref.
In ethanol 92 % positive	0.025 µg/cm ²	observed	(238)
In ethanol 100% positive	0.125 µg/cm ²	observed	(238)
PATCH TEST			

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ED10%	0.0004 µg/cm ²	estimated	(238)
ED50%	0.0045 µg/cm ²	estimated	(238)
Cinnamal			
ROAT			
In ethanol no effect	0.02%	observed	(101)
In ethanol 44 % positive	0.1%	observed	(101)
In ethanol 72 % positive	0.8%	observed	(101)
Deodorant matrix 11% positive	0.26 µg/cm ² (0.01%)	observed	(103)
Deodorant matrix 41% positive	0.84 µg/cm ² (0.032%)	observed	(103)
Deodorant matrix 82% positive	2.63 µg/cm ² (0.1%)	observed	(103)
PATCH TEST			
ED50%	96 µg/cm ²	estimated	(101)
No effect level	0.4 µg/cm ² (0.01%)	observed	(101)
No effect level	NG (0.002%)	observed	(103)
HICC			
ROAT			
In a cream base ED10%	4.9 µg/cm ²	interpolated	(105)
In a perfume (ethanol) ED10%	1.2 µg/cm ²	interpolated	(105)
In ethanol 61% positive	15.3 µg/cm ² (3.4-22.2)	observed	(224)
In ethanol 89% positive	126.2 µg/cm ² (40.5-226.2)	observed	(224)
In ethanol/water no response	0.0357 µg/cm ²	observed	(263)
In ethanol/water ED10%	0.064 µg/cm ²	estimated	(263)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm ² (median)	observed	(102)
PATCH TEST			
ED10% (95% CI)	0.662 µg/cm ² (0.052-2.35)	estimated	(263)
ED10%	0.75 µg/cm ²	estimated	(102)
ED10%	0.9 µg/cm ² 29 (7-69) ppm	estimated	(224)
ED50% (95% CI)	11.1 µg/cm ² (3.41- 33.1)	estimated	(263)
ED50% (95% CI)	18.3 µg/cm ² (3.41- 33.1)	estimated	(102)
ED50% (95% CI)	20 µg/cm ² 662 (350-1250) ppm	estimated	(224)
No effect level	<0.0022 µg/cm ²	observed	(263)
Hydroxycitronellal			
ROAT			
Deodorant matrix 57 % positive	0.94 µg/cm ² (0.032%)	observed	(104)
Deodorant matrix 71 % positive	2.94 µg/cm ² (0.1%)	observed	(104)
Deodorant matrix 100 % positive	9.40 µg/cm ² (0.32%)	observed	(104)
PATCH TEST			

No effect level	<0.00012 % (=0.036 µg/cm ²)* (*calculated)	observed	(104)
Isoeugenol			
ROAT			
in ethanol 63% positive	5.6 µg/cm ²	observed	(100)
in ethanol 42% positive	2.2 µg/cm ²	observed	(264)
in ethanol 67% positive	9.0 µg/cm ²	observed	(264)
Deodorant matrix 23 % positive	0.167 µg/cm ²	observed	(279)
Deodorant matrix 69 % positive	0.53 µg/cm ²	observed	(279)
Deodorant matrix 77 % positive	1.67 µg/cm ²	observed	(279)
PATCH TEST			
ED50% (in petrolatum)	32 µg/cm ²	estimated	(100)
No effect (in ethanol)	<0.0005% (0.15 µg/cm ²)	observed	(264)
No effect (in petrolatum)	<0.4 µg/cm ²	observed	(100)

Summary of results for specific fragrance ingredients

Chloroatranol (constituent of *Evernia prunastri*)

In ROAT a dose of 0.025 µg/cm² to 0.125 µg/cm² in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm².

Cinnamal

In ROAT a dose of 0.26 µg/cm² gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm².

The ED50 in patch testing was 96 µg/cm².

HICC

In ROAT a dose of 0.0357 µg/cm² gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm² to 1.2 µg/cm². The dose in a cream base was 4.9 µg/cm².

In ROAT a dose of 15.3 µg/cm² to 126.2 µg/cm² in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm².

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm² gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm².

The no-effect level in patch testing was below 0.036 µg/cm².

Isoeugenol

In ROAT a dose of 2.2 µg/cm² a response in 42% and 9.0 µg/cm² in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm² caused a response in 23% and 77% reacted to 1.67 µg/cm².

The ED50 in patch testing was 32 µg/cm².

The no-effect in patch testing was below 0.15 µg/cm².

Elicitation levels have been studied for cinnamal, isoeugenol and hydroxycitronellal which are established contact allergens in humans and which already have given rise to a significant number of cases (> 100, see chapter 7). Further HICC has been studied extensively, but is considered in a separate section (chapter 11.3) of this opinion. It is however not possible to derive a safe threshold directly from the data of cinnamal, isoeugenol and hydroxycitronellal. The main reasons are that many of the test subjects reacted to all the tested doses in ROAT, which is a simulation of every day exposures. Thus it was not possible to determine the dose only eliciting responses in a few, e.g. 10% of the subjects and that only a limited number of exposure scenarios were studied.

The studies have covered few product types: hydro-alcoholic products, e.g. perfumes and deodorant roll-on matrix. The vehicle is one of many factors which influence the thresholds of allergic reactions. Also the presence of irritants and other allergens can influence the elicitation level. This means that the currently available studies do not cover all the relevant exposure scenarios. However, taking into account that dose-response investigations in sensitised patients are very complex to perform, it is not likely that much more data will become available in the near future. It is therefore necessary to exploit the full pool of elicitation data, also covering chemicals other than fragrance ingredients, to derive a more general threshold which could be used when no or insufficient data exist to set a specific threshold for a substance of concern.

General thresholds

The methodology of the different experiments has varied to some extent as different anatomical sites of exposure have been employed, different vehicles, exposure periods and cut-off points. The reason is that the studies have been performed to investigate various clinical and scientific aspects of allergic contact reactions and not for formal regulatory requirements. Some studies are small and for this reason the precision of the estimates of thresholds is limited. In spite of this, the results of the various experiments are reasonably uniform, except for chloroatranol which had very low threshold reactions, and show that low concentrations may elicit allergic reactions.

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent “meta-analysis” of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED₁₀ at patch testing varied by a factor of 7 from the lowest to the highest value and the median was 0.82 µg/cm² if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 µg/cm² if included (see Table 11-6 and Figure 11-2 below: (280)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (280) and guinea pig QSAR evidence (281). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of 0.8 µg/cm² for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED₁₀ value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions: 0.8 µg/cm² (280).

- Exposure doses and exposure areas from SCCS notes of guidance 7th revision (282) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (274).

Equation:

Safe concentration in product = (Generally safe exposure level ($0.8 \mu\text{g}/\text{cm}^2$)/daily exposure to product ($\mu\text{g}/\text{cm}^2/\text{day}$)) x 100 (for %).

Table 11-5: Concentration limits in different product types based on $0.8 \mu\text{g}/\text{cm}^2$ allergen as a 'generally safe exposure level', if specific dose-response data are unavailable.

	Estimated daily exposure level (g) (Table 3 SCCS NoG)	Mean exposed skin surface (cm^2) (Table 2 SCCS NoG)	Exposure $/\text{cm}^2/\text{day}$ in grams	Exposure $/\text{cm}^2/\text{day}$ in μg ($1\text{g} = 1 \times 10^6 \mu\text{g}$)	Concentration limit in product % in product: (GEL/daily exposure) x 100
Body lotion	7.82 g	15,670 cm^2	0.000499	499	0.16%
Face cream	1.54 g	565 cm^2	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 cm^2	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221 ¹⁾	2210	0.04%

Note: 1) $2.21 \text{ mg}/\text{cm}^2/\text{day}$ from Technical dossier Quantitative Risk Assessment.

The estimated daily use of the various product categories in Table 11-5 are based on the SCCS Notes of Guidance (see above), except for perfume, for which no value is given. This value is taken from the Technical Dossier on Quantitative Risk Assessment from RIFM.

Generally the estimated use of different products is higher in the IFRA/RIFM assessments than in SCCS Notes of Guidance.

Table 11-6: Overview of dose-response studies and thresholds for eight allergens, after (280).

ED₁₀ patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (283), MCI/MI (Kathon TM CG) (284), nickel (285), methylidibromo glutaronitrile (MDBGN) (286), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (102, 224, 263), isoeugenol (264, 279) and formaldehyde (287). The shaded values were considered as outliers.

Study	Number of patients	ED₁₀ ($\mu\text{g}/\text{cm}^2$)	95 % interval
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59

Study	Number of patients	ED ₁₀ (µg/cm ²)	95 % interval
Nickel 1999	26	7.49	2.42–14.5
Nickel 2005	13	0.74	0.066–2.38
Nickel 2007	20	0.82	0.13–2.37
Cobalt 2005	11	0.44	0.033–1.3
Chromium	17	1.04	0.0033–5.55
Isoeugenol 2001	24	1.48	0.22–4.74
Isoeugenol 2005	13	0.23	0.0073–1.32
HICC 2003	18	0.85	0.062–3.26
HICC 2007	14	1.17	0.043–5.05
HICC 2009	17	0.66	0.052–2.35
MDBGN 2004	19	0.025	0.00021–0.19
MDBGN 2008	18	0.50	0.052–1.69

Note: The ED₁₀ value is the concentration which elicits an allergic reaction in 10% of a group of sensitised individuals under patch test conditions.

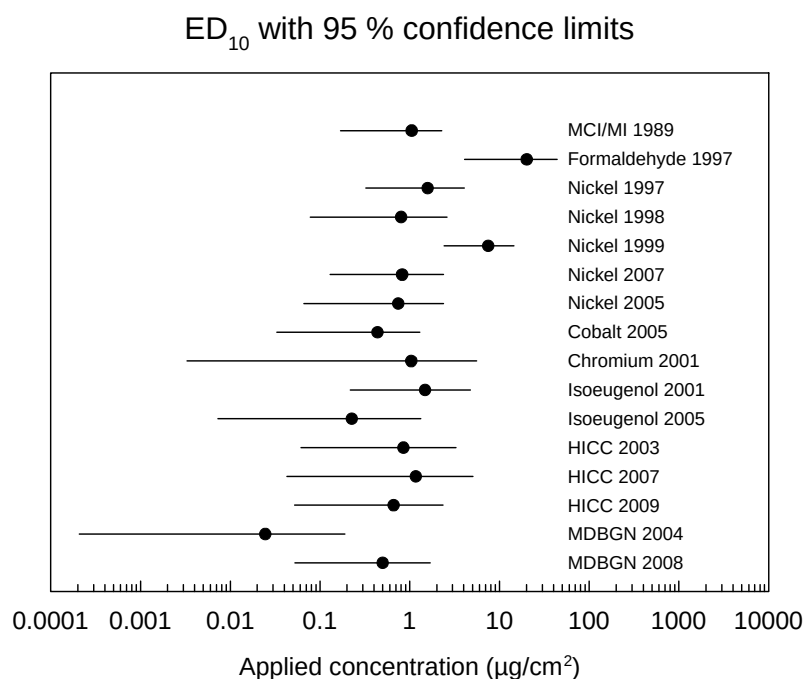


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

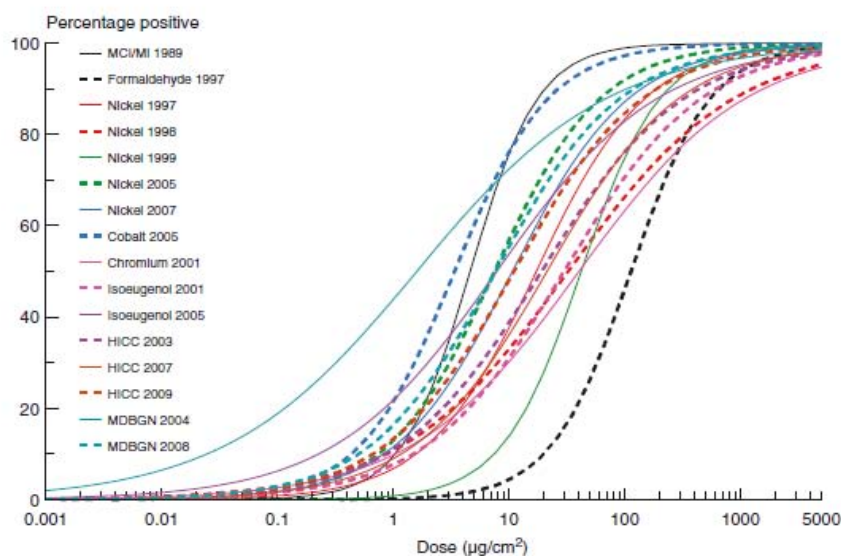


Figure 11-3: The fitted dose-response curves from the studies in Table 11-6, which are the basis for estimation of the ED10 value, after (280).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was $0.8 \mu\text{g}/\text{cm}^2$. In the model data for the fragrance substances isoeugenol and HICC was included. The two studies on isoeugenol and the three studies on HICC gave an average ED10 value of $0.85 \mu\text{g}/\text{cm}^2$ and $0.89 \mu\text{g}/\text{cm}^2$ with a range 0.23-1.48. This means that even if the model was used for these substances individually the result would be very similar to the general threshold value.

The data from cinnamal and hydroxycitronellal studies was not incorporated in the model because: (i) serial dilution patch testing was done in petrolatum for cinnamal, making the dosing less exact; (ii) and only seven patients participated in the hydroxycitronellal study, while a criteria for inclusion in the model was ten participants (280).

According to the above calculations, a limit of $0.8 \mu\text{g}/\text{cm}^2$ for the product types of most importance for fragrance allergy corresponds to concentrations of 100 to 400 ppm (0.01-0.04%) for deodorants, perfume spray, hand and face lotions. For body lotion the general threshold was 0.16%. However, it does not seem meaningful in the context of contact allergy to distinguish between different types of creams, as a body cream would be applied with the hands and the relevant parameter in contact allergy is dose per area skin and not total dose.

A general threshold would have to take into consideration the uncertainties in quantification of exposure and safe thresholds as well as the possibilities of aggregate exposures and exposure to chemically similar substances. Therefore in setting one general threshold the product category carrying the highest risk of sensitisation and elicitation, which is deodorants, was chosen to drive the generation of the threshold. This means that a threshold of $0.8 \mu\text{g}/\text{cm}^2$ is equal to 0.01% or 100 ppm (see Table Table 11-1 and the related text), the lowest of the threshold values derived.

The approach taken by the SCCS is based on scientific evidence published in peer-reviewed journals (283)(284)(285)(286)(102, 224, 263)(264, 279)(287) in the past 20 years. The meta-analysis deriving the general threshold limit at $0.8 \mu\text{g}/\text{cm}^2$ limit has been published (280) in a peer-reviewed journal. The use of threshold limits based on elicitation data is a well established methodology which has been applied (with success) in EU to prevent further cases of induction and elicitation (primary and secondary prevention) in the case of nickel allergy, chromium in cement, chromium in shoes in

Germany, dimethyl fumurate in consumer items and also in part in IFRA guidelines e.g. concerning HICC.

The elicitation threshold model is based on 16 studies of 8 allergens, two of which are fragrance ingredients. It includes data from moderate to extreme allergens with a median EC3 value of 1.2.

The 11 fragrance allergens to which the limit is suggested to apply range from extreme to moderate with median EC3 value of 4.8, although in the case of coumarin an EC3 value could not be established.

Thus in general the potency profile of the fragrance substances of concern is not very different from those included in the model to provide the suggested general safe threshold.

The approach is targeting the relevant end-point, namely, allergic contact dermatitis. The mere consideration of potency of the allergen, according to the LLNA (EC3), is insufficient in identifying the size of the problems of contact allergy/allergic contact dermatitis. Additional information is needed from clinical and epidemiological studies, exposure assessment and dose-elicitation studies. For instance, the elicitation thresholds of e.g. HICC (EC3: 17.1) and isoeugenol (EC3: 0.54) are very similar (0.85 µg/cm² and 0.89 µg/cm², respectively) despite very different potencies. Both are frequent causes of contact allergy.

It should be noted that the general threshold is only suggested to be used for substances of concern if no specific data of sufficient quality exist to set an individual safe threshold. In cases where specific data of sufficient quality are available, these data should be used to set an individual safe threshold.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (288) and in a reduction in morbidity, i.e. elicitation (289). Another example is restriction of chromium VI in cement (290).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens (239). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most

frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (291)) (for more studies see chapter 4.3.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (69). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (105). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (102).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (292). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see 11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: "The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material" (293). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (293).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/ antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcohols for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcohols for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02

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Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.

QRA Quantitative risk assessment.

* Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the pre-consultation version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany (294)) and 2003-2011 (Danish contact dermatitis group (295)), respectively. The first analysis identified a slight decrease, which was considered “not overwhelming in absolute terms”, namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

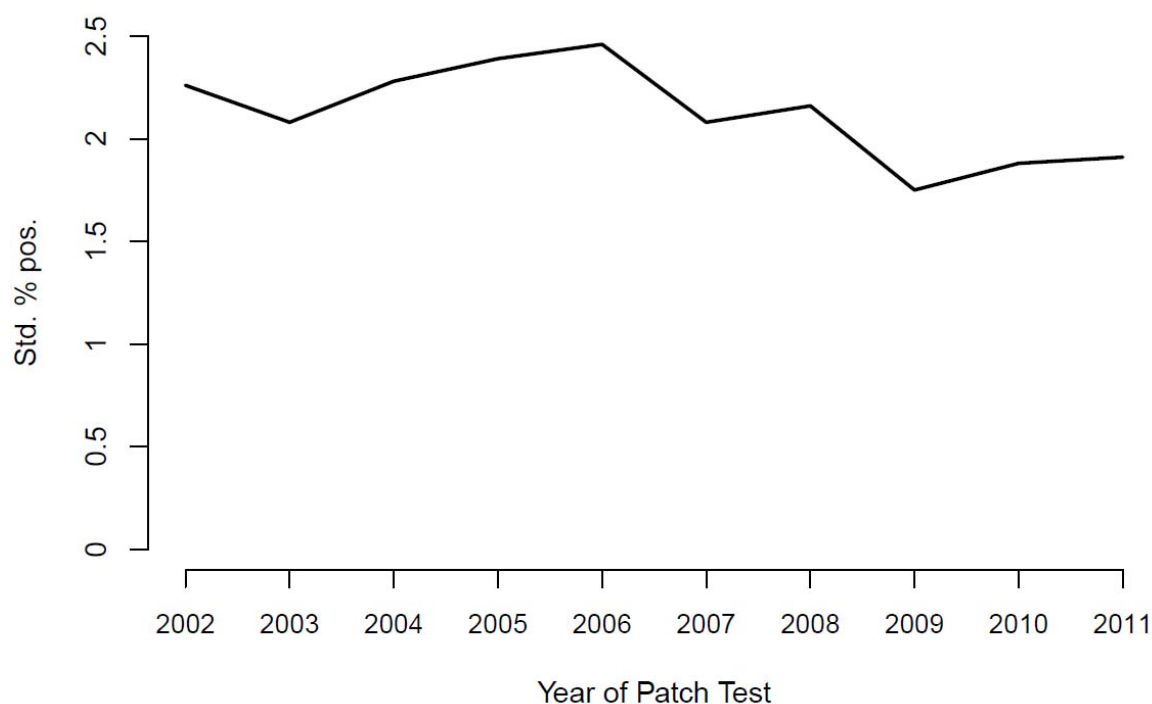


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

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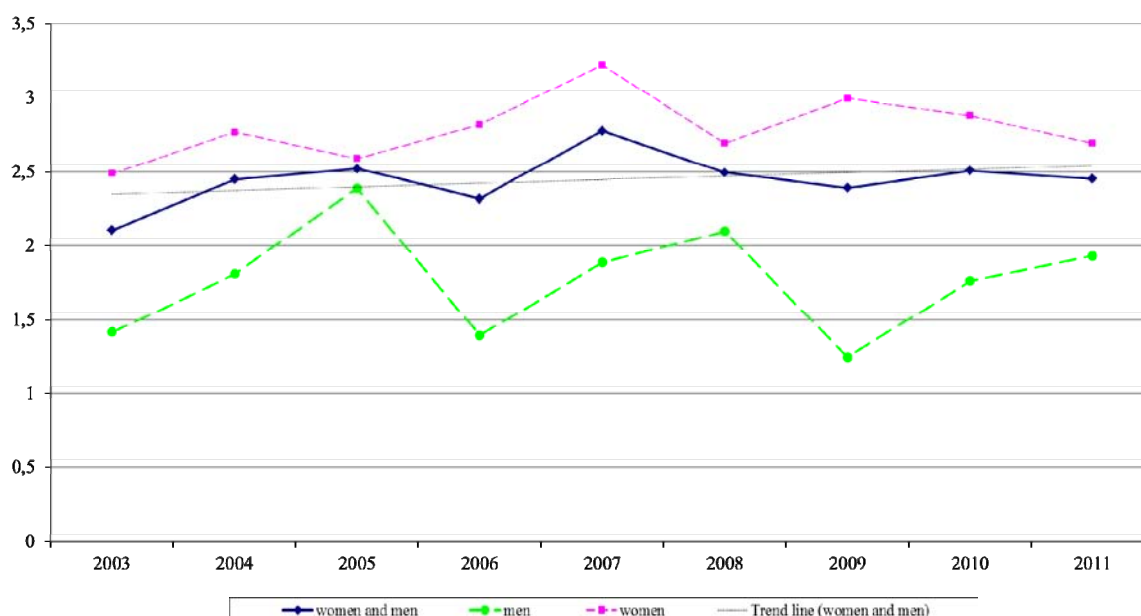


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: "There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."
- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, "safe use" means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of $0.8 \mu\text{g}/\text{cm}^2$ was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.

- It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.
- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.
- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.
- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents, despite efforts to reduce the allergen content (296).

12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up 10% of the European population carry mutations, which impairs the skin barrier and which seem to increase the risk of fragrance allergy. Data are needed to qualify and quantify the increase in risk of susceptible groups in order to provide a better protection of all consumers.
- Aberrant enzyme activity in certain individuals, often related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations. More research into the role of relevant traits is needed.
- Dose-response data from clinical studies are available for only a few allergens. To establish individual safe levels such data are required for all established allergens of concern and covering an appropriate range of product types. This would also consolidate the basis of the use of a general threshold for safe use of fragrance allergens.
- Data on human exposure to fragrances from the use of different product categories is very scarce and therefore does not provide an optimal basis of risk assessment, e.g. exposure data on use for perfume/eau de cologne are lacking.
- Most experimental studies are done on individual fragrance ingredients, while exposure to allergens in cosmetic products is usually to mixtures of allergens. The risk of sensitisation and elicitation may depend on the mixture of substances, but very few studies on this exist. It is necessary to improve the knowledge base on cocktail effects on sensitisation/elicitation to improve the basis of risk assessment and management.
- Screening in dermatitis patients should be performed with air exposed samples of such fragrance substances that in experimental studies have been demonstrated to act as prehaptens, i.e. autoxidise and form oxidation mixtures containing allergenic oxidation products.
- Patch testing should if possible, be performed with the isolated true haptens formed from prehaptens and prohaptens to increase the possibility to diagnose allergy from these type of substances.
- There is a need for more experimental research to further establish the impact of the behaviour of fragrance substances when applied on the skin (including factors such as volatility, autoxidation, skin penetration, reactivity in skin and bioactivation).

12.2. Non-human studies

- Several studies in the industry submission (164) were of insufficient quality, not following the OECD guidelines.
- In some cases it was found that either very few concentrations points had been used in LLNAs, or concentrations were insufficient for achieving a 3-fold increase of the SI.

A sufficient number of doses (concentrations) should be applied in LLNAs (at least 5) so that interpolation (for deriving an EC3 value) can rely on more than two or three actual data points to be more reliable. SCCS therefore suggests a change in the OECD guideline 429. (It is important to remember that the production of unreliable data is a waste of animals.) Moreover, the maximum concentration should be high enough to achieve a > 3-fold increase in SI, as far as this is possible with the substance/vehicle combination chosen.

- Data on experimental results are often not published, but available only on file in the companies having performed the tests. Access to such results would be important for the scientific community, e.g. in the context of REACH, or independently, either to the public domain, or to a Public Trustee.
- The OECD guideline 429 recommends several vehicles. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus, as an additional control, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.
- As long as no validated *in vitro* method exists, more research is needed. Until one or more method(s) have been decided to fulfil the requirements for substituting *in vivo* testing, the *in vivo* testing for prediction of skin sensitisation has to be used.
- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances. Therefore, further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is needed to increase the safety for the consumer, i.e. experimental studies which include air oxidation and bioactivation.
- Further experimental investigations of the sensitisation potential of fragrance substances are needed to determine the impact of the volatility of the substance as well as the effect of the vehicle on skin penetration/absorption and reactivity.
- From a clinical perspective it is important for the individual who is sensitised to one fragrance substance to know if they must also avoid other fragrance substances that can cause allergic contact dermatitis due to cross-reactivity with the original sensitiser. Prediction of risks for cross-reactivity requires sound application of theoretical principles in combination with well-designed experimental studies. This is a field that has not been studied very much so far and needs to be focused on much more in the future.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods as possible. Prediction of different activation pathways should be included.
- Effect estimates such as proportions of sensitised humans or animals, or mean stimulation indices, EC3 values and other derivations should ideally be accompanied by an interval estimate (confidence interval) to address precision (297).

13. Opinion

Contact allergy to fragrances is a common, significant and relevant problem in Europe. The studies since the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 26 fragrance allergens, identified by the SCCNFP, are still relevant fragrance allergens for consumers because of their exposure from cosmetic products. Additional exposure to many of these 26 fragrance allergens also occurs from the use of other consumer products, such as detergents, toys, etc. Some of these fragrance substances are also used as preservatives.

The overall trend of fragrance contact allergy appears to have been stable for the last 10 years, as some causes of fragrance allergy have decreased and others increased. From the few population-based studies, it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. This is based on the limited testing with eight common fragrance allergens (FM I) out of the approximately 2500 fragrance ingredients listed in CosIng and indicative of the substances that may be present in fragrance compounds. However, the real prevalence of contact allergy to fragrance substances may be higher if the testing were to be performed with the full spectrum of fragrance allergens, including oxidised substances, where relevant.

Among eczema patients in the European population, around 16% are sensitised to fragrance ingredients. The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Contact sensitisation, and its clinical manifestation, allergic contact dermatitis, can be prevented if the exposure to known contact allergens is reduced or abolished (primary prevention). Experiences so far, have indicated that not all substances that later turned out to be significant contact allergens after human exposure, were predicted by experimental studies, e.g. the preservative methyldibromo glutaronitrile and the fragrance chemical HICC. Thus, a significant exposure of the population may occur before a substance is established as an important contact allergen in man.

Elicitation of allergic contact dermatitis occurs when a consumer sensitised to a certain substance is re-exposed to the substance in question. Prevention at this stage, termed secondary prevention, can be achieved if use of the allergen in products is eliminated or reduced to a tolerable level (general prevention), or if the patients succeed in avoiding all sources of exposure (individual prevention). Ingredient listing of individual fragrance allergens has been shown to be an important tool to enable consumers with an identified allergy to reduce/avoid relevant exposures. Moreover, ingredient listing is also of great importance to ensure that an adequate diagnosis of fragrance contact allergy can be made without undue delay. If the information given on the presence of fragrance allergens is incomplete, diagnosis of fragrance contact allergy may be missed.

The SCCNFP, in its 1999 opinion, identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the European Cosmetics legislation (298) as ingredient labelling of these 26 fragrance substances (Annex III, entries 67-92). However, safe use concentrations for these substances in cosmetic products have not yet been determined and much new evidence concerning fragrance allergy has been published since 1999. The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature up to October 2010. This review addresses the issue of contact allergy to fragrance substances, including natural extracts and updates the list of fragrance allergens relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of: (i) established fragrance allergens; (ii) likely fragrance allergens; and (iii) possible fragrance allergens. The review also includes fragrances, which on modification by oxidation or by enzyme mediated processes, can produce allergens. Available dose-response data have been

examined to answer whether safe thresholds can be established for the most frequent fragrance allergens.

13.1. Question 1

Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?

In order to answer this question, the SCCS has used clinical and epidemiological data to identify known fragrance allergens. These were categorised as *established contact allergens in humans* (see Table 13-1).

Where sufficient animal evidence was present, these substances were categorised as established contact allergens in animals (Table 13-2). For a number of other fragrance substances, combinations of limited clinical data together with SAR considerations have been applied to indicate likely fragrance allergens in man (Table 13-3). Finally, SAR has also been applied to substances that lack human data to identify fragrance chemicals that have the structural potential to be contact allergens. Substances with insufficient human data were also considered as possible fragrance allergens. For these further tests (experimental/clinical data) are required (Table 13-4).

Table 13-1: Established contact allergens in humans.

For categorisation of importance (+ to +++) see chapter 7.1. Allergens of special concern are substances where between 100 and 1,000 cases (+++) and more than 1,000 (+++++) have been published. These are set in bold. Fragrance substances identified as allergens in the 1999 opinion of SCCNFP (1) are marked with an asterisk.

“ox.” = oxidised; “non-ox.” = non-oxidised; “r.t.” = rarely tested (see chapter 7)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
Individual chemicals		
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL *	122-40-7	++
AMYL CINNAMYL ALCOHOL *	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISE ALCOHOL *	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL *	100-51-6	++
BENZYL BENZOATE *	120-51-4	++
BENZYL CINNAMATE *	103-41-3	++
BENZYL SALICYLATE *	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL *	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL*	104-55-2	+++
CINNAMYL ALCOHOL*	104-54-1	+++
CITRAL*	5392-40-5	+++
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN*	91-64-5	+++
(DAMASCENONE) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL*	97-53-0	+++
FARNESOL*	4602-84-0	++ - +++
GERANIOL*	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
HYDROXYISOHEXYL CARBOXALDEHYDE (HICC)* 3-CYCLOHEXENE	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL*	107-75-5	+++
ISOEUGENOL*	97-54-1	+++
alpha-ISOMETHYL IONONE*	127-51-5	++
(DL)-LIMONENE*	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL*	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-	67801-20-1	++ (r.t.)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CYCLOPENTENYL)PENT-4-EN-2-OL		
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	+
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++
Natural extracts		
CANANGA ODORATA and Ylang-ylang oil	83863-30-3; 8006-81-3	+++
CEDRUS ATLANTICA BARK OIL	92201-55-3; 8000-27-9	++
CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
CITRUS AURANTIUM AMARA FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
CITRUS BERGAMIA PEEL OIL EXPRESSED	89957-91-5	+ (r.t.)
CITRUS LIMONUM PEEL OIL EXPRESSED	84929-31-7	++
CITRUS SINENSIS (syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED	97766-30-8; 8028-48-6	++
CYMBOPOGON CITRATUS / SCHOENANTHUS OILS	89998-14-1; 8007-02-1; 89998-16-3	++
EUCALYPTUS SPP. LEAF OIL	92502-70-0; 8000-48-4	++
EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL	8000-34-8	+++
EVERNIA FURFURACEA EXTRACT*	90028-67-4	+++
EVERNIA PRUNASTRI EXTRACT*	90028-68-5	+++
JASMINUM GRANDIFLORUM / OFFICINALE	84776-64-7; 90045-94-6; 8022-96-6	+++
JUNIPERUS VIRGINIANA	8000-27-9;	++

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
	85085-41-2	
<i>LAURUS NOBILIS</i>	8002-41-3; 8007-48-5; 84603-73-6	++
<i>LAVANDULA HYBRIDA</i>	91722-69-9	+ (r.t.)
<i>LAVANDULA OFFICINALIS</i>	84776-65-8	++
<i>MENTHA PIPERITA</i>	8006-90-4; 84082-70-2	++
<i>MENTHA SPICATA</i>	84696-51-5	++
MYROXYLON PEREIRAE	8007-00-9;	++++
<i>NARCISSUS SPP.</i>	diverse	++
<i>PELARGONIUM GRAVEOLENS</i>	90082-51-2; 8000-46-2	++
<i>PINUS MUGO/PUMILA</i>	90082-72-7 / 97676-05-6	++
<i>POGOSTEMON CABLIN</i>	8014-09-3; 84238-39-1	++
<i>ROSE FLOWER OIL (ROSA SPP.)</i>	Diverse	++
SANTALUM ALBUM	84787-70-2; 8006-87-9	+++
TURPENTINE (oil)	8006-64-2; 9005-90-7; 8052-14-0	++++
VERBENA ABSOLUTE	8024-12-2	++

Table 13-2: Fragrance substances categorised as established contact allergens in animals.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Individual chemicals			
Allyl phenoxyacetate	7493-74-5	none	3.1
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0	none	4.3
CYCLAMEN ALDEHYDE	103-95-7	none	22
Dibenzyl ether	103-50-4	none	6.3
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	limited	7.5
trans-2-Hexenal	6728-26-3	none	2.6
2-Hexylidene cyclopentanone	17373-89-6	none	2.4
HEXYL SALICYLATE	6259-76-3	negative	0.18
p-Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	none	9.5

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Isocyclocitral	1335-66-6	none	7.3
α -Methyl cinnamic aldehyde	101-39-3	none	4.5
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0	none	16.4
METHYLUNDECANAL	110-41-8	none	10
2-Methoxy-4-methylphenol	93-51-6	none	5.8
4-Methoxy- α -methyl benzenpropanal	5462-06-6	none	23.6
METHYL OCTINE CARBONATE	111-80-8	limited	2.5
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	none	8.1
PHENYLACETALDEHYDE	122-78-1	limited	3
Natural extracts			
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	none	35.4

Table 13-3: Fragrance substances categorised as likely contact allergens by combination of evidence.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
AMBRETTOLIDE	7779-50-2	limited	none	+
CARVACROL	499-75-2	limited	none	+
Citrus paradisi §	8016-20-4	none	R43	n.a.
CUMINALDEHYDE	122-03-2	limited	none	+
CYCLOPENTADECANONE	502-72-7	limited	none	+
trans-trans-delta-DAMASCONE	71048-82-3	limited	none	+
2,4-dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	none	R43	+
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	limited	none	+
ETHYL VANILLIN	121-32-4	limited	none	+
HELIOTROPINE	120-57-0	limited	none	+
ISOAMYL SALICYLATE	87-20-7	limited	none	++
ISOLONGIFOLENEKETONE	33407-62-4	limited	none	+
Longifolene §	475-20-7	none	R43	+
Mentha arvensis §	68917-18-0	none	R43	n.a.
METHOXYCITRONELLAL	3613-30-7	limited	none	+
METHYL CINNAMATE	103-26-4	limited	none	++
METHYLIONANTHEME	55599-63-8	limited	none	+

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
5-METHYL-alpha-IONONE	79-69-6	limited	none	+
MYRCENE	123-35-3	limited	none	++
MYRTENOL	515-00-4	limited	none	+
NEROL	106-25-2	limited	none	++
Nerolidol (isomer not specified)	7212-44-4	limited	none	++
NOPYL ACETATE	128-51-8	limited	none	+
PHYTOL	150-86-7	limited	none	+
RHODINOL	6812-78-8	limited	none	+
trans-ROSE KETONE-5	39872-57-6	limited	none	++

§ Substances/natural mixtures were classified as R43, according to the submission by IFRA. The evidence on which this classification was based was not available to the SCCS, so the validity of classification cannot be assessed. Nevertheless, the four substances/substance mixtures should be treated as *likely contact allergens*.

n.a.: not applicable (natural mixture)

Table 13-4: Fragrance substances categorised as possible contact allergens.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
Individual chemicals				
CYCLOHEXYL ACETATE	622-45-7	limited	none	0
ETHYLENE DODECANEDIOATE	54982-83-1	limited	none	0
HYDROXYCITRONELLOL	107-74-4	limited	none	0
METHOXYTRIMETHYLHEPTANOL	41890-92-0	limited	none	0
METHYL p-ANISATE	121-98-2	limited	none	0
METHYL DIHYDROJASMONATE	24851-98-7	limited	none	0
PHENETHYL ALCOHOL	60-12-8	limited	none	0
PHENYLPROPANOL	122-97-4	limited	none	0
AMYL CYCLOPENTANONE	4819-67-4	negative	none	+
BENZYL ACETATE	140-11-4	negative	none	+
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	negative	none	+
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	negative	none	+
alpha-IONONE	127-41-3	negative	none	+
beta-IONONE	79-77-6	negative	none	+
METHYL IONONE (mixture of	1335-46-2	negative	none	+

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC value (min; %)	SAR
isomers)				
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	negative	none	+
alpha-TERPINYL ACETATE	80-26-2	negative	none	+
CITRONELLYL NITRILE	51566-62-2	none	none	++
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0	none	none	+
DECANAL	112-31-2	none	none	++
DIHYDROMYRCENOL	18479-58-8	none	none	+
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6	none	none	++
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6	none	none	+
GERANYL ACETATE	105-87-3	none	none	++
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0	none	none	+
IONONE isomeric mixture	8013-90-9	none	none	+
ISOBERGAMATE	68683-20-5	none	none	+
METHYL DECENOL	81782-77-6	none	none	+
TRICYCLODECENYL PROPIONATE	17511-60-3	none	none	+
OXACYCLOHEXADECENONE	34902-57-3	none	none	++
VERDYL ACETATE	2500-83-6/ 5413-60-5	none	none	+
trans-beta-Damascone	23726-91-2	none	none	+
gamma-Damascone	35087-49-1	none	none	+
Citronellal	106-23-0	none	none	++
Phenethyl salicylate	87-22-9	none	none	++
Natural extracts				
ACORUS CALAMUS ROOT OIL	84775-39-3	Limited	none	
CEDRUS DEODARA WOOD OIL	91771-47-0	Limited	none	
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Limited	none	
CITRUS TANGERINA ...	223748-44-5	Limited	none	
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Limited	none	
ILLICIAM VERUM FRUIT OIL	84650-59-9	Limited	none	
LAVANDULA SPICA	97722-12-8	Limited	none	

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
LITSEA CUBEBA	90063-59-5	Limited	none	
PELARGONIUM ROSEUM	90082-55-6	Limited	none	
SALVIA spp.	Diverse	Limited	none	
TAGETES PATULA	91722-29-1	Limited	none	
THYMUS spp.	84929-51-1	Limited	none	
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	Limited	none	

Regarding the above categorisation of fragrance substances, the following aspects need to be considered when interpreting an outcome other than established contact allergen in humans:

- If human evidence is negative, there is still a potential sensitisation risk, as in this set of substances the number of (consecutive) patients tested was low, i.e. up to a few hundred.
- If EC3 values are given as higher (>) than a certain value (see 8.3), an exact EC3 could not be established, as the substance had been tested in too low concentration(s). In these cases, the substances have not been categorised as 'established contact allergen in animals'.
- For SAR, the categories of prediction are: non-sensitiser (0); possible-sensitiser (+); predicted sensitiser (++); and not predictable (n.p.). (For details see Table 9-3 and Table 9-4). SAR predictions are only considered when human and animal data are limited or missing.
- Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I to this opinion, these substances have not further been evaluated.

Fragrance ingredients listed in Table 13-1 clearly have caused disease in man, and based on the clinical experience alone, these 82 substances were classified as established contact allergens in humans, 54 individual chemicals and 28 natural extracts (mixtures of chemicals), including all 26 fragrance allergens identified by SCCNFP in 1999. For a number of other substances, no patch test data were available, but positive animal data, obtained by a validated guideline method (LLNA) addressing hazard, indicate that a – yet not quantified – risk for humans is very likely to exist, given sufficient exposure. In other cases only in a relatively small number of patients has been tested positively ('limited human evidence'). Here, combination with SAR analyses corroborates the conclusion that these substances, too, are sufficiently qualified to be regarded as 'likely fragrance allergens'.

Of those 82 substances identified as established contact allergens in humans, 12 chemicals (listed in Table 13-5) and eight natural extracts are considered of special concern as they have given rise to at least 100 reported cases. These substances pose a particularly high risk of sensitisation to the consumer and are further considered in the answer of question 2. One substance, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), was shown to be the cause of allergic contact dermatitis in more than 1500 reported cases since 1999. The number of cases is only those reported in scientific publications, and therefore the actual number of cases is severely under-estimated.

Table 13-5: Established fragrance contact allergens of special concern (single chemicals only).

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)

*including their respective esters

The established contact allergens in animals (Table 13-2) and the likely contact allergens, identified based on a combination of limited evidence from man together with positive SAR predictions (Table 13-3), are predicted to cause disease in man given sufficient exposure.

Information on the presence of all the substances given in Table 13-1, Table 13-2 and Table 13-3 in cosmetic products is important in order to enable aimed testing of patients with contact dermatitis and to diagnose fragrance allergy without delay. Further, this information is important to the sensitised consumer as it will enable them to avoid cosmetic products, which they may not tolerate.

Substances given in Table 13-4 are possible contact allergens and further data are required to judge if these are contact allergens in humans and give rise to contact allergy in consumers.

Conclusions - Question 1

The studies since the SCCNFP Opinion on fragrance allergy in consumers (1) have confirmed that the fragrance allergens currently listed in Annex III, entries 67-92 are still relevant fragrance allergens for the consumers from their exposure to cosmetic products.

The review of the clinical and experimental data shows that many more fragrance substances than those identified in the SCCNFP opinion of 1999 have been shown to be sensitisers in humans. A comprehensive list of established contact allergens in humans is given in Table 13-1.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking.

Additionally, limited human and/or animal evidence together with structure activity relationship analysis suggests that other fragrance ingredients may be a cause of concern with regard to their potential of causing contact allergy in humans.

Ingredient listing is important in clinical practice for the management of patients who are allergic to one or more of the listed fragrance chemicals. It is also important for the

patients in order to avoid future exposure to fragrance contact allergens which they may not tolerate.

The SCCS considers that those substances itemised in Table 13-1, Table 13-2 and Table 13-3 represent those fragrance ingredients that the consumer should be made aware of when present in cosmetic products.

Substances known to be transformed (e.g. hydrolysis of esters) to known contact allergens should be treated as equivalent to these known contact allergens. The combined concentration of the alcohol and its ester must be considered regarding exposure. Important indicative, but not exhaustive, examples include isoeugenol and its esters, geraniol and its esters, eugenol and its esters, and linalool and its esters.

13.2. Question 2

Can the SCCS establish any threshold for their safe use based on the available scientific data?

Dose-response relationships exist between exposure to contact allergens and the proportion of consumers who will become sensitised to an allergen (i.e. induction), as well as the proportion who will suffer from allergic contact dermatitis (elicitation). For a number of recognised contact allergens in man, dose-elicitation studies on sensitised individuals are available. These studies indicate that it is in principle possible to derive exposure levels that the majority of sensitised individuals will tolerate. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy and limit the risk of induction.

Among the established chemical fragrance allergens, 12 were identified as posing a high risk of sensitisation to the consumer (Table 13-5), i.e. more than 100 reported cases. For these substances, limitation of exposure would help to protect sensitised consumers from developing allergic contact dermatitis.

In cases where specific data of sufficient quality on threshold levels for a particular allergen are available, these data should be used to set an individual safe threshold. However, when such quality data are not available and a substance has been identified to pose a high risk of sensitisation to the consumer, a general threshold limit can be applied.

Dose-response studies have been performed with only four of these fragrance substances (HICC, isoeugenol, cinnamal and hydroxycitronellal). In addition, such a study has also been performed on chloroatranol, a potent allergen in *Evernia prunastri* and *Evernia furfuracea*. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.

If no adequate data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 µg/cm² has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 µg/cm² under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

The model providing the general threshold of 100 ppm has been based on single substances only and no general safe level for the natural extracts of concern can be

identified, but the maximum use concentration applies to the identified fragrance allergens also when present in the natural extract.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of more than 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I), which will severely underestimate the actual prevalence in the population. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. The SCCP concluded in 2003 that 200 ppm of HICC would be tolerated by the majority of sensitised individuals and this level of exposure would have a low potential to induce sensitisation (241). Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Chloroatranol and atranol are the main allergenic components of *Evernia prunastri* and *Evernia furfuracea*. The SCCS concluded in 2004 (239) that these should not be present in cosmetic products, due to their exceptionally high sensitisation potential. Attempts to effectively reduce the content of these compounds in “oak moss abs.” (300) have largely failed to reduce contact allergy to *Evernia prunastri* and *Evernia furfuracea* and the data presented in this opinion show that the number of cases remains high.

Conclusions - Question 2

There are two components to the safety of fragrance ingredients in terms of contact allergy. First, the need to eliminate or reduce induction of contact allergy (primary prevention), which, when it occurs, is life long. Secondly, the need to eliminate or reduce elicitation reactions (secondary prevention) on the skin of those individuals who are already sensitised. Human dose elicitation experiments have hitherto been performed only for a very small number of substances. It is unlikely that more of these studies will be performed due to experimental and subject recruitment difficulties.

For individual substances, no levels that could be considered safe for the majority of consumers could be established from the available data.

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 µg/cm² (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

Such a threshold based on elicitation levels in sensitised individuals will be sufficiently low to protect both sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy.

The SCCS is of the opinion that for substances identified as posing a high risk to the consumer and for which no individual thresholds could be derived (Table 13-5), the general threshold of 0.01% would limit the problem of fragrance allergy in the consumer significantly.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the above identified fragrance allergens also when present in the natural extract. This will also reduce the risk of sensitisation and elicitation from natural extracts.

It is important to stress that this general threshold, although limiting the problem, does not preclude that the most sensitive segment of the population may react upon exposure to these levels. Hence, this threshold does not remove the necessity for providing information to the consumer concerning the presence of the fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, in 2003 the SCCP suggested that levels of up to 200 ppm would be tolerated by the majority of sensitised individuals. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized. The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to allergenic constituents, despite efforts to reduce the allergen content (296). The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

13.3. Question 3

Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation, and thus increasing the risk of sensitisation.

Experimental and clinical studies have shown that there are fragrance substances that act as prehaptens, i.e. their sensitisation potency is markedly increased by air exposure due to oxidation (autooxidation). Non/low-sensitising compounds are thereby transformed into more potent sensitisers. Limonene, linalool, linalyl acetate, alpha-terpinene and geraniol have all been identified as prehaptens. These fragrance substances are common in scented cosmetics as well as in household products. The clinical studies show that the exposure to allergens formed due to autooxidation causes significant contact allergy in consumers. Patch testing with oxidised limonene and oxidised linalool shows that these substances rank among the most common contact allergens.

In the SAR analyses performed in this work by the SCCS, fragrance compounds with structural alerts that indicate that they are possible prehaptens have been identified (Table 9-1, Table 9-2). In such cases further thorough investigations are needed. It is also important to investigate the stability of the primary oxidation products (the hydroperoxides) formed from various structures of fragrance compounds. The stability of these compounds can have great impact on the sensitisation potency of the oxidised compound as they are strong sensitisers. However, the secondary oxidation products (aldehydes and epoxides) can also be important sensitisers depending on the overall structure of the compound as was demonstrated for oxidised geraniol.

Air oxidation of prehaptens can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autooxidation rate depends not only on the compound itself, but also on its purity. The prevention of autooxidation using antioxidants

needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation. As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

It should be noted that, to decrease the risk for sensitisation in the population, the possibility to reduce the sensitisation potency by preventing autoxidation is important also for a direct acting hapten or prohaptens, if a further activation by air oxidation to more allergenic compounds has been shown.

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 µg/cm² (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

Compounds that are bioactivated by metabolising enzymes to haptens are referred to as prohaptens. Established prohaptens of clinical importance are cinnamyl alcohol, geranial, geraniol, eugenol, isoeugenol and alpha-terpinene.

Table 13-6: Known prehaptens and prohaptens.

Fragrance substance	Activation by air oxidation	Bioactivation (oxidation)	Bioactivation (hydrolysis)
Cinnamyl alcohol		x	
Eugenol		x	
Eugenyl acetate		x	x
Geranial	x	x	
Geraniol	x	x	
Geranyl acetate	x	x	x
Isoeugenol		x	
Isoeugenyl acetate		x	x
Limonene	x		
Linalool	x		
Linalyl acetate	x		
alpha-Terpinene.	x	x	

When bioactivation occurs, the risk of cross-reactivity should be considered. An increased complexity in the cross-reactivity pattern is obtained when a compound could act both as a prehaptens and a prohaptens. For instance, it is known that cinnamyl alcohol and cinnamal can cross-react due to the formation of common sensitising substances. The same applies to geraniol and citral.

In case derivatives of a fragrance substance are used, it must be taken into account that the derivative could be transformed into the parent or a cross-reacting compound. For such derivatives the same rules as for the corresponding parents should apply, unless the

stability of the derivative has been demonstrated. In particular, hydrolysis of esters to the corresponding alcohols can cause cross-reactions. Acetate esters of eugenol, isoeugenol and geraniol are frequently used in cosmetics.

To be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests.

Activation of individual compounds to various haptens increases the risks of cross-reactivity between chemicals and also causes difficulties in prediction of these risks. Prediction of risks requires sound application of theoretical principles in combination with well designed experimental studies. Based on the acquired knowledge, qualified suggestions using structure activity relationship (SAR) regarding many fragrance substances have been made (Table 9-1 to Table 9-3). However, as the stability of formed oxidation products (mainly hydroperoxides) is important for the sensitisation potency, the SAR hypotheses must be followed by experimental investigations for the actual compounds.

Conclusions - Question 3

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation. Fragrances with published data showing the formation of sensitising compounds by autoxidation, bioactivation or both include the following (see also Table 13-6).

Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation are limonene, linalool, and linalyl acetate.

Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation are cinnamyl alcohol, eugenol, isoeugenol and isoeugenyl acetate.

Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation are geraniol and alpha -terpinene.

A fragrance substance that sensitises without activation but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is geranial (one isomer of citral).

In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

The possibility to reduce the sensitisation potency by preventing air oxidation is important also for a direct acting hapten or prohaptens, if a further activation by air oxidation to more allergenic compounds has been shown.

In the case of prohaptens, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geranial (citral) and between cinnamyl alcohol and cinnamal.

Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenyl acetate, eugenyl acetate and geranyl acetate which all are known to be used as fragrance ingredients.

The substances presented above are based on current knowledge and should be seen as indicative and illustrative of the general problem. As substances with structural alerts for acting as pro- and/or prehapten are quite common among the fragrance substances listed (see Tables 9-1 and 9-2), the possibility for activation to generate new potent allergens should be considered.

The SCCS is of the opinion that substances known to be transformed (e.g. by oxidation either via air oxidation or via bioactivation) to known contact allergens should be treated as equivalent to these contact allergens, i.e the same restrictions and other regulatory requirements should apply, unless specific data exist that allow for an individual assessment. Important indicative examples include limonene, linalool, linalyl acetate, geraniol, geranial, alpha-terpinene, eugenol, isoeugenol and cinnamyl alcohol.

14. List of abbreviations

ACD	Allergic contact dermatitis
alc.	Alcohol (as vehicle)
CI	Confidence interval
CLP	Classification, labelling and packaging
coloph.	Colophonium
DCs	Dendritic cells
EC	European Commission
ESSCA	European Surveillance System on Contact Allergies
EDT	Eau de toilette
EDP	Eau de perfume
EU	European Union
FM	Fragrance mix
GC	Gas chromatography
GPMT	Guinea pig maximisation test
HICC	Hydroxyisohexyl 3-cyclohexene carboxaldehyde
HRIPT	Human repeat insult patch test
IFRA	International Fragrance Association (www.ifraorg.org)
IVDK	Information Network of Departments of Dermatology (www.ivdk.gwdg.de)
INCI	International Nomenclature on Cosmetic Ingredients
LCs	Langerhans cells
LLNA	Local lymph node assay
MPR	<i>Myroxylon pereirae</i> resin
NACDG	North American Contact Dermatitis Group
OECD	Organization of Economic Co-operation and Development
ox.	oxidised
pet.	Petrolatum (as vehicle)
ppm	parts per million (10000 ppm = 1%)
PPV	Positive predictive value
PR	Prevalence ratio
PT(ed)(ing)	Patch test(ed) (ing)
QMM	Quantitative mechanistic model
QRA	Quantitative risk assessment
(Q)SAR	(Quantitative) structure activity relationship
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
RIFM	Research Institute for Fragrance Materials (www.rifm.org/)

Opinion on fragrance allergens in cosmetic products

ROAT	Repeated open application test
SC	Single constituents (of one of the fragrance mixes)
SCCS	Scientific Committee on Consumer Safety
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SCCP	Scientific Committee on Consumer Products
UK	United Kingdom
US(A)	United States (of America)
UV	Ultraviolet

15. References

1. SCCNFP. The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers: Opinion concerning Fragrance Allergy in Consumers. A Review of the Problem. Analysis of the Need for appropriate Consumer Information and Identification of Consumer Allergens, adopted 8 December 1999. *SCCNFP/0017/98 Final* 1999:
2. Rustemeyer T, van Hoogstraten I M W, von Blomberg M E, Scheper R J. Mechanisms in Allergic Contact Dermatitis. In: Frosch P J, Menné T, Lepoittevin J P, eds. *Contact Dermatitis*. Heidelberg: Springer, 2006:
3. Johansen J D, Andersen T F, Kjoller M, Veien N, Avnstorp C, Andersen K E, Menne T. Identification of risk products for fragrance contact allergy: a case-referent study based on patients' histories. *Am J Contact Dermat* 1998; 9: 80-86.
4. Marks J G, Belsito D V, DeLeo V A, et al. North American Contact Dermatitis Group patch test results for the detection of delayed-type hypersensitivity to topical allergens. *J Am Acad Dermatol* 1998; 38: 911-918.
5. Johansen J D, Rastogi S C, Menné T. Contact allergy to popular perfumes: assessed by patch test, use test and chemical analysis. *Br J Dermatol* 1996; 135: 419-422.
6. Johansen J D, Rastogi S C, Andersen K E, Menne T. Content and reactivity to product perfumes in fragrance mix positive and negative eczema patients. A study of perfumes used in toiletries and skin-care products. *Contact Dermatitis* 1997; 36: 291-296.
7. de Groot A C, Frosch P J. Adverse reactions to fragrances. A clinical review. *Contact Dermatitis* 1997; 36: 57-86.
8. Cronin E. Contact Dermatitis. *Churchill Livingstone, Edinburgh* 1980:
9. Johansen J D, Andersen T F, Veien N, Avnstorp C, Andersen K E, Menne T. Patch testing with markers of fragrance contact allergy. Do clinical tests correspond to patients' self-reported problems? *Acta Derm Venereol* 1997; 77: 149-153.
10. Johansen J D, Rastogi S C, Bruze M, Andersen K E, Frosch P, Dreier B, Lepoittevin J P, White I, Menne T. Deodorants: a clinical provocation study in fragrance-sensitive individuals. *Contact Dermatitis* 1998; 39: 161-165.
11. Lammintausta K, Kalimo K, Havu V K. Occurrence of contact allergy and hand eczemas in hospital wet work. *Contact Dermatitis* 1982; 8: 84-90.
12. Meding B. Epidemiology of Hand Eczema in an Industrial City. *Acta Dermatol Venerol (Stockh) Suppl* 1990; 153: 2-43.
13. Heydorn S, Johansen J D, Andersen K E, Bruze M, Svedman C, White I R, Basketter D A, Menne T. Fragrance allergy in patients with hand eczema - a clinical study. *Contact Dermatitis* 2003; 48: 317-323.
14. Buckley D A, Rycroft R J, White I R, McFadden J P. Contact allergy to individual fragrance mix constituents in relation to primary site of dermatitis. *Contact Dermatitis* 2000; 43: 304-305.
15. Heydorn S, Menne T, Johansen J D. Fragrance allergy and hand eczema - a review. *Contact Dermatitis* 2003; 48: 59-66.
16. Wöhrl S, Hemmer W, Focke M, Götz M, Jarisch R. The significance of fragrance mix, balsam of Peru, colophony and propolis as screening tools in the detection of fragrance allergy. *Br J Dermatol* 2001; 145: 268-273.

17. Edman B. The influence of shaving method on perfume allergy. *Contact Dermatitis* 1994; 31: 291-292.
18. Heydorn S, Menne T, Andersen K E, Bruze M, Svedman C, White I R, Basketter D A. Citral a fragrance allergen and irritant. *Contact Dermatitis* 2003; 49: 32-36.
19. Rothenborg H W, Menne T, Sjolín K E. Temperature dependent primary irritant dermatitis from lemon perfume. *Contact Dermatitis* 1977; 3: 37-48.
20. Tanaka S, Matsumoto Y, Dlova N, Ostlere L S, Goldsmith P C, Rycroft R J, Basketter D A, White I R, Banerjee P, McFadden J P. Immediate contact reactions to fragrance mix constituents and Myroxylon pereirae resin. *Contact Dermatitis* 2004; 51: 20-21.
21. Hausen B M. Contact allergy to balsam of Peru. II. Patch test results in 102 patients with selected balsam of Peru constituents. *Am J Contact Dermat* 2001; 12: 93-102.
22. Katsarou A, Armenaka M, Ale I, Koufou V, Kalogeromitros D. Frequency of immediate reactions to the European standard series. *Contact Dermatitis* 1999; 41: 276-279.
23. Nakayama H. Perfume allergy and cosmetic dermatitis (in Japanese). *Jpn J Dermatol* 1974; 84: 659-667.
24. Nakayama H, Harada R, Toda M. Pigmented cosmetic dermatitis. *Int J Dermatol* 1981; 15: 673-675.
25. Cronin E. Photosensitivity to musk ambrette. *Contact Dermatitis* 1984; 11: 88-92.
26. Darvay A, White I R, Rycroft R J, Jones A B, Hawk J L, McFadden J P. Photoallergic contact dermatitis is uncommon. *Br J Dermatol* 2001; 145: 597-601.
27. Wang L, Sterling B, Don P. Berloque dermatitis induced by "Florida water". *Cutis* 2002; 70: 29-30.
28. Elberling J, Linneberg A, Dirksen A, Johansen J D, Frolund L, Madsen F, Nielsen N H, Mosbech H. Mucosal symptoms elicited by fragrance products in a population-based sample in relation to atopy and bronchial hyper-reactivity. *Clin Exp Allergy* 2005; 35: 75-81.
29. Kumar P, Caradonna-Graham V M, Gupta S, Cai X, Rao P N, Thompson J. Inhalation challenge effects of perfume scent strips in patients with asthma. *Ann Allergy Asthma Immunol* 1995; 75: 429-433.
30. Millqvist E, Bende M, Lowhagen O. Sensory hyperreactivity--a possible mechanism underlying cough and asthma-like symptoms. *Allergy* 1998; 53: 1208-1212.
31. Elberling J, Linneberg A, Mosbech H, Dirksen A, Frolund L, Madsen F, Nielsen N H, Johansen J D. A link between skin and airways regarding sensitivity to fragrance products? *Br J Dermatol* 2004; 151: 1197-1203.
32. Lindberg M, Matura M. Patch Testing. In: Johansen J D, Frosch P, Lepoittevin J P, eds. *Contact Dermatitis*. Heidelberg etc., : Springer, 2011: 439-464.
33. Basketter D. Diagnostic patch testing - does it have a wider relevance? *Contact Dermatitis* 2012; 67: 1-2.
34. Larsen W G. Perfume Dermatitis. A Study of 20 Patients. *Arch Dermatol* 1977; 113: 623-626.
35. Frosch P J, Pirker C, Rastogi S C, Andersen K E, Bruze M, Svedman C, Goossens A, White I R, Uter W, Arnau E G, Lepoittevin J P, Menne T, Johansen J D. Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix. *Contact Dermatitis* 2005; 52: 207-215.

36. Frosch P J, Rastogi S C, Pirker C, Brinkmeier T, Andersen K E, Bruze M, Svedman C, Goossens A, White I R, Uter W, Arnau E G, Lepoittevin J P, Johansen J D, Menne T. Patch testing with a new fragrance mix - reactivity to the individual constituents and chemical detection in relevant cosmetic products. *Contact Dermatitis* 2005; 52: 216-225.
37. Bruze M, Andersen K E, Goossens A. Recommendation to include fragrance mix 2 and hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall) in the European baseline patch test series. *Contact Dermatitis* 2008; 58: 129-133.
38. Lindberg M, Edman B, Fischer T, Stenberg B. Time trends in Swedish patch test data from 1992 to 2000. A multi-centre study based on age- and sex-adjusted results of the Swedish standard series. *Contact Dermatitis* 2007; 56: 205-210.
39. Temesvari E, Nemeth I, Baló-Banga M J, Husz S, Kohanka V, Somos Z, Judak R, Remenyik E V, Szegedi A, Nebenfuhrer L, Meszaros C, Horvath A. Multicentre study of fragrance allergy in Hungary. Immediate and late type reactions. *Contact Dermatitis* 2002; 46: 325-330.
40. Machovcova A, Dastychova E, Kostalova D, Vojtechovska A, Reslova J, Smejkalova D, Vaneckova J, Vocilkova A. Common contact sensitizers in the Czech Republic. Patch test results in 12,058 patients with suspected contact dermatitis*. *Contact Dermatitis* 2005; 53: 162-166.
41. Lunder T, Kansky A. Increase in contact allergy to fragrances: patch-test results 1989-1998. *Contact Dermatitis* 2000; 43: 107-109.
42. Schnuch A, Lessmann H, Geier J, Frosch P J, Uter W. Contact allergy to fragrances: frequencies of sensitization from 1996 to 2002. Results of the IVDK*. *Contact Dermatitis* 2004; 50: 65-76.
43. Uter W, Geier J, Frosch P J, Schnuch A. Contact allergy to fragrances: current patch test results (2005 to 2008) from the IVDK network. *Contact Dermatitis* 2010; 63: 254-261.
44. van Oosten E J, Schuttelaar M L, Coenraads P J. Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. *Contact Dermatitis* 2009; 61: 217-223.
45. deGroot A C, Coenraads P J, Bruynzeel D P, Jagtman B A, van Ginkel C J W, Noz K, van der Valk P G M, Pavel S, Vink J, Weyland J W. Routine patch testing with fragrance chemicals in The Netherlands. *Contact Dermatitis* 2000; 42: 184-185.
46. Hendriks S A, van Ginkel C J. Evaluation of the fragrance mix in the European standard series. *Contact Dermatitis* 1999; 41: 161-162.
47. Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. *Contact Dermatitis* 2008; 58: 134-141.
48. Brites M M, Goncalo M, Figueiredo A. Contact allergy to fragrance mix--a 10-year study. *Contact Dermatitis* 2000; 43: 181-182.
49. Cuesta L, Silvestre J F, Toledo F, Lucas A, Perez-Crespo M, Ballester I. Fragrance contact allergy: a 4-year retrospective study. *Contact Dermatitis* 2010; 63: 77-84.
50. Katsarma G, Gawkrödger D J. Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. *Contact Dermatitis* 1999; 41: 193-197.
51. Buckley D A, Basketter D A, Kan-King-Yu D, White I R, White J L, McFadden J P. Atopy and contact allergy to fragrance: allergic reactions to the fragrance mix I (the Larsen mix). *Contact Dermatitis* 2008; 59: 220-225.
52. Thyssen J P, Carlsen B C, Menne T, Johansen J D. Trends of contact allergy to fragrance mix I and Myroxylon pereirae among Danish eczema patients tested between 1985 and 2007. *Contact Dermatitis* 2008; 59: 238-244.

53. Uter W, Hegewald J, Aberer W, Ayala F, Bircher A J, Brasch J, Coenraads P J, Schuttelaar M L, Elsner P, Fartasch M, Mahler V, Belloni Fortina A, Frosch P J, Fuchs T, Johansen J D, Menne T, Jolanki R, Krecisz B, Kiec-Swierczynska M, Larese F, Orton D, Peserico A, Rantanen T, Schnuch A. The European standard series in 9 European countries, 2002/2003 - First results of the European Surveillance System on Contact Allergies. *Contact Dermatitis* 2005; 53: 136-145.
54. Hegewald J, Uter W, Aberer W, Ayala F, Beliauskienė A, Belloni Fortina A, Bircher A, Brasch J, Chowdhury M M, Coenraads P J, Schuttelaar M-L, Elsner P, English J, Fartasch M, Mahler V, Frosch P J, Fuchs T, Gawkrödger D J, Giménez-Arnau A M, Green C M, Johansen J D, Menné T, Jolanki R, King C M, Krecisz B, Kiec-Swierczynska M, Larese F, Ormerod A D, Orton D, Peserico A, Rantanen T, Rustemeyer T, Sansom J E, Statham B N, Corradin M T, Wallnofer W, Wilkinson M, Schnuch A. The European Surveillance System of Contact Allergies (ESSCA): results of patch testing the standard series, 2004. *J Eur Acad Dermatol Venereol* 2008; 22: 174–181.
55. Uter W, Räscher C, Aberer W, Ayala F, Balato A, Beliauskienė A, Fortina A B, Bircher A, Brasch J, Chowdhury M M, Coenraads P J, Schuttelaar M L, Cooper S, Corradin M T, Elsner P, English J S, Fartasch M, Mahler V, Frosch P J, Fuchs T, Gawkrödger D J, Gimenez-Arnau A M, Green C M, Horne H L, Jolanki R, King C M, Krecisz B, Kiec-Swierczynska M, Ormerod A D, Orton D I, Peserico A, Rantanen T, Rustemeyer T, Sansom J E, Simon D, Statham B N, Wilkinson M, Schnuch A. The European baseline series in 10 European Countries, 2005/2006--results of the European Surveillance System on Contact Allergies (ESSCA). *Contact Dermatitis* 2009; 61: 31-38.
56. An S, Lee A Y, Lee C H, Kim D W, Hahm J H, Kim K J, Moon K C, Won Y H, Ro Y S, Eun H C. Fragrance contact dermatitis in Korea: a joint study. *Contact Dermatitis* 2005; 53: 320-323.
57. Hussain I, Rani Z, Rashid T, Haroon T S. Suitability of the European standard series of patch test allergens in Pakistani patients. *Contact Dermatitis* 2002; 46: 50-51.
58. Gupta N, Shenoi S D, Balachandran C. Fragrance sensitivity in allergic contact dermatitis. *Contact Dermatitis* 1999; 40: 53-54.
59. Freireich-Astman M, David M, Trattner A. Standard patch test results in patients with contact dermatitis in Israel: age and sex differences. *Contact Dermatitis* 2007; 56: 103-107.
60. Lazarov A. European Standard Series patch test results from a contact dermatitis clinic in Israel during the 7-year period from 1998 to 2004. *Contact Dermatitis* 2006; 55: 73-76.
61. Kashani M N, Gorouhi F, Behnia F, Nazemi M J, Dowlati Y, Firooz A. Allergic contact dermatitis in Iran. *Contact Dermatitis* 2005; 52: 154-158.
62. Akyol A, Boyvat A, Pekşari Y, Gurgey E. Contact sensitivity to standard series allergens in 1038 patients with contact dermatitis in Turkey. *Contact Dermatitis* 2005; 52: 333-337.
63. Lu X, Li L F, Wang W, Wang J. A clinical and patch test study of patients with positive patch test reactions to fragrance mix in China. *Contact Dermatitis* 2005; 52: 188-191.
64. Belsito D V, Fowler J F, Jr., Sasseville D, Marks J G, Jr., De Leo V A, Storrs F J. Delayed-type hypersensitivity to fragrance materials in a select North American population. *Dermatitis* 2006; 17: 23-28.
65. Zug K A, Warshaw E M, Fowler J F, Jr., Maibach H I, Belsito D L, Pratt M D, Sasseville D, Storrs F J, Taylor J S, Mathias C G, Deleo V A, Rietschel R L, Marks J. Patch-test results of the North American Contact Dermatitis Group 2005-2006. *Dermatitis* 2009; 20: 149-160.

66. Bruynzeel D P, Diepgen T L, Andersen K E, Brandao F M, Bruze M, Frosch P J, Goossens A, Lahti A, Mahler V, Maibach H I, Menne T, Wilkinson J D. Monitoring the European standard series in 10 centres 1996-2000. *Contact Dermatitis* 2005; 53: 146-149.
67. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. I. Reactivity to 14 frequently used chemicals. *Contact Dermatitis* 2002; 47: 78-85.
68. Krautheim A, Uter W, Frosch P, Schnuch A, Geier J. Patch testing with fragrance mix II: results of the IVDK 2005-2008. *Contact Dermatitis* 2010; 63: 262-269.
69. Heisterberg M V, Andersen K E, Avnstorp C, al. e. Fragrance mix II in the baseline series contributes significantly to detection of fragrance allergy. *Contact Dermatitis* 2010: (accepted):
70. Frosch P J, Pilz B, Andersen K E, Burrows D, Camarasa J G, et al. Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 1995; 33: 333-342.
71. Frosch P J, Johansen J D, Menne T, Rastogi S C, Bruze M, Andersen K E, Lepoittevin J P, Gimenez Arnau E, Pirker C, Goossens A, White I R. Lyr al is an important sensitizer in patients sensitive to fragrances. *Br J Dermatol* 1999; 141: 1076-1083.
72. Beliauskienė A, Valiukeviciene S, Uter W, Schnuch A. The European baseline series in Lithuania: results of patch testing in consecutive adult patients. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2011; 25: 59-63.
73. Geier J, Brasch J, Schnuch A, Lessmann H, Pirker C, Frosch P J. Lyr al has been included in the patch test standard series in Germany. *Contact Dermatitis* 2002; 46: 295-297.
74. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Dermatitis* 2007; 57: 1-10.
75. Api A M. Only Peru Balsam extracts or distillates are used in perfumery. *Contact Dermatitis* 2006; 54: 179.
76. Avalos-Peralta P, Garcia-Bravo B, Camacho F M. Sensitivity to Myroxylon pereirae resin (balsam of Peru). A study of 50 cases. *Contact Dermatitis* 2005; 52: 304-306.
77. Cachao P, Menezes Brandao F, Carmo M, Frazao S, Silva M. Allergy to oil of turpentine in Portugal. *Contact Dermatitis* 1986; 14: 205-208.
78. Lear J T, Heagerty A H M, Tan B B, et al. Transient re-emergence of oil of turpentine allergy in the pottery industry. *Contact Dermatitis* 1996; 34: 169-172.
79. Treudler R, Richter G, Geier J, Schnuch A, Orfanos C E, Tebbe B. Increase in sensitization to oil of turpentine: recent data from a multicenter study on 45,005 patients from the German-Austrian Information Network of Departments of Dermatology (IVDK). *Contact Dermatitis* 2000; 42: 68-73.
80. Schäfer T, Böhler E, Ruhdorfer S, Weigl L, Wessner D, Filipiak B, Wichmann H E, Ring J. Epidemiology of contact allergy in adults. *Allergy* 2001; 56: 1192-1196.
81. Meding B, Swanbeck G. Occupational hand eczema in an industrial city. *Contact Dermatitis* 1990; 22: 13-23.
82. Nielsen N H, Menné T. Allergic contact sensitization in an unselected Danish population - the Glostrup allergy study, Denmark. *Acta Dermatol Venerol (Stockh)* 1992; 72: 456-460.

83. Meneghini C L, Sertoli A, Nava C, Angelini G, Francalani S, Foti C, Moroni P. Irritant contact dermatitis of the hands in housewives. In: Elsner P, Maibach H I, eds. *Irritant Dermatitis New Clinical and Experimental Aspects Curr Probl Dermatol*. Basel: Karger, 1995: 41-48.
84. Seidenari S, Manzini B M, Danese P, Motolese A. Patch and prick test study of 593 healthy subjects. *Contact Dermatitis* 1990; 23: 162-167.
85. Mørtz C G, Bindslev-Jensen C, Lauritsen J, Andersen K E. Allergic contact sensitization in 8th grade school children in Odense, Denmark. . *Abstract presented at the Jadassohn Centenary Congress, London 9-12 Oct 1996* 1996:
86. Guin J D, Berry V K. Perfume sensitivity in adult females. A study of contact sensitivity to a perfume mix in two groups of student nurses. *J Am Acad Dermatol* 1980; 3: 299-302.
87. Nielsen N H, Linneberg A, Menne T, Madsen F, Frolund L, Dirksen A, Jorgensen T. Allergic contact sensitization in an adult Danish population: two cross-sectional surveys eight years apart (the Copenhagen Allergy Study). *Acta Derm Venereol* 2001; 81: 31-34.
88. Thyssen J P, Linneberg A, Menne T, Nielsen N H, Johansen J D. The prevalence and morbidity of sensitization to fragrance mix I in the general population. *Br J Dermatol* 2009; 161: 95-101.
89. Buckley D A, Rycroft R J, White I R, McFadden J P. The frequency of fragrance allergy in patch-tested patients increases with their age. *Br J Dermatol* 2003; 149: 986-989.
90. Uter W, Schnuch A, Geier J, Pfahlberg A, Gefeller O. Association between occupation and contact allergy to the fragrance mix: a multifactorial analysis of national surveillance data. *Occup Environ Med* 2001; 58: 392-398.
91. Dotterud L K, Smith-Sivertsen T. Allergic contact sensitization in the general adult population: a population-based study from Northern Norway. *Contact Dermatitis* 2007; 56: 10-15.
92. Smith-Sivertsen T, Dotterud L K, Lund E. Nickel allergy and its relationship with local nickel pollution, ear piercing, and atopic dermatitis: a population-based study from Norway. *J Am Acad Dermatol* 1999; 40: 726-735.
93. White J M, Gilmour N J, Jeffries D, Duangdeeden I, Kullavanijaya P, Basketter D A, McFadden J P. A general population from Thailand: incidence of common allergens with emphasis on para-phenylenediamine. *Clin Exp Allergy* 2007; 37: 1848-1853.
94. Bruze M. What is a relevant contact allergy? *Contact Dermatitis* 1990; 23: 224-225.
95. Ale I, Maibach H I. Clinical Relevance in Allergic Contact Dermatitis. An algorithmic approach. *Derm Beruf Umwelt* 1995; 43: 119-121.
96. Wahlberg J E, Lindberg M. Patch Testing. In: Frosch P J, Menné T, Lepoittevin J P, eds. *Contact Dermatitis*. Berlin: Springer, 2006: 365-390.
97. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. II. Reactivity to essential oils. *Contact Dermatitis* 2002; 47: 279-287.
98. Rothenborg H W, Hjorth N. Allergy to perfumes from toilet soaps and detergents in patients with dermatitis. *Arch Dermatol* 1968; 97: 417-421.
99. Hannuksela M, Kousa M, Pirila V. Allergy to ingredients of vehicles. *Contact Dermatitis* 1976; 2: 105-110.
100. Johansen J D, Andersen K E, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. *Contact Dermatitis* 1996; 34: 414-418.

101. Johansen J D, Andersen K E, Rastogi S C, Menne T. Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. *Contact Dermatitis* 1996; 34: 165-171.
102. Jorgensen P H, Jensen C D, Rastogi S, Andersen K E, Johansen J D. Experimental elicitation with hydroxyisohexyl-3-cyclohexene carboxaldehyde-containing deodorants. *Contact Dermatitis* 2007; 56: 146-150.
103. Bruze M, Johansen J D, Andersen K E, Frosch P, Lepoittevin J P, Rastogi S, Wakelin S, White I, Menne T. Deodorants: an experimental provocation study with cinnamic aldehyde. *J Am Acad Dermatol* 2003; 48: 194-200.
104. Svedman C, Bruze M, Johansen J D, Andersen K E, Goossens A, Frosch P J, Lepoittevin J P, Rastogi S, White I R, Menne T. Deodorants: an experimental provocation study with hydroxycitronellal. *Contact Dermatitis* 2003; 48: 217-223.
105. Schnuch A, Uter W, Dickel H, Szliska C, Schliemann S, Eben R, Rueff F, Gimenez-Arnau A, Löffler H, Aberer W, Frambach Y, Worm M, Niebuhr M, Hillen U, Martin V, Jappe U, Frosch P J, Mahler V. Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients. *Contact Dermatitis* 2009; 61: 152-162.
106. Uter W, Geier J, Schnuch A, Frosch P J. Patch test results with patients' own perfumes, deodorants and shaving lotions: results of the IVDK 1998-2002. *J Eur Acad Dermatol Venereol* 2007; 21: 374-379.
107. Basra M K, Fenech R, Gatt R M, Salek M S, Finlay A Y. The Dermatology Life Quality Index 1994-2007: a comprehensive review of validation data and clinical results. *Br J Dermatol* 2008; 159: 997-1035.
108. Skoet R, Zachariae R, Agner T. Contact dermatitis and quality of life: a structured review of the literature. *Br J Dermatol* 2003; 149: 452-456.
109. Moberg C, Alderling M, Meding B. Hand eczema and quality of life: a population-based study. *Br J Dermatol* 2009; 161: 397-403.
110. Meding B, Swanbeck G. Consequences of having hand eczema. *Contact Dermatitis* 1990; 23: 6-14.
111. Agner T, Andersen K E, Brandao F M, Bruynzeel D P, Bruze M, Frosch P, Goncalo M, Goossens A, Le Coz C J, Rustemeyer T, White I R, Diepgen T. Contact sensitisation in hand eczema patients-relation to subdiagnosis, severity and quality of life: a multi-centre study. *Contact Dermatitis* 2009; 61: 291-296.
112. Lysdal S H, Johansen J D. Fragrance contact allergic patients: strategies for use of cosmetic products and perceived impact on life situation. *Contact Dermatitis* 2009; 61: 320-324.
113. Meding B, Wrangsjo K, Jarvholm B. Fifteen-year follow-up of hand eczema: predictive factors. *J Invest Dermatol* 2005; 124: 893-897.
114. Hald M, Agner T, Blands J, Ravn H, Johansen J D. Allergens associated with severe symptoms of hand eczema and a poor prognosis. *Contact Dermatitis* 2009; 61: 101-108.
115. Wijnhoven S W P, Ezendam J, Schuur A G, van Loveren H, van Engelen J G M. *Allergens in consumer products. RIVM Reprot 320025001*. Bilthoven: Institute for Public Health and the Environment, 2008.
116. Schnuch A, Aberer W, Agathos M, Becker D, Brasch J, Elsner P, Frosch P J, Fuchs T, Geier J, Hillen U, Löffler H, Mahler V, Richter G, Szliska C. Durchführung des Epikutantests mit Kontaktallergenen. Leitlinien der Deutschen Dermatologischen Gesellschaft; Deutschen Gesellschaft für Allergie und klinische Immunologie. *J Dtsch Dermatol Ges* 2008; 6: 770-775.

117. Scheinmann P L. The foul side of fragrance-free products: What every clinician should know about managing patients with fragrance allergy. *J Am Acad Dermatol* 1999; 41: 1020-1024.
118. Hagvall L, Backtorp C, Norrby P O, Karlberg A T, Borje A. Experimental and theoretical investigations of the autoxidation of geranial: a dioxolane hydroperoxide identified as a skin sensitizer. *Chemical research in toxicology* 2011; 24: 1507-1515.
119. Hagvall L, Backtorp C, Svensson S, Nyman G, Borje A, Karlberg A T. Fragrance compound geraniol forms contact allergens on air exposure. Identification and quantification of oxidation products and effect on skin sensitization. *Chem Res Toxicol* 2007; 20: 807-814.
120. Hagvall L, Baron J M, Borje A, Weidolf L, Merk H, Karlberg A T. Cytochrome P450-mediated activation of the fragrance compound geraniol forms potent contact allergens. *Toxicol Appl Pharmacol* 2008; 233: 308-313.
121. Brared Christensson J, Matura M, Backtorp C, Borje A, Nilsson J L, Karlberg A T. Hydroperoxides form specific antigens in contact allergy. *Contact Dermatitis* 2006; 55: 230-237.
122. Karlberg A T, Bergstrom M A, Borje A, Luthman K, Nilsson J L. Allergic contact dermatitis--formation, structural requirements, and reactivity of skin sensitizers. *Chem Res Toxicol* 2008; 21: 53-69.
123. Karlberg A T, Boman A, Melin B. Animal experiments on the allergenicity of d-limonene--the citrus solvent. *Ann Occup Hyg* 1991; 35: 419-426.
124. Karlberg A T, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 1992; 26: 332-340.
125. Karlberg A T, Shao L P, Nilsson U, Gafvert E, Nilsson J L. Hydroperoxides in oxidized d-limonene identified as potent contact allergens. *Arch Dermatol Res* 1994; 286: 97-103.
126. Sköld M, Börje A, Matura M, Karlberg A T. Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis* 2002; 46: 267-272.
127. Sköld M, Börje A, Harambasic E, Karlberg A T. Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. *Chem Res Toxicol* 2004; 17: 1697-1705.
128. Sköld M, Hagvall L, Karlberg A T. Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis* 2008; 58: 9-14.
129. Rudback J, Bergstrom M A, Borje A, Nilsson U, Karlberg A T. alpha-Terpinene, an antioxidant in tea tree oil, autoxidizes rapidly to skin allergens on air exposure. *Chem Res Toxicol* 2012; 25: 713-721.
130. Karlberg A T, Dooms-Gossens A. Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis* 1997; 36: 201-206.
131. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjo K, Karlberg A T. Oxidized citrus oil (R-limonene): a frequent skin sensitizer in Europe. *J Am Acad Dermatol* 2002; 47: 709-714.
132. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjo K, Karlberg A T. Patch testing with oxidized R-(+)-limonene and its hydroperoxide fraction. *Contact Dermatitis* 2003; 49: 15-21.
133. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Selected oxidized fragrance terpenes are common contact allergens. *Contact Dermatitis* 2005; 52: 320-328.

134. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Not only oxidized R-(+)- but also S-(-)-limonene is a common cause of contact allergy in dermatitis patients in Europe. *Contact Dermatitis* 2006; 55: 274-279.
135. Christensson J B, Matura M, Gruvberger B, Bruze M, Karlberg A T. Linalool--a significant contact sensitizer after air exposure. *Contact Dermatitis* 2010; 62: 32-41.
136. Sköld M, Karlberg A T, Matura M, Börje A. The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. *Food Chem Toxicol* 2006; 44: 538-545.
137. Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. *Contact Dermatitis* 1987; 16: 93-95.
138. Fregert S, Hjorth N. Results of Standard Patch Tests with Substances Abandoned. *Contact Dermatitis Newsletter* 1969; 5: 85-86.
139. de Groot A C, Liem D H, Nater J P, van Ketel W G. Patch tests with fragrance materials and preservatives. *Contact Dermatitis* 1985; 12: 87-92.
140. Hagvall L, Skold M, Brared-Christensson J, Borje A, Karlberg A T. Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis* 2008; 59: 143-150.
141. Berglund V. *Master Thesis University of Gothenburg*. 2011.
142. Ruberto G, Baratta M T, Deans S G, Dorman H J. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Med* 2000; 66: 687-693.
143. Kim H J, Chen F, Wu C, Wang X, Chung H Y, Jin Z. Evaluation of antioxidant activity of Australian tea tree (*Melaleuca alternifolia*) oil and its components. *J Agric Food Chem* 2004; 52: 2849-2854.
144. Foti M C, Ingold K U. Mechanism of inhibition of lipid peroxidation by gamma-terpinene, an unusual and potentially useful hydrocarbon antioxidant. *J Agric Food Chem* 2003; 51: 2758-2765.
145. Buckley D A. Allergy to oxidized linalool in the UK. *Contact Dermatitis* 2011; 64: 240-241.
146. Smith C K, Hotchkiss S A. Enzymes and mechanisms of xenobiotic metabolism. In: editor?)) w i, eds. *Allergic Contact Dermatitis Chemical and Metabolic Mechanisms*. Taylor and Francis, London and New York, 2001: 45-87.
147. Kalgutkar A S, Gardner I, Obach R S, Shaffer C L, Callegari E, Henne K R, Mutlib A E, Dalvie D K, Lee J S, Nakai Y, O'Donnell J P, Boer J, Harriman S P. A comprehensive listing of bioactivation pathways of organic functional groups. *Curr Drug Metab* 2005; 6: 161-225.
148. Nilsson A M, Bergstrom M A, Luthman K, Nilsson J L, Karlberg A T. A conjugated diene identified as a prohaptens: contact allergenic activity and chemical reactivity of proposed epoxide metabolites. *Chem Res Toxicol* 2005; 18: 308-316.
149. Bergström M A, Luthman K, Nilsson J L, Karlberg A T. Conjugated dienes as prohaptens in contact allergy: in vivo and in vitro studies of structure-activity relationships, sensitizing capacity, and metabolic activation. *Chem Res Toxicol* 2006; 19: 760-769.
150. Bergström M A, Ott H, Carlsson A, Neis M, Zwadlo-Klarwasser G, Jonsson C A, Merk H F, Karlberg A T, Baron J M. A skin-like cytochrome P450 cocktail activates prohaptens to contact allergenic metabolites. *J Invest Dermatol* 2007; 127: 1145-1153.

151. Basketter D A. Skin Sensitization to Cinnamic Alcohol: The Role of Skin Metabolism. *Acta Derm Venereol* 1992; 72: 264-265.
152. Smith C K, Moore C A, Elahi E N, Smart A T, Hotchkiss S A. Human skin absorption and metabolism of the contact allergens, cinnamic aldehyde, and cinnamic alcohol. *Toxicol Appl Pharmacol* 2000; 168: 189-199.
153. Cheung C, Hotchkiss S A, Pease C K. Cinnamic compound metabolism in human skin and the role metabolism may play in determining relative sensitisation potency. *J Dermatol Sci* 2003; 31: 9-19.
154. Elahi E N, Wright Z, Hinselwood D, Hotchkiss S A, Basketter D A, Pease C K. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. *Chem Res Toxicol* 2004; 17: 301-310.
155. Ott H, Wiederholt T, Bergstrom M A, Heise R, Skazik C, Czaja K, Marquardt Y, Karlberg A T, Merk H F, Baron J M. High-resolution transcriptional profiling of chemical-stimulated dendritic cells identifies immunogenic contact allergens, but not prohaptens. *Skin Pharmacol Physiol* 2010; 23: 213-224.
156. Bertrand F, Basketter D A, Roberts D W, Lepoittevin J P. Skin sensitization to eugenol and isoeugenol in mice: possible metabolic pathways involving ortho-quinone and quinone methide intermediates. *Chemical research in toxicology* 1997; 10: 335-343.
157. Rastogi S C, Johansen J D. Significant exposures to isoeugenol derivatives in perfumes. *Contact Dermatitis* 2008; 58: 278-281.
158. Flyvholm M A, Andersen K E, Baranski B, Sarlo K. *Criteria for classification of skin- and airway-sensitizing substances in the work and general environments*. Regional Office for Europe: WHO, 1996.
159. Basketter D A, Flyvholm M A, Menne T. Classification criteria for skin-sensitizing chemicals: a commentary. *Contact Dermatitis* 1999; 40: 175-182.
160. Schnuch A, Lessmann H, Schulz K H, Becker D, Diepgen T L, Drexler H, Erdmann S, Fartasch M, Greim H, Kricke-Helling P, Merget R, Merk H, Nowak D, Rothe A, Stropp G, Uter W, Wallenstein G. When should a substance be designated as sensitizing for the skin ('Sh') or for the airways ('Sa')? *Hum Exp Toxicol* 2002; 21: 439-444.
161. Basketter D A, Andersen K E, Liden C, Van Loveren H, Boman A, Kimber I, Alanko K, Berggren E. Evaluation of the skin sensitizing potency of chemicals by using the existing methods and considerations of relevance for elicitation. *Contact Dermatitis* 2005; 52: 39-43.
162. Anonymous. *Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures*. ECHA Reference: ECHA-11-G-06-EN. Date: 04/2011 http://echa.europa.eu/documents/10162/17217/clp_en.pdf 2011.
163. Lalko J, Api A M, Politano V T, Letizia C. Quantitative risk assessment for dermal sensitization to fragrance ingredients: The utility of LLNA data in the weight of evidence approach to identifying thresholds. *46th Congress of the European Societies of Toxicology, September 13-16 2009, Dresden, Germany* 2009:
164. RIFM. Local lymph node assay (LLNA) protocol summaries: Data presented at the 46th Congress of the European Societies of Toxicology. *Research Institute for Fragrance Materials, Inc* 2009:
165. Gerberick G F, Kern P S, Schlatter H, Dearman R J, Kimber I, Patlewicz G Y, Basketter D A. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods *Dermatitis* 2005; 16: 157-202.
166. Kern P S, Gerberick G F, Ryan C A, Kimber I, Aptula A, Basketter D A. Local lymph node data for the evaluation of skin sensitization alternatives: a second compilation. *Dermatitis* 2010; 21: 8-32.

167. Arnau E G, Andersen K E, Bruze M, Frosch P J, Johansen J D, Menne T, Rastogi S C, White I R, Lepoittevin J P. Identification of Lilial as a fragrance sensitizer in a perfume by bioassay-guided chemical fractionation and structure-activity relationships. *Contact Dermatitis* 2000: 43: 351-358.
168. Lepoittevin J P. Metabolism versus chemical transformation or pro- versus prehapten? *Contact Dermatitis* 2006: 54: 73-74.
169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensitizing cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:
170. Heisterberg M V, Menne T, Johansen J D. Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. *Contact Dermatitis* 2011: 65: 266-275.
171. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Jr., Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis: a worldwide multicenter investigation (Part II). *Contact Dermatitis* 2001: 44: 344-346.
172. Andersen A. Final report on the safety assessment of sodium p-chloro-m-cresol, p-chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol. *Int J Toxicol* 2006: 25 Suppl 1: 29-127.
173. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis - a worldwide multicenter investigation (Part III). *Contact Dermatitis* 2002: 46: 141-144.
174. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on trans,trans-delta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S211-215.
175. Letizia C S, Cocchiara J, Wellington G A, Funk C, Api A M. Food and chemical toxicology. *Food Chem Toxicol* 2000: 38 Suppl 3: S1-236.
176. Mitchell D M, Beck M H. Contact allergy to benzyl alcohol in a cutting oil reodorant. *Contact Dermatitis* 1988: 18: 301-302.
177. Mitchell J C. Contact hypersensitivity to some perfume materials. *Contact Dermatitis* 1975: 1: 196-199.
178. Malten K E, van Ketel W G, Nater J P, Liem D H. Reactions in selected patients to 22 fragrance materials. *Contact Dermatitis* 1984: 11: 1-10.
179. Mitchell J C, Calnan C D, Clendenning W E, Cronin E, Hjorth N, Magnusson B, Maibach H I, Meneghini C L, Wilkinson D S. Patch testing with some components of balsam of Peru. *Contact Dermatitis* 1976: 2: 57-58.
180. Bernaola G, Escayol P, Fernandez E, de Corres L F. Contact dermatitis from methylionone fragrance. *Contact Dermatitis* 1989: 20: 71-72.
181. English J S, Rycroft R J. Allergic contact dermatitis from methyl heptene and methyl octene carbonates. *Contact Dermatitis* 1988: 18: 174-175.
182. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on myrtenol. *Food Chem Toxicol* 2008: 46 Suppl 11: S237-240.
183. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on nerolidol (isomer unspecified). *Food Chem Toxicol* 2008: 46 Suppl 11: S247-250.
184. Sanchez-Politta S, Campanelli A, Pashe-Koo F, Saurat J H, Piletta P. Allergic contact dermatitis to phenylacetaldehyde: a forgotten allergen? *Contact Dermatitis* 2007: 56: 171-172.

185. McGinty D, Letizia C S, Api A M. Fragrance material review on phytol. *Food Chem Toxicol* 2010: 48 Suppl 3: S59-63.
186. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on rhodinol. *Food Chem Toxicol* 2008: 46 Suppl 11: S259-262.
187. Lapczynski A, Lalko J, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-isodamascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S267-271.
188. Trattner A, David M. Patch testing with fine fragrances: comparison with fragrance mix, balsam of Peru and a fragrance series. *Contact Dermatitis* 2003: 49: 287-289.
189. Surburg H, Panten J. *Common fragrance and flavor materials: preparation, properties and uses*. Weinheim: Wiley-VCH, 2006.
190. Schmidt E. Production of Essential Oils. In: Husnu Can Baser K, Buchbauer G, eds. *Handbook of Essential Oils - Science, Technology, and Applications*. Boca Raton: CRC Press, 2010: 88-95.
191. Trattner A, David M, Lazarov A. Occupational contact dermatitis due to essential oils. *Contact Dermatitis* 2008: 58: 282-284.
192. Jung P, Sesztak-Greinecker G, Wantke F, Gotz M, Jarisch R, Hemmer W. Mechanical irritation triggering allergic contact dermatitis from essential oils in a masseur. *Contact Dermatitis* 2006: 54: 297-299.
193. Bilsland D, Strong A. Allergic contact dermatitis from the essential oil of French marigold (*Tagetes patula*) in an aromatherapist. *Contact Dermatitis* 1990: 23: 55-56.
194. Cockayne S E, Gawkrödger D J. Occupational contact dermatitis in an aromatherapist. *Contact Dermatitis* 1997: 37: 306-307.
195. Boonchai W, Iamtharachai P, Sunthonpalin P. Occupational allergic contact dermatitis from essential oils in aromatherapists. *Contact Dermatitis* 2007: 56: 181-182.
196. Keane F M, Smith H R, White I R, Rycroft R J. Occupational allergic contact dermatitis in two aromatherapists. *Contact Dermatitis* 2000: 43: 49-51.
197. Selvaag E, Holm J O, Thune P. Allergic contact dermatitis in an aroma therapist with multiple sensitizations to essential oils. *Contact Dermatitis* 1995: 33: 354-355.
198. Romaguera C, Vilaplana J. Occupational contact dermatitis from ylang-ylang oil. *Contact Dermatitis* 2000: 43: 251.
199. Rudzki E, Grzywa Z. Allergy to perfume mixture. *Contact Dermatitis* 1986: 15: 115-116.
200. Rudzki E, Grzywa Z, Bruo W S. Sensitivity to 35 essential oils. *Contact Dermatitis* 1976: 2: 196-200.
201. Vilaplana J, Romaguera C. Contact dermatitis from the essential oil of tangerine in fragrance. *Contact Dermatitis* 2002: 46: 108.
202. Rudzki E, Grzywa Z. Sensitizing and irritating properties of star anise oil. *Contact Dermatitis* 1976: 2: 305-308.
203. Sugiura M, Hayakawa R, Kato Y, Sugiura K, Hashimoto R. Results of patch testing with lavender oil in Japan. *Contact Dermatitis* 2000: 43: 157-160.
204. Lalko J, Api A M. Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. *Food Chem Toxicol* 2006: 44: 739-746.
205. SCCP. *Memorandum Classification and categorization of skin sensitizers and grading of test reactions (SCCP/0919/05)*. Scientific Committee for on Consumer Protection, adopted 20 September 2005. 2005.

206. SCCP. *Memorandum on Hair Dye Substances and their Skin Sensitising Properties. Scientific Committee on Consumer Protection, adopted 19 December 2006.* 2006.
207. Christensson J B, Johansson S, Hagvall L, Jonsson C, Borje A, Karlberg A T. Limonene hydroperoxide analogues differ in allergenic activity. *Contact Dermatitis* 2008; 59: 344-352.
208. Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. *J Exp Med* 1936; 64: 625-629.
209. Ridriguez E, Towers G H N, Mitchell J C. Biological aspects of sesquiterpene lactones. *Phytochemistry* 1976; 15: 1573-1580.
210. Roberts D W, Goodwin B F J, Williams D L, Jones K, Johnson A W, Alderson J C E. Correlation between skin sensitization potential and chemical reactivity for nitrobenzyl compounds. *Food Chem Toxicol* 1984; 21: 811-813.
211. Dupuis G, Benezra C. *Allergic contact dermatitis to simple chemicals: a molecular approach* New York: Marcel Dekker, 1982.
212. Smith C K, Hotchkiss S A. *Allergic Contact Dermatitis, Chemical and Metabolic Mechanisms.* London: Taylor and Francis, 2001.
213. Roberts D W, Lepoittevin J P. Hapten-Protein Interactions. In: Lepoittevin J P, Basketter D, Goossens A, Karlberg A T, eds. *Allergic Contact Dermatitis The Molecular Basis.* Heidelberg: Springer, 1998:
214. Sykes P. *A guidebook to mechanism in organic chemistry* Edinburgh: Pearson, 1961.
215. Aptula A O, Roberts D W. Mechanistic applicability domains for nonanimal-based prediction of toxicological end points: general principles and application to reactive toxicity. *Chem Res Toxicol* 2006; 19: 1097-1105.
216. Gerberick G F, Vassallo J D, Bailey R E, Chaney J G, Morrall S W, Lepoittevin J P. Development of a peptide reactivity assay for screening contact allergens. *Toxicological sciences : an official journal of the Society of Toxicology* 2004; 81: 332-343.
217. Natsch A, Gfeller H, Rothaupt M, Ellis G. Utility and limitations of a peptide reactivity assay to predict fragrance allergens in vitro. *Toxicology in vitro : an international journal published in association with BIBRA* 2007; 21: 1220-1226.
218. Gerberick G F, Troutman J A, Foertsch L M, Vassallo J D, Quijano M, Dobson R L, Goebel C, Lepoittevin J P. Investigation of peptide reactivity of pro-hapten skin sensitizers using a peroxidase-peroxide oxidation system. *Toxicological sciences : an official journal of the Society of Toxicology* 2009; 112: 164-174.
219. Troutman J A, Foertsch L M, Kern P S, Dai H J, Quijano M, Dobson R L M, Lalko J F, Lepoittevin J-P, Gerberick G F. The incorporation of lysine into the peroxidase peptide reactivity assay for skin sensitization assessments. *Toxicol Sci* 2011; 122: 422-436.
220. Roberts D W, Aptula A O, Patlewicz G. Mechanistic applicability domains for non-animal based prediction of toxicological endpoints. QSAR analysis of the schiff base applicability domain for skin sensitization. *Chem Res Toxicol* 2006; 19: 1228-1233.
221. Roberts D W, Aptula A O, Patlewicz G Y. Chemistry-Based Risk Assessment for Skin Sensitization: Quantitative Mechanistic Modeling for the S(N)Ar Domain. *Chem Res Toxicol* 2011:
222. Johansen J D. Contact allergy to fragrances: clinical and experimental investigations of the fragrance mix and its ingredients. *Contact Dermatitis* 2002; 46 (suppl. 3): 4-31.
223. Fenn R S. Aroma chemical usage trends in modern perfumery. *Perfumer Flavorist* 1989; 14: 1-10.

224. Johansen J D, Frosch P J, Svedman C, Andersen K E, Bruze M, Pirker C, Menne T. Hydroxyisohexyl 3-cyclohexene carboxaldehyde- known as Lyrall: quantitative aspects and risk assessment of an important fragrance allergen. *Contact Dermatitis* 2003; 48: 310-316.
225. Rastogi S C, Johansen J D, Menne T. Natural ingredients based cosmetics. Content of selected fragrance sensitizers. *Contact Dermatitis* 1996; 34: 423-426.
226. Müller P M, Lamparsky D. *Perfumes: Art Science and Technology*. London: Elsevier Applied Science, 1991.
227. Poulsen P B, Schmidt A. *A survey and health assessment of cosmetic products for children. Survey of Chemical Substances in Consumer Products, No. 88*. Copenhagen: Danish Environmental Protection Agency, 2007.
228. Rastogi S C, Jensen G H, Johansen J D. *Survey and risk assessment of chemical substances in deodorants. Survey of Chemical Substances in Consumer Products, No. 86*. Copenhagen: Danish Environmental Protection Agency, 2007.
229. Buckley D A. Fragrance ingredient labelling in products on sale in the U.K. *Br J Dermatol* 2007; 157: 295-300.
230. Rastogi S C, Lepoittevin J P, Johansen J D, Frosch P J, Menne T, Bruze M, Dreier B, Andersen K E, White I R. Fragrances and other materials in deodorants: search for potentially sensitizing molecules using combined GC-MS and structure activity relationship (SAR) analysis. *Contact Dermatitis* 1998; 39: 293-303.
231. Rastogi S C, Johansen J D, Frosch P, Menne T, Bruze M, Lepoittevin J P, Dreier B, Andersen K E, White I R. Deodorants on the European market: quantitative chemical analysis of 21 fragrances. *Contact Dermatitis* 1998; 38: 29-35.
232. Rastogi S C, Menne T, Johansen J D. The composition of fine fragrances is changing. *Contact Dermatitis* 2003; 48: 130-132.
233. Rastogi S C, Johansen J D, Bossi R. Selected important fragrance sensitizers in perfumes--current exposures. *Contact Dermatitis* 2007; 56: 201-204.
234. Rastogi S C, Bossi R, Johansen J D, Menne T, Bernard G, Gimenez-Arnau E, Lepoittevin J P. Content of oak moss allergens atranol and chloroatranol in perfumes and similar products. *Contact Dermatitis* 2004; 50: 367-370.
235. Rastogi S C, Johansen J D, Menne T, Frosch P, Bruze M, Andersen K E, Lepoittevin J P, Wakelin S, White I R. Contents of fragrance allergens in children's cosmetics and cosmetic-toys. *Contact Dermatitis* 1999; 41: 84-88.
236. Rastogi S C. *Contents of selected fragrance materials in cleaning products and other consumer products. Survey of chemical compounds in consumer products, No. 8*. Copenhagen: Danish Environmental Protection Agency, 2002.
237. Bernard G, Gimenez-Arnau E, Rastogi S C, Heydorn S, Johansen J D, Menne T, Goossens A, Andersen K, Lepoittevin J P. Contact allergy to oak moss: search for sensitizing molecules using combined bioassay-guided chemical fractionation, GC-MS, and structure-activity relationship analysis. *Arch Dermatol Res* 2003; 295: 229-235.
238. Johansen J D, Andersen K E, Svedman C, Bruze M, Bernard G, Gimenez-Arnau E, Rastogi S C, Lepoittevin J P, Menne T. Chloroatranol, an extremely potent allergen hidden in perfumes: a dose-response elicitation study. *Contact Dermatitis* 2003; 49: 180-184.
239. SCCP. *Opinion on Atranol and Chloroatranol present in natural extracts (e.g. oak moss and tree moss extract)*. Scientific Committee on Consumer Products, adopted 7 December 2004. 2004.

240. White I R, Johansen J D, Arnau E G, Lepoittevin J P, Rastogi S, Bruze M, Andersen K E, Frosch P J, Goossens A, Menne T. Isoeugenol is an important contact allergen: can it be safely replaced with isoeugenyl acetate? *Contact Dermatitis* 1999; 41: 272-275.
241. SCCP. *Opinion on Hydroxyisohexyl 3-cyclohexene carboxaldehyde (sensitisation only)*. Scientific Committee on Consumer Products. Adopted 7 December 2004. 2004.
242. Nardelli A, D'Hooghe E, Drieghe J, Dooms M, Goossens A. Allergic contact dermatitis from fragrance components in specific topical pharmaceutical products in Belgium. *Contact Dermatitis* 2009; 60: 303-313.
243. Fisher A A. Cosmetic dermatitis in childhood. *Cutis* 1995; 55: 15-16.
244. Corea N V, Basketter D A, Clapp C, Van Asten A, Marty J P, Pons-Guiraud A, Laverdet C. Fragrance allergy: assessing the risk from washed fabrics. *Contact Dermatitis* 2006; 55: 48-53.
245. Hartmann K, Hunzelmann N. Allergic contact dermatitis from cinnamon as an odour-neutralizing agent in shoe insoles. *Contact Dermatitis* 2004; 50: 253-254.
246. Murphy L A, White I R. Contact dermatitis from geraniol in washing-up liquid. *Contact Dermatitis* 2003; 49: 52.
247. Foti C, Zambonin C G, Conserva A, Casulli C, D'Accolti L, Angelini G. Occupational contact dermatitis to a limonene-based solvent in a histopathology technician. *Contact Dermatitis* 2007; 56: 109-112.
248. Topham E J, Wakelin S H. D-Limonene contact dermatitis from hand cleansers. *Contact Dermatitis* 2003; 49: 108-109.
249. Wakelin S H, McFadden J P, Leonard J N, Rycroft R J. Allergic contact dermatitis from d-limonene in a laboratory technician. *Contact Dermatitis* 1998; 38: 164-165.
250. Rastogi S C, Heydorn S, Johansen J D, Basketter D A. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 2001; 45: 221-225.
251. Yazar K, Johnsson S, Lind M L, Boman A, Liden C. Preservatives and fragrances in selected consumer-available cosmetics and detergents. *Contact Dermatitis* 2011; 64: 265-272.
252. Api A M, Bredbenner A, McGowen M, Niemiera D, Parker L, Renskers K, Selim S, Sgaramella R, Signorelli R, Tedrow S, Troy W. Skin contact transfer of three fragrance residues from candles to human hands. *Regul Toxicol Pharmacol* 2007; 48: 279-283.
253. Nadiminti H, Ehrlich A, Udey M C. Oral erosions as a manifestation of allergic contact sensitivity to cinnamon mints. *Contact Dermatitis* 2005; 52: 46-47.
254. Hoskyn J, Guin J D. Contact allergy to cinnamal in a patient with oral lichen planus. *Contact Dermatitis* 2005; 52: 160-161.
255. Silvestre J F, Albares M P, Blanes M, Pascual J C, Pastor N. Allergic contact gingivitis due to eugenol present in a restorative dental material. *Contact Dermatitis* 2005; 52: 341.
256. Guarneri F, Barbuzza O, Vaccaro M, Galtieri G. Allergic contact dermatitis and asthma caused by limonene in a labourer handling citrus fruits. *Contact Dermatitis* 2008; 58: 315-316.
257. Wallenhammar L M, Ortengren U, Adreasson H, Barregard L, Björkner B, Karlsson S, et al. Contact allergy and hand eczema in Swedish dentists. *Contact Dermatitis* 2000; 43: 192-199.
258. Geier J, Lessmann H, Schnuch A, Uter W. Contact sensitizations in metalworkers with occupational dermatitis exposed to water-based metalworking fluids: results of the research project "FaSt". *Int Arch Occup Environ Health* 2004; 77: 543-551.

259. Decapite T J, Anderson B E. Allergic contact dermatitis from cinnamic aldehyde found in an industrial odour-masking agent. *Contact Dermatitis* 2004; 51: 312-313.
260. Schubert H J. Skin diseases in workers at a perfume factory. *Contact Dermatitis* 2006; 55: 81-83.
261. Heydorn S, Andersen K E, Johansen J D, Menne T. A stronger patch test elicitation reaction to the allergen hydroxycitronellal plus the irritant sodium lauryl sulfate. *Contact Dermatitis* 2003; 49: 133-139.
262. Hannuksela M. Sensitivity of Various Skin Sites in the Repeated Open Application Test. *Am J Contact Dermatitis* 1991; 2: 102-104.
263. Fischer L A, Menné T, Avnstorp C, Kasting G B, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2009; 161: 560-567.
264. Andersen K E, Johansen J D, Bruze M, Frosch P J, Goossens A, Lepoittevin J P, Rastogi S, White I, Menne T. The time-dose-response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001; 170: 166-171.
265. DeGroot A C, Frosch P J. Adverse reactions to fragrances. A clinical review. *Contact Dermatitis* 1997; 36: 57-86.
266. Schnuch A, Geier J, Uter W, Frosch P J. Another look on allergies to fragrances: frequencies of sensitisation to the fragrance mix and its constituents. Results from the IVDK. *Exog Dermatol* 2002; 1: 231-237.
267. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Contact allergy to farnesol in 2021 consecutively patch tested patients. Results of the IVDK. *Contact Dermatitis* 2004; 50: 117-121.
268. Uter W, Schnuch A, Gefeller O. Guidelines for the descriptive presentation and statistical analysis of contact allergy data. *Contact Dermatitis* 2004; 51: 47-56.
269. Uter W, Gefeller O, Geier J, Lessmann H, Pfahlberg A, Schnuch A. *Untersuchungen zur Abhängigkeit der Sensibilisierung gegen wichtige Allergene von arbeitsbedingten sowie individuellen Faktoren. Schriftenreihe der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Forschung, Fb 949.* Bremerhaven: 2002.
270. van Loveren H, Cockshott A, Gebel T, Gundert-Remy U, de Jong W H, Matheson J, McGarry H, Musset L, Selgrade M K, Vickers C. Skin sensitization in chemical risk assessment: report of a WHO/IPCS international workshop focusing on dose-response assessment. *Regulatory toxicology and pharmacology : RTP* 2008; 50: 155-199.
271. Kimber I, Dearman R J, Basketter D A, Ryan C A, Gerberick G F, McNamee P M, Lalko J, Api A M. Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. *Regulatory toxicology and pharmacology : RTP* 2008; 52: 39-45.
272. Paramasivan P, Lai C, Pickard C, Ardern-Jones M, Healy E, Friedmann PS. Repeated low-dose skin exposure is an effective sensitizing stimulus, a factor to be taken into account in predicting sensitization risk. *Br J Dermatol.* 2010; 162: 594-7
273. Suskind R R. The hydroxycitronellal story: What can we learn from it? In: Frosch P J, Johansen J D, White I R, eds. *Fragrances Beneficial and adverse effects*. Berlin, Heidelberg, New York: Springer, 1988: 159-165.
274. Api A M, Basketter D, Cadby P A, Cano M-F, Ellis G, Gerberick F, Griem P, McNamee P M, Ryan C A, Safford B. *Dermal Sensitization Quantitative Risk Assessment (QRA) For Fragrance Ingredients Technical Dossier. June 22, 2006 QRA Expert Group.* <http://www.ifraorg.org/en-us/search/tags/21261> (last accessed 2011-11-27). 2006.

275. SCCP. Opinion on Dermal Sensitisation Quantitative Risk Assessment (Citral, Farnesol and Phenylacetaldehyde). Scientific Committee for on Consumer Protection, adopted 24 June 2008. 2008:
276. Fischer L A, Voelund A, Andersen K E, Menne T, Johansen J D. The dose-response relationship between the patch test and ROAT and the potential use for regulatory purposes. *Contact Dermatitis* 2009: 61: 201-208.
277. Roberts D W. QSAR for upper-respiratory tract irritation. *Chem Biol Interact* 1986: 57: 325-345.
278. Roberts D W, Natsch A. High throughput kinetic profiling approach for covalent binding to peptides: application to skin sensitization potency of Michael acceptor electrophiles. *Chem Res Toxicol* 2009: 22: 592-603.
279. Bruze M, Johansen J D, Andersen K E, Frosch P, Goossens A, Lepoittevin J P, Rastogi S C, White I, Menne T. Deodorants: an experimental provocation study with isoeugenol. *Contact Dermatitis* 2005: 52: 260-267.
280. Fischer L A, Menne T, Voelund A, Johansen J D. Can exposure limitations for well-known contact allergens be simplified? An analysis of dose-response patch test data. *Contact Dermatitis* 2011: 64: 337-342.
281. Franot C, Roberts D W, Basketter D A, Benezra C, Lepoittevin J P. Structure-activity relationships for contact allergenic potential of gamma,gamma-dimethyl-gamma-butyrolactone derivatives. 2. Quantitative structure-skin sensitization relationships for alpha-substituted-alpha-methyl-gamma,gamma-dimethyl-gamma-butyrolactone s. *Chem Res Toxicol* 1994: 7: 307-312.
282. SCCS. *Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 7th revision*. Scientific Committee for Consumer Safety, adopted 14 December 2010. 2010.
283. Basketter D, Horev L, Slodovnik D, Merimes S, Trattner A, Ingber A. Investigation of the threshold for allergic reactivity to chromium. *Contact Dermatitis* 2001: 44: 70-74.
284. Pasche F, Hunziker N. Sensitization to Kathon CG in Geneva and Switzerland. *Contact Dermatitis* 1989: 20: 115-119.
285. Fischer L A, Johansen J D, Menne T. Nickel allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2007: 157: 723-729.
286. Fischer L A, Johansen J D, Menne T. Methyldibromoglutaronitrile allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2008: 159: 1138-1143.
287. Flyvholm M A, Hall B M, Agner T, et al. Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. Relationship to repeated open application test with a product containing formaldehyde releaser. *Contact Dermatitis* 1997: 36: 26-33.
288. Thyssen J P, Johansen J D, Menne T, Nielsen N H, Linneberg A. Nickel allergy in Danish women before and after nickel regulation. *The New England journal of medicine* 2009: 360: 2259-2260.
289. Thyssen J P, Linneberg A, Menne T, Nielsen N H, Johansen J D. The association between hand eczema and nickel allergy has weakened among young women in the general population following the Danish nickel regulation: results from two cross-sectional studies. *Contact Dermatitis* 2009: 61: 342-348.
290. Zachariae C O, Agner T, Menné T. Chromium allergy in consecutive patients in a country where ferrous sulfate has been added since 1981. *Contact Dermatitis* 1996: 35: 83-85.

-
291. Braendstrup P, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall) is still a frequent allergen. *Contact Dermatitis* 2008; 59: 187-188.
292. SCCNFP. *Opinion on hydroxyisohexyl 3-cyclohexene carboxaldehyde. The Scientific Committee on Cosmetic products and Non-Food Products Intended for Consumers, adopted 9 December 2003.* 2003.
293. Api A M, Vey M. A new IFRA Standard on the fragrance ingredient, hydroxyisohexyl 3-cyclohexene carboxaldehyde. *Contact Dermatitis* 2010; 62: 254-255.
294. Schnuch A, Geier J, Uter W. Is hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation declining in central Europe? *Contact Dermatitis* 2012; 67: 47-49.
295. Heisterberg M V, Laurberg G, Veien N, Menné T, Avnstorp C, Kaaber K, Andersen K A, Sommerlund M, Danielsen A, Andersen B, Kristensen B, Kristensen O, Nielsen N H, Thormann J, Vissing S, Johansen J D. Prevalence of allergic contact dermatitis caused by hydroxyisohexyl 3-cyclohexene carboxaldehyde has not changed in Denmark. *Contact Dermatitis* 2012; 67: 49-51.
296. Nardelli A, Gimenez-Arnau E, Bernard G, Lepoittevin J P, Goossens A. Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals? *Contact Dermatitis* 2009; 60: 91-95.
297. Anonymous. *OECD Guidelines for the Testing of Chemicals / Section 4: Health Effects. Test No. 429: Skin Sensitisation (Local Lymph Node Assay).* Paris: OECD, 2002.
298. Anonymous. 76/768/EEC - Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal L* 1976; 262, 27/09/1976: 169.
299. Vigan M. Contact dermatitis sentinel network by GERDA. *Nouv Dermatol* 1996; 15: 677-678.
300. SCCP. *Opinion on Oak moss/Tree moss (sensitisation only) Scientific Committee on Consumer Products, adopted 15 April 2008.* 2008.

Annex I - Catalogue of fragrance allergens**Contents**

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Single chemicals

Often, results with the single constituents of the FM I or, yet more rarely, FM II, are presented in one paper. As the main ordering of this annex is by allergen, core information on these studies is presented in a tabular format and referenced by a unique acronym in the single sections, to avoid redundancy. Regarding nomenclature, terms which are often not officially an INCI Name but Perfuming Name as listed by CosIng are used. "Current Regulation" refers to the EU Cosmetics Directive only.

Table 55: Background information on studies reporting results with (all) single constituents of the FM I (**amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, isoeugenol, EVERNIA PRUNASTRI**)

Reference	Country	Study period, Patients	Comments by reviewers
Larsen 2002 c (1)	7 industrial countries worldwide	Prior to 2002 n=218 patients with known contact allergy to fragrance ingredients	Test concentrations identified as non-irritating in serial dilution testing in 20 healthy volunteers
Utrecht 1999 (2)	Utrecht, The Netherlands	1994-1998 n=757 patients with suspected ACD to cosmetics	All patients tested with FM I and single constituents
Sheffield 1999 (3)	UK	1994-1995 n=744, 40 of these positive to FM I and tested with single constituents	
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
Hungary 2002 (5)	Hungary, multicentre study,	1998-1999, n=3604 patients	recruitment not clear, presumably consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients
IVDK 2010 (7)	Germany, Switzerland and one centre in	2005-2008 n=36961 tested with FM I, n=4167 with FM II and	

	Austria	all SC	

Table 56: Results of PTing with single constituents of the FM I in patients positive to the FM I (as percent)

N(pos) to FM I, ref.	<i>Evernia prun.</i>	Isoeu g.	Hydroxy citron.	Cinna mal	Cinnamy I alcohol	Eugen ol	Gera-niol	Alpha-amyl cinnam al
N=160 (5)	13.1%	14.8 %	2.5%	8.1%	20.6%	8.8%	7.5%	5.0%
N= 991 (8)	18.4%	11.2 %	10.1%	6.1%	6.1%	6.6%	4.6%	2.4%
N=50 (2)	19.6%	14.3 %	8.9%	8.9%	7.1%	5.4%	2.7%	0%
n=40 Sheffield 1999 (3)	30%	20%	2.5%	12.5 %	10%	5%	0%	0%
N=226 Coimbra 2000 (9)	22.1%	19.9 %	6.6%	13.3 %	7.9%	14.6 %	8.4%	4.4%
N=655 IVDK 2010 (7)	29.8%	18.0 %	12.8%	11.6 %	9.6%	6.7%	4.7%	2.8%

Table 57: Background information on studies reporting results with (all) single constituents of the FM II (**citronellol, citral, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), Farnesol, alpha-Hexyl-cinnamic aldehyde**)

Reference	Country	Study period, Patients	Comments by reviewers
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
EU 2005 (10)	6 European centres	10/2002 – 06/2003, n=1701	Applied in consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients

IVDK 2010b (11)	Germany, Switzerland and one centre in Austria	2005-2008 n=35633 tested with FM II, n=2217 with all SC	

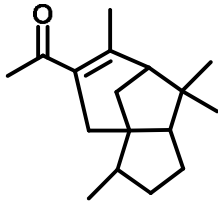
Table 58: Background information on studies reporting results with several fragrance compounds not, or only partly, corresponding to mixes (later created) or with essential oils

Reference	Country	Study period, Patients
deGroot 2000 (12)	The Netherlands (multicentre)	09/1998-04/1999 n=1825 consecutive patients
An 2005 (13)	South Korea (multicentre)	04/2002 – 06/2003 n=422 consecutive patients
Sugiura 2000 (14)	Nagoya, Japan	1990-1998 n=1483 patients with suspected cosmetic dermatitis
Frosch 1995 (15)	11 European depts.	Prior to 1995 n=1069 consecutive patients
Frosch 2002 a (16)	6 European depts.	10/1997-10/1998 n=1855 consecutive patients
Frosch 2002 b (17)	6 European depts.	Prior to 2002 n=1606 consecutive patients
Coimbra 2000 (9)	Portugal	07/1989-06/1999 n=226 with FM I SC n=67 also with other fragrances
Larsen 1977 (18)	US	1977 n=20 “perfume-sensitive patients”
Larsen 2001 (19)	worldwide multicentre	? (prior to 2001) n=178 patients with known contact allergy to fragrance ingredients
Belsito 2006 (20)	North American (5 US, 1 Canadian) depts.	2003 n=1603 patients
NACDG 2009 (21)	US and Canada	2005-2006 n= 4454 patients
Wöhrl 2001 (22)	“FAZ” clinic Vienna	1997-2000 n=747 of 2660 consecutive patients tested with special series
EECDRG 1995 (15)	European, multicentre	Different fragrances, tested in 2 concentrations, in sets of about 100 patients each in different centres
Goossens 1997 (23)	Leuven, Belgium	1978-1987 n=111 “Japanese perfume series” (highly selected patients)

Opinion on fragrance allergens in cosmetic products

Reference	Country	Study period, Patients
Malten 1984 (24)	Dutch multicentre	N=182 patients with suspected cosmetic dermatitis tested with 22 fragrance compounds
DeGroot 1985 (25)	Dutch	N=179 patients with suspected cosmetic dermatitis tested with 16 fragrance compounds
Rudzki 1976 (26)	Warsaw, Poland	N=200 consecutive patients
Rudzki 1986 (27)	Warsaw, Poland	N=86 patients of 299 (of 5315) patients with positive reaction to FM I tested with essential oils series
Santucci 1987 (28)	Rome, Italy	N=1500 consecutive patients; n=63 reacting positively to FM I re-tested with extended fragrance series
Nakayama 1974 (after (29))	Japan	N=183 patients with cosmetic dermatitis
IVDK 2010c (30)	Germany, Switzerland and one centre in Austria	15682 patients tested with at least one essential oil in different test series
Trattner/David (31)	Tel Aviv, Israel	N=641 consecutive patients

Catalogue of single chemicals evaluated

ACETYLCEDRENE	
CAS # 32388-55-9	
EC # 251-020-3	
1-[(3R,3aR,7R,8aS)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl]-ethanone	
Other names 1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-, [3R-(3a,3aβ,7β,8aα)]-Ethanone; 1H-3a,7-Methanoazulene, Ethanone deriv.; Acetyl-α-cedrene; Lixetone; Vertofix	

Current regulation: /

Clinical

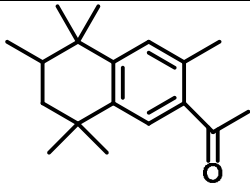
data:

In the Frosch 2002 a study, a total of 0.2% had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction to 1% and none to 5% "Vertofix ®" in pet., tested in 100 consecutive patients in Stockholm, were observed (15). In a case report, a 28-year-old patient with axillary dermatitis after using 2 different deodorants tested positive not only to HICC, but also to acetyl cedrene (tested 10.8% in diisopropylene glycol (20 healthy controls negative) (32). In this case report it is stated that "Acetyl cedrene (Vertofix Coeur) is a complex reaction mixture of which a principal constituent is methyl cedryl ketone".

Additional

information:

Acetyl cedrene (Vertofix®, IFF) is a complex mixture obtained from cedar wood oil by the acetylation of terpenes. The principal component of acetyl cedrene is methyl cedryl ketone (CAS 32388-55-9). It is a "top 100" substance (IFRA, pers. comm.2010)

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	
CAS # 21145-77-7	
EC # 216-133-4 / 244-240-6	
1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone	
AHMT (perfume), AHTN, Extralide, Fixolide, Musk tonalid, NSC 19550, Tentarome, Tetralide, Tonalid, Tonalide.	

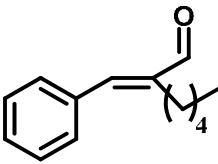
Current regulation: Annex III, part 1, entry 182

Clinical

data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Tonalide ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

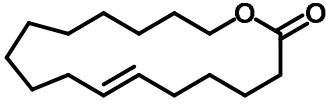
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; Jasmine aldehyde; NSC 6649; Pentylcinnamaldehyde; α-Amyl-β-phenylacrolein; α-Amylcinnamal; α-Amylcinnamaldehyde; α-Pentylcinnamaldehyde	

Current regulation: Annex III, part 1, entry 67

Clinical data:
In the “background information” section of the 1999 opinion (33), amyl cinnamal (synonymous: alpha amyl cinnamaldehyde) has been classified as frequently reported contact allergen because it has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=4, i.e., 0.2% (95% CI: 0.1 – 0.5%) positive reactions to this compound (1% pet.) in 2062 consecutively PTed patients (4). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed (6). The Larsen 2001 study yielded 2.3% positive reactions in 178 patients with known contact allergy to fragrance ingredients (test concentration: 5% pet.) (19). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to amyl cinnamal (22). The IVDK 2010 study, 0.26% (95% CI: 0 – 0.60%) of 1214 consecutively tested patients reacted to the compound, while 0.61% (95% CI: 0.36 – 0.86%) of 4375 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7).

Additional information:
It is a “top 100” substance and classified as R43 (IFRA, pers. comm. 2010).

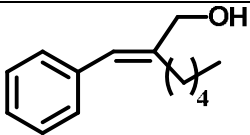
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

Current regulation: /

Clinical data:
The Larsen 2001 study, using omega-6-hexadecenlactone (HDL, 5% pet.) as test concentration, diagnosed 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Ambrettolide is 1 of 2 components of Ambrette seed oil (obtained from *Hibiscus abelmoschus* L., *Malvaceae*) responsible for the musk odour. In Surburg/Panten, the compound has the chemical name (Z)-7-hexadecen-16-olide (or Hexadec-7-en-16-olide according to CosIng), CAS 123-69-3 (34).

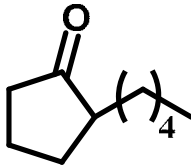
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol, 2-Benzylidene- (6Cl,8Cl)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

Current regulation: Annex II, Part 1, entry 74

Clinical data:
In the “background information” section of the 1999 opinion, amyl cinnamyl alcohol is mentioned to cross-react with amyl cinnamal. Moreover, this compound has been identified as a cause of allergic reactions in a notable number of persons with eczema from the use of cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.4% (95% CI: 0.1 – 0.7%) positive reactions in 1977 consecutively PTed patients (4). The IVDK 2010 study, 0.79% (95% CI: 0.54 – 1.04%; percentages standardised for age and sex) of 5650 patients PTed reacted to the compound (7). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6).

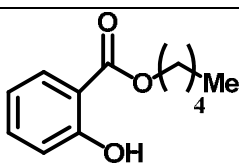
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

AMYLCYCLOPENTANONE	
CAS # 4819-67-4	
EC # 225-392-2	
2-Pentylcyclopentanone 2-Pentyl-1-cyclopentanone; 2-Pentylcyclopentanone; 2-Pentylcyclopenten-1-one; 2-n-Amylcyclopentanone; 2-n-Pentyl cyclopentanone; Delphone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

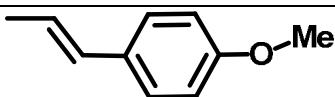
AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

Current regulation: /

Clinical data:

In the Frosch 2002 a study, a total of n=3 (0.2%) had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% amyl salicylate and 1 positive reaction to 5% amyl salicylate were observed in 100 consecutive patients patch tested in Stockholm (15).

Additional information:
A RIFM review is available (36). It is a “top 100” substance (IFRA, pers. comm.2010)

trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, EINECS); E-Anethole (INCI); 1-Methoxy-4-[(1E)-1-propenyl]benzene; (E)-1-Methoxy-4-(1-propenyl)-benzene (CosIng); NSC 209529; trans-1-(4-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)propene; trans-1-p-Anisylpropene; trans-4-(1-Propenyl)anisole; trans-Anethol; trans-Anethole; trans-p-Anethole; trans-p-Methoxy-β-methylstyrene	

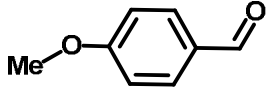
Current regulation: /

Clinical data:

A case of a 64 year old patient, who developed severe cheilitis and a loss of taste has been described (37). Both were reversible after the cessation of use of previous toothpastes. The patch test was strongly positive to anethole (isoform not given) 5% pet.; this was found an ingredient of the causative toothpaste. Two cases of occupational allergic contact dermatitis occurring in a traditional cake factory due to anise oil have been described, both testing (strongly) positive to anise oil (5% o.o.) and anethole (5% pet.) (38).

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). trans-Anethole can be purified from star anise oil (34, 39), see 3.2., and is the main component of anise, star anise

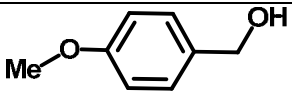
and fennel oils (38)

ANISALDEHYDE	
CAS # 123-11-5	
EC # 204-602-6	
4-Methoxy-benzaldehyde	
p-Methoxybenzaldehyde; p-Anisaldehyde; 4-Anisaldehyde; Aubepine; Crategine; NSC 5590; Obepin; p-Anisic aldehyde; Anisic aldehyde; p-Formylanisole.	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

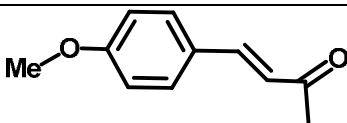
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy- α -hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methoxy)phenyl]methanol; p-(Methoxyphenyl)methanol; p-Anisalcohol; p-Anisyl alcohol; p-Methoxybenzyl alcohol	

Current regulation: Annex III, part 1, n° 80

Clinical data:
In the “background information” section of the 1999 opinion, anisyl alcohol is classified as “less frequently reported allergen”; 2 studies were identified where 3 and 4 cases, respectively, with cosmetic dermatitis due to contact allergy to anisyl alcohol had been reported (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded $n=1$, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2004 consecutively PTed patients, patch test concentration: 1% pet. (4). Similar results were obtained in the following period, with $n=1$ (and $n=3$ irritant and $n=6$ doubtful) reactions in 986 patients tested with 1% in pet. (30). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). This test concentration has been regarded as relatively high by Hostynek and Maibach (40). The test concentration of Anisyl Alcohol has been further validated by Bruze et al. and 10% in pet was recommended as a non-irritant concentration for routine investigations (40a).

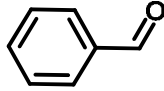
Additional information: /

ANISYLIDENE ACETONE	
CAS # 943-88-4	
EC # 213-404-9	
4-(4-Methoxyphenyl)-3-Buten-2-one	
1-(p-Methoxyphenyl)-1-buten-3-one; 4-(4-Methoxyphenyl)-3-buten-2-one; 4-(p-Methoxyphenyl)-3-buten-2-one; 4-Methoxybenzalacetone; 4-Methoxybenzylideneacetone; 4-Methoxystyryl methyl ketone; 4'-Methoxybenzylideneacetone; Anisalacetone; Methyl p-methoxystyryl ketone; NSC 31752; NSC 7946; p-Anisalacetone; p-Methoxybenzalacetone; p-Methoxybenzylideneacetone; p-Methoxystyryl methyl ketone	

Current regulation: Annex III, part 1, n° 443

Clinical data:
In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to anisylidene acetone 2% pet. (24)

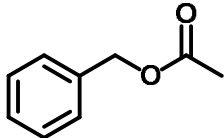
Additional information: /

BENZALDEHYDE	
CAS # 100-52-7	
EC # 202-860-4	
Benzaldehyde	
Artificial Almond Oil; Benzaldehyde FFC; Benzenecarbonal; Benzenecarboxaldehyde; Benzoic acid aldehyde; Benzoic aldehyde; NSC 7917; Phenylformaldehyde; Phenylmethanal	

Current regulation: /

Clinical data:
 In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions to benzaldehyde 5% pet. (22). The IVDK 2010 study, 6 weak positive reactions were observed, i.e., 0.16% (95% CI: 0.03 – 0.29%; percentages standardised for age and sex) of 2820 patients PTed reacted to the compound (7). A review is available in the Int. J. Toxicol. (41). In the case of a 19 year old pastry maker, Seite-Bellezza et al. report on immediate reactions to MP, cinnamal and benzaldehyde (tested at 5% pet.) subsiding after a few hours, in line with the patient's history (42).

Additional information: /

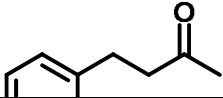
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl acetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

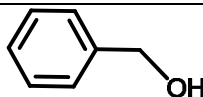
Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl acetate in pet., tested in 100 consecutive patients in Odense, DK, were observed (15). Benzyl acetate is a component of several natural mixtures, for example a major constituent of Narcissus abs., and a minor constituent of Jasmine abs. (17).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

BENZYL ACETONE	
CAS # 2550-26-7	

EC # 219-847-4	
4-Phenyl-2-butanone	
4-Phenylbutan-2-one (REACH, EINECS); Benzylacetone; Methyl 2-phenylethyl ketone; Methyl phenethyl ketone; NSC 44829; NSC 813M; Phenethyl methyl ketone; 1-Phenyl-3-butanone; 2-Phenylethyl methyl ketone	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (43).	

BENZYL ALCOHOL	
CAS # 100-51-6	
EC # 202-859-9	
Phenylmethanol	
Benzyl alcohol; (Hydroxymethyl)benzene; Benzenecarbinol; Benzylic alcohol; NSC 8044; Phenylcarbinol; Benzenemethanol; Phenylmethyl alcohol; Sunmorl BK 20; TB 13G; α-Hydroxytoluene; α-Toluenol	
Current regulation: Annex III, part 1, n° 45; Annex VI, part1, n ° 34	

Clinical data:
In the “background information” section of the 1999 opinion, benzyl alcohol is classified as allergen frequently causing allergic reactions. It has been found to cause allergic reactions in 1.2 to 15% of patients with eczema from cosmetic products (33). A CIR expert panel review is available in the Int. J. Toxicol. (44).

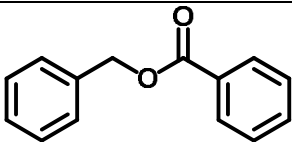
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

Both in terms of case reports (45-47) and clinical epidemiology data (0.22 % [95% CI: 0.16 – 0.28%] positive tested with benzyl alcohol in the context of a “topical drugs” series, n=26448 (7)) the relevance of this alternative exposure is highlighted. In a study from Alicante, Spain, 86 selected patients were tested with benzyl alcohol, yielding 2 positive reactions (48).

After application of saline soaks preserved with benzyl alcohol onto his stasis dermatitis, a 53 year old patient developed a rash, which was, according to test results obtained by J. D. Guin and J. Goodman, at least partly due to an immediate hypersensitivity to benzyl alcohol, as verified by an intense urticarial reaction at the test site lasting several days (49). According to 2 cases reported by A. A. Fisher, PT-proven, relevant delayed type hypersensitivity is not associated with immediate reactions in scratch or intradermal tests (50). D. W. Shaw describes a patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications (51). Another contribution points to covert exposures to benzyl alcohol even in products labelled “fragrance free” (52) probably because benzyl alcohol is used as preservative, or an essential oil containing benzyl alcohol is used as cosmetic ingredient.

Additional information:

Benzyl alcohol is a component of several natural mixtures, including Myroxylon pereirae resin, which have been used for extraction, but is nowadays synthesised (53). It is permitted in certain foodstuffs (liquors: < 100 mg/l, sweets and cakes: < 250 mg/kg) under the coding “E 1519” (http://www.zusatzstoffe-online.de/zusatzstoffe/317.e1519_benzylalkohol.html, last accessed 2009-11-27). In addition to being a fragrance compound (which may be used, even in relatively high concentration, to scent topical medications (54)), benzyl alcohol is used as antioxidant in topical therapeutics or cosmetics. The German “Rote Liste” (<http://www.rote-liste.de>, last accessed 2009-11-11), for instance, lists 205 specialties containing benzyl alcohol. Benzyl alcohol may be used up to 1.0% as a preservative in cosmetic products according to the Cosmetic Directive 76/768/EEC

BENZYL BENZOATE	
CAS # 120-51-4	
EC # 204-402-9	
Benzyl benzoate	
Benzyl ester benzoic acid; Ascabin; Ascabiol; Benylate; Benzyl benzenecarboxylate; Benzyl benzoate; Benzyl phenylformate; Benzylets; Colebenz; NSC 8081; Nicca Sunsolt LM 7EX; Novoscabin; Pelemol B66; Peruscabin; Phenylmethyl benzoate; Scabagen; Scabanca; Scabcare BB; Scabide; Scabiozon; Scobenol; Vanzoate; Venzonate	

Current regulation: Annex III, part 1, n° 85

Clinical data:

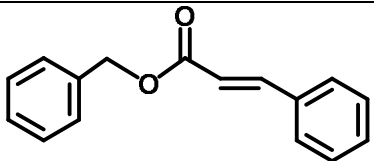
In the “background information” section of the 1999 opinion, benzyl benzoate is classified as “less frequently reported allergen”; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2003 consecutively PTed patients, test concentration 1% pet. (4). In the subsequent period (2005-2008), n=1062 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). Thus, the pooled proportion of positive patch test reactions is 1 / 3385 (0.03%, exact upper 1-sided 95% CI: 0.14%)

Additional information:

Benzyl benzoate naturally occurs in MP resin and ylang-ylang oil. Nowadays it is synthesised and used for a variety of purposes (53). These include use as a scabicide (one brand specialty on the German market, using a concentration of 10% for children and 25% for adults), possibly with some differences among European countries. In France, a combination of benzyl benzoate 10% and sulfiram 2% is reported to be used most often (55). Hausen et al. review the older literature and mention a study identifying 1 sensitised patient in 73 patients treated for scabies (details not given) (53). According to the mandatory factsheet (see PDF “benzylbenzoate_infosheet_DE.pdf”) dermatitis after anti-scabies treatment is “rare”, in a range between 1:1000 and 1:10000.

It is a “top 100” substance (IFRA, pers. comm.2010).

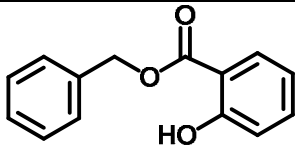
BENZYL CINNAMATE	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

Current regulation: Annex III, part 1, n° 81

Clinical data:
In the “background information” section of the 1999 opinion, benzyl cinnamate (synonymous: benzyl 3-phenyl-2-propenoate, cinnamein) is classified as “less frequently reported allergen”; one study of patients with contact allergy to cosmetic products was identified and further a study where benzyl cinnamate associated with contact sensitisation to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2042 consecutively PTed patients, test concentration 5% pet. (4). The IVDK 2010 study, n=4 weak positive were observed, amounting to 0.12% (95% CI: 0 – 0.25%; percentages standardised for age and sex) of 2872 patients PTed reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, using the same test concentration, were observed in 320 patients (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22).

Additional information: A RIFM review is available (56).

BENZYL SALICYLATE	
CAS # 118-58-1	
EC # 204-262-9	
Benzyl 2-hydroxybenzoate	
Salicylic acid, Benzyl ester; Benzoic acid, 2-Hydroxy-, phenylmethyl ester; Benzyl o-hydroxybenzoate; NSC 6647	

Current regulation: Annex III, part 1, n° 75

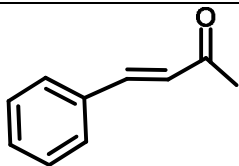
Clinical data:

In the “background information” section of the 1999 opinion (33), benzyl salicylate is classified among the frequent allergens, with 0.2 to 10% of patients with eczema from cosmetic products testing positively. In one study, benzyl salicylate accounted for 75% of reactions to commercial products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=2, i.e. 0.1% (95% CI: 0.01 – 0.4%) positive reactions in 2041 consecutively PTed patients (test concentration 1% pet.) (4). The IVDK 2010 study, 2 of 3775 patients PTed reacted weakly positive to the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at 2% pet. (6). In the deGroot 2000 study, 10 of 1825 consecutive patients tested positive to benzyl salicylate (2% pet.), of these, 3 were not detected by the FM I (12). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22). Trattner/David found 2 positive cases in 641 consecutive eczema patients (31). In a study from Alicante, Spain, 86 selected patients were tested with benzyl salicylate, yielding 2 positive reactions (48).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010). A RIFM review is available, including internal results on, e.g. HRIPT, and a review of LLNA results, where benzyl salicylate is classified as “weak” allergen (57).

BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; Acetocinnamone; Benzalacetone; Benzylideneacetone; Methyl 2-phenylvinyl ketone; Methyl phenylvinyl ketone; Methyl styryl ketone; Methyl β-styryl ketone; NSC 5605; Styryl methyl ketone	

Current regulation: Annex II, n° 356

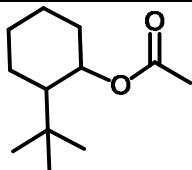
Clinical

data:

In the Malten 1984 study, none of 182 patients displayed a positive PT reaction to

benzylidene acetone 0.5% pet. (24).

Additional information: /

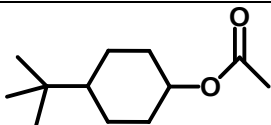
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Verdox ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (58).

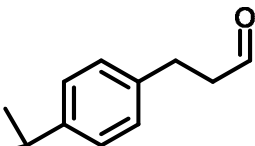
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinal A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; NSC 163103; Oryclone, Oryclone special, Oryclon extra; p-t-BCHA; p-tert-Butylcyclohexyl acetate; para-tert-Butylcyclohexyl acetate; PTBCHA; Velvetone; Verbeniax; Vertenex; Vertinate; Vertopol; Ylanate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Vertenex ®” in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (59).

p-tert -Butyldihydrocinnamaldehyde	
CAS # 18127-01-0	
EC # 242-016-2	

4-(1,1-Dimethylethyl)-benzenepropanal	
p-tert-Butyl-hydrocinnamaldehyde; 3-(4-tert-Butylphenyl)propanal; Bourgeonal; p-tert-Butyldihydrocinnamaldehyde	

Current regulation: III/155

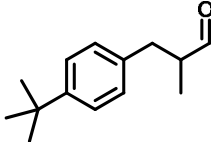
Clinical data: /

Additional

information:

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=39132

BUTYLPHENYL METHYLPROPIONAL (Lilial®)	
CAS # 80-54-6	
EC # 201-289-8	
3-(4-tert-Butylphenyl)-2-methylpropanal	
p-t-Butyl-alpha-methylhydrocinnamic aldehyde; 2-(4-tert-Butylbenzyl)propionaldehyde (REACH, EINECS); 4-(1,1-Dimethylethyl)-α-methyl-benzenepropanal; Hydrocinnamaldehyde, p-tert-Butyl-α-methyl-; (±)-2-Methyl-3-(4-tert-butylphenyl)propanal; 2-Methyl-3-(4-tert-butylphenyl)propanal; 2-[(4-tert-Butylphenyl)methyl]propanal; 3-(4-tert-Butylphenyl)-2-methylpropanal; 3-(p-tert-Butylphenyl)-2-methylpropionaldehyde; 3-(p-tert-Butylphenyl)isobutylaldehyde; 4-(1,1-Dimethylethyl)-α-methylbenzenepropanal; 4-tert-Butyl-α-methylhydrocinnamic aldehyde; Lilestralis; Lilial; Lysmeral; NSC 22275; lilestral; p-tert-Butyl-α-methylhydrocinnamaldehyde; p-tert-Butyl-α-methylhydrocinnamic aldehyde; pt-Bucinal; α-Methyl-p-tert-butylhydrocinnamaldehyde; β-Lilial	

Current regulation: Annex III, part 1, n° 83

Clinical data:

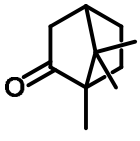
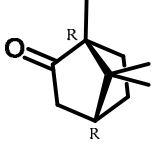
In the “background information” section of the 1999 opinion, lilial is classified as “less frequently reported allergen”; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were considered to possibly have been false positive (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002a study yielded 0.2% positive reactions to Lilial® (10% pet.) among the 1855 consecutive patients tested (16). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2004 patients consecutively tested (4). The IVDK 2010 study, 0.62% (95% CI: 0.04 – 1.21%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In the deGroot 2000 study, 9 of 1825 consecutively tested patients had a positive reaction to lilial® (5%

pet.) (12). Lilial® has been identified as constituent of perfumes used by a patient, causing ACD (60).

Additional information:

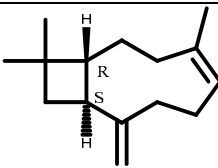
It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2
CAS # 76-22-2 / 464-49-3	
EC # 207-355-2 / 200-945-0	
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (76-22-2) (1R,4R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (464-49-3)	
76-22-2: DL-Bornan-2-one (REACH, EINECS); 2-Bornanone; Bornan-2-one, INCI name according to CAS; CAMPHOR/DL-bornan-2-one; Camphor; (±)-Camphor; DL-Camphor; 1,7,7-Trimethylnorcamphor; 2-Camphanone; Alphanon; Borneo camphor; Root bark oil; Spirit of camphor 464-49-3: (1R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one; (1R,4R)-(+)-Camphor; (+)-2-Bornanone; (+)-Camphor; (1R)-(+)-Camphor; (1R)-Camphor; (1R,4R)-(+)-Camphor; (R)-(+)-Camphor; (R)-Camphor; Camphor; D-Camphor; D-(+)-Camphor; Alcanfor; Japanese camphor.	 464-49-3

Current regulation: /

Clinical data:
From the UK, a case of allergic contact dermatitis after application of Earex ® ear drops due to rectified camphor oil (tested 10% pet.) was reported (61). Application of a liquid rubefacient of Asian origin caused allergic contact dermatitis in a 58-year-old patient, according to the positive PT result with 10% camphor ("alcaonfor") in pet. due to this ingredient (62). In the US, a case of contact dermatitis due to "Vics VapoRub" has been reported (63).

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

beta-CARYOPHYLLENE	
CAS # 87-44-5	
EC # 201-746-1	
(1R,4E,9S)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	
(E)-(1R,9S)-(-)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; [1R-(1R*,4E,9S*)]-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; (-)-(E)-Caryophyllene; (-)-Caryophyllene; (-)-E-Caryophyllene; (-)-trans-Caryophyllene; (-)-β-Caryophyllene; (E)-Caryophyllene; Caryophyllene; Caryophyllene B; NSC 11906; l-Caryophyllene; trans-Caryophyllene; β-Caryophyllen; β-Caryophyllene; (-)-β-Caryophyllene	

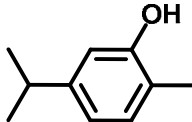
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% positive reactions to caryophyllene (5% pet.) in 1606 consecutive were observed (17).

Additional information:

beta-Caryophyllene autoxidizes at air exposure. As the primary oxidation products, the hydroperoxides, are very unstable and immediately form epoxides with low sensitizing capacity, the increase in allergenic activity caused by autoxidation is comparably low (64). A multicenter study identified 0.5% positive reactions to oxidized *beta*-caryophyllene (3.0% pet.) in 1511 consecutive patients (65). Of these, 2 patients (0.1%) reacted to the major oxidation product (caryophyllene oxide) (3.9% pet.).

CARVACROL	
CAS # 499-75-2	
EC # 207-889-6	
2-Methyl-5-(1-methylethyl)-phenol	
2-Hydroxy-1-methyl-4-(1-methylethyl)benzene; 2-Hydroxy-p-cymene; 2-Methyl-5-(1-methylethyl)phenol; 2-Methyl-5-isopropylphenol; 3-Isopropyl-6-methylphenol; 5-Isopropyl-2-methylphenol; 5-Isopropyl-o-cresol; 6-Methyl-3-isopropylphenol; Antioxine; Dentol; Isopropyl o-cresol; Isothymol; NSC 6188; p-Cymen-2-ol	

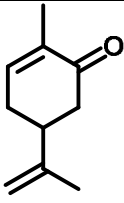
Current regulation: /

Clinical data:

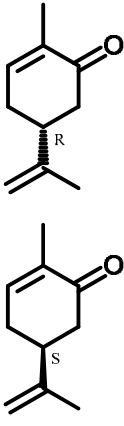
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an “excited back syndrome” and are thus of limited evidence (25). Meynadier et al.¹¹ patch tested 28 patients with contact allergy to fragrance ingredients using 2% carvacrol in pet. Positive reactions were observed in 3 of 28 patients (after (66)).

Additional information:

Carvacrol is derived from p-cymene by sulfonation followed by alkali fusion. Carvacrol can also be derived from savory, thyme, marjoram, oregano, lovage root, and Spanish origanum oil (66). Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001, according to (66)).

CARVONE	
CAS # 99-49-0 / 6485-40-1 / 2244-16-8	
EC # 202-759-5 / 229-352-5 / 218-827-2	
2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (99-49-0)	

¹¹ Meynadier, J. M., J. Meynadier, J. L. Peyron, and L. Peyron. 1986. Clinical forms of skin manifestations in allergy to perfume. *Ann. Dermatol. Venerol.* 113:31–39.

(5R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (6485-40-1) (5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (2244-16-8)	 6485-40-1 2244-16-8
99-49-0: p-Mentha-6,8-dien-2-one; (±)-Carvone; 2-Methyl-5-isopropenyl-2-cyclohexenone; 5-Isopropyl-2-methyl-2-cyclohexen-1-one; Carvone; DL-Carvone; Karvon; Limonen-6-one; NSC 6275; p-Mentha-1(6),8-dien-2-one 6485-40-1: R)-(-)-p-Mentha-6,8-dien-2-on); (-)-(5R)-Carvone; (-)-(R)-Carvone; (-)-Carvone; (-)-p-Mentha-6,8-dien-2-one; (4R)-(-)-Carvone; (R)-(-)-Carvone; (R)-Carvone; L-(-)-Carvone; L-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; l-Carvone 2244-16-8: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone	

Current regulation: /

Clinical data:

Cases of allergic contact cheilitis due to L-carvone in toothpastes have been reported (67-69). In an earlier study, 15 of 541 (2.8%) of consecutive PT patients tested also with L-Carvone (5% pet.) exhibited positive reactions, which were (i) associated with positive PT results to *Compositae* mix and (ii) mostly were not considered clinically relevant. Upon re-testing with lower concentrations (2% and 1% pet.) only 2 of 8 patients thus tested were positive (70).

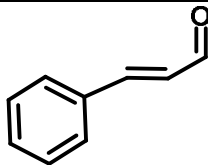
“Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis on the head. She had used a hair conditioner with a “mint” scent, and the dermatitis resolved when she discontinued using this product. While the manufacturer would not confirm carvone as an ingredient, the clinical course, patch-test results, and ingredient list strongly suggest that this was a relevant allergen in this case of allergic contact dermatitis”¹²

Additional information:

D-Carvone occurs in caraway seed oil and dill oil in a concentration of up to 60%. L-Carvone is a component of the oil from *Mentha spicata* (spearmint).

R-Carvone is identified as a secondary oxidation product in autoxidized limonene (71). However, it is not a major allergen in this oxidation mixture and only one of 30 patients with known contact allergy to oxidized R- limonene reacted when tested with carvone (3% pet.) (72). Experimental findings in guinea pigs show no cross reactivity between R- and S carvone, but both enantiomers were found to be equally strong sensitizers (73).

¹² <http://www.ncbi.nlm.nih.gov/pubmed/20233552>

CINNAMAL	
CAS # 104-55-2	
EC # 203-213-9	
3-Phenyl-2-propenal	
Cinnamaldehyde; 3-Phenyl-2-propen-1-al; 3-Phenyl-2-propenaldehyde; 3-Phenylacrolein; 3-Phenylacrylaldehyde; 3-Phenylpropenal; Abion CA; Benzylideneacetaldehyde; Cassia aldehyde; Cinnacure; Cinnamal; Cinnamic aldehyde; Cinnamite; Cinnamyl aldehyde; NSC 16935; NSC 40346; Phenylacrolein; Zimtaldehyde; β-Phenylacrolein	

Current regulation: Annex III, part 1, n° 76

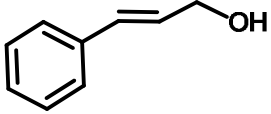
Clinical data:
In the “background information” section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.0% (95% CI: 0.6 – 1.6%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.6% (95% CI: 0.5 – 3.6%) had positive reactions to cinnamal (6). In a study by the North American Contact Dermatitis Group, no significant trend of cinnamal contact sensitisation in the consecutive patients analysed was observed between 1984 (5.9% pos.) and 2000 (3.6% pos.); tested at 1% pet. (74). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded 1.7% positive reactions. In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.9% positive reactions (22). The NACDG study found 3.1% positive reactions in 4435 patients tested (21). The IVDK 2010 study, 1.43% (95% CI: 0.67 – 2.18%) of 1214 consecutively tested patients reacted to the compound, while 2.64% (95% CI: 2.16 – 3.13%) of 4527 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=7 reacted positively to cinnamal (48).

While, in addition to typical ACD due to contact sensitisation, immediate reactions to some fragrance compounds (and MPR, see below) are observed not infrequently, such immediate type reactions may rarely be very severe (anaphylaxis) and possibly immunologically mediated, as illustrated by the case of a 42 year old nurse with anaphylaxis (maximum grade of contact urticaria syndrome) 20 min after application of cinnamal (75). Following industrial use as “odour masking” agent, cinnamal caused occupational ACD in an exposed worker (76).

Additional information:

A specific RIFM review is available (77); another RIFM review addresses several cinnamic compounds (78).

CINNAMYL ALCOHOL	
CAS # 104-54-1	
EC # 203-212-3	
3-Phenyl-2-propen-1-ol	

Opinion on fragrance allergens in cosmetic products

Cinnamyl alcohol; 1-Phenyl-3-hydroxy-1-propene; 1-Phenylprop-1-en-3-ol; 3-Hydroxy-1-phenylprop-1-ene; 3-Phenyl-2-propenol; 3-Phenylallyl alcohol; Cinnamic alcohol; NSC 623440; NSC 8775; Styrene; Styryl alcohol; Styryl carbinol; γ -Phenylallyl alcohol	

Current regulation: Annex III, part 1, n° 69

Clinical data:

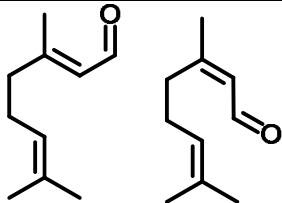
In the “background information” section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to cinnamyl alcohol, tested at 2% pet., i.e., twice the commonly used concentration (6). As test concentrations of up to 5% are apparently non-irritating (de Groot et al. after (33)), the latter data can be regarded as valid. In the An 2005 study, 13 of 422 consecutive patients, i.e., 3.1%, had positive reaction (13) (test concentration 2%). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 0.73% (95% CI: 0.17 – 1.30%) of 1214 consecutively tested patients reacted to the compound, while 2.36% (95% CI: 1.89 – 2.83%) of 4502 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to cinnamyl alcohol (48).

Additional information:

In a recent experimental study protein-cinnamal adducts were detected in skin homogenates treated with cinnamal and cinnamyl alcohol but not with alpha-amyl cinnamal. This suggests that there is a common hapten involved in cinnamal and cinnamyl alcohol sensitization, in line with the observation of a marked concordance upon patch testing (7, 79), and that metabolic activation (to cinnamal) is involved in the latter. Conversely, there does not appear to be a common hapten for cinnamal and alpha-amyl cinnamal (80), again in line with the observations in the IVDK 2010 study (7).

A RIFM review is available (81)

CITRAL	 Citral = isomeric mixture of Geranial and Neral
CAS # 5392-40-5	
EC # 226-394-6	
3,7-Dimethyl-2,6-octadienal	
3,7-Dimethyl-2,6-octadien-1-al; Citral; Citral PQ Extra; Lemarome N; Lemsyn GB; NSC 6170	

Current regulation: Annex III, part 1, n° 70

Clinical data:

In the “background information” section of the previous opinion (33), citral is classified as frequent allergen, causing about 1% allergic reactions in consecutive PT patients, and being a proven cause of contact allergic reactions in 2.6% patients with eczema from

cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002 a study yielded 1.1% positive (and 1.3% doubtful) reactions among the 1855 consecutive patients tested (16). In a study on 586 consecutive patients with hand eczema it has been noted that citral (2% pet.) not only caused (mostly weak) positive PT reactions, but far more often irritant reactions (n=82 vs. n=28). It was hypothesised that this very property could contribute to citral's sensitising potential (82). In the EU 2005 study, 12 of 1701 patients (0.7%, 95% CI: 0.4 – 1.2%) reacted positively to 2% citral in pet. (10). The IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2021 consecutively PTed patients; 10 of 13 citral positive patients also reacted positively to geraniol (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 multicentre study, 19 of 1825 consecutive patients tested positively to citral (2% pet.), 4 of whom did not react positively to the FM I (12). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reaction (13) (test concentration 2%). In the Malten 1984 study, neral at 1% in pet. yielded 2.6% positive reactions in 182 patients (24). In a study from Alicante, Spain, 86 selected patients were tested with citral, yielding 2 positive reactions (48).

Citral in a lip salve has been reported to have caused longstanding, recurrent allergic contact cheilitis in a 30 year old female patient, diagnosed by a strong positive reaction to the FM II, followed by a strong positive reaction to citral (83).

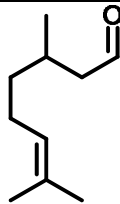
Additional information:

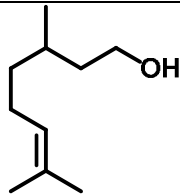
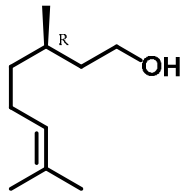
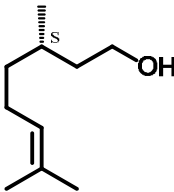
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

Geranial forms oxidation product with increased sensitizing capacity both via spontaneous autoxidization at air exposure and via metabolic oxidation (Hagvall L. Thesis 2009: <http://hdl.handle.net/2077/18951>).

Geranial and neral have been identified as secondary oxidation products when geraniol autoxidizes (84). They have also been identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLAL	
CAS # 106-23-0	
EC # 203-376-6	
3,7-Dimethyl-6-octenal	
(±)-Citronellal; 2,3-Dihydrocitral; 3,7-Dimethyloct-6-en-1-al; Citronellal; NSC 46106; Rhodinal; dl-Citronellal; β-Citronellal	
Current regulation: /	
Clinical /	data:
Additional information: A compound of essential oils of citrus fruits, namely grapefruit, but also contained in “citronella oil” and oil of Melissa.	

CITRONELLOL			
CAS # 106-22-9 / 1117-61-9 / 7540-51-4			
EC # 247-737-6 / 214-250-5 / 231-415-7			
3,7-Dimethyl-6-octen-1-ol (106-22-9); (3R)-3,7-Dimethyl-6-octen-1-ol (1117-61-9); (3S)-3,7-Dimethyl-6-octen-1-ol (7540-51-4)			106-22-9
106-22-9: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronello 950; DL-Citronellol; Dihydrogeraniol; NSC 8779; Rodinol; dl-Citronellol; β-Citronellol			
1117-61-9: (R)-3,7-Dimethyl-6-octen-1-ol; (R)-(+)-3,7-Dimethyl-6-octen-1-ol; (+)-(R)-Citronellol; (+)-Citronellol; (+)-β-Citronellol; (3R)-(+)-β-Citronellol; (R)-(+)-Citronellol; (R)-(+)-β-Citronellol; (R)-Citronellol; (R)-β-Citronellol; D-Citronellol; d-Citronellol	1117-61-9	7540-51-4	
7540-51-4: (-)-3,7-Dimethyl-6-octen-1-ol; (-)-(S)-Citronellol; (-)-Citronellol; (-)-β-Citronellol; (S)-(-)-Citronellol; (S)-(-)-β-Citronellol; (S)-3,7-Dimethyl-6-octen-1-ol; (S)-Citronellol; (S)-β-Citronellol; L-Citronellol; l-Citronellol			

Current regulation: Annex III, part 1, n° 86

Clinical data:

In the “background information” section of the 1999 opinion, citronellol is classified as “less frequently reported allergen”; with few cases of contact allergy reported in the literature (33).

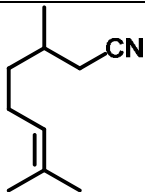
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronello“ (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%, 95% CI: 0.06 – 0.6%) reacted positively to 1%

citronellol in pet.; at the same concentration, n=23 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2003 patients consecutively PTed (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at only 2% pet. (6). The Larsen 2001 study yielded 5.6% positive reactions to l-citronellol (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Citronellol autoxidizes spontaneously in contact with air in the same way as linalool forming allergenic primary oxidation products, hydroperoxides (AT Karlberg, personal communication, 2011).

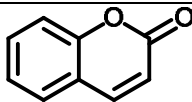
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLYL NITRILE	
CAS # 51566-62-2	
EC # 257-288-8	
3,7-Dimethyl-6-octenenitrile	
3,7-Dimethyl-6-octenenitrile (REACH, EINECS, INCI); Agrunitril; Agrunitrile; Citronellyl nitrile	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010)

COUMARIN	
CAS # 91-64-5	
EC # 202-086-7	
2H-1-Benzopyran-2-one	
1,2-Benzopyrone; 2-Chromenone; 2-Propenoic acid, 3-(2-hydroxyphenyl)-, δ -lactone; 5,6-Benzo-2-pyrone; Benzo- α -pyrone; Coumarinic anhydride; NSC 8774; Rattex; Tonka bean camphor; cis-o-Coumarinic acid lactone; o-Hydroxycinnamic acid lactone	

Current regulation: Annex III, part1, n° 77

Clinical data:

In the “background information” section of the previous opinion (33), coumarin is classified as frequent allergen, causing allergic reactions in about 0.4 – 0.8% in consecutive PT patients, and causing contact allergic reactions in 0.8-10% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the EU 2005 study, none of the

1701 patients reacted positively to 5% coumarin in pet., while 7 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2020 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 study, 13 of 1825 consecutive patients reacted positively to coumarin (5% pet.) (12).

V. Mutterer et al. present the case of a 44 year old patient in whom coumarin was identified as culprit allergen by controlled ROAT testing with 1%, after having caused dermatitis by the use of a deodorant containing coumarin at 0.23% and an EdT (90).

Additional information:

Coumarin is found in several plant families, including *Melilotus* and *Galium*, e.g., *Galium odoratum* (sweet woodruff), however, also in oil of lavender, lovage and others (53).

Researchers from INSERM and “Rhodia Organique, Lyon , France” observed that pure coumarin is not an allergen in the LLNA, however, commercially available materials, containing “contaminants” (3,4-dihydrocoumarin, 6-chlorocoumarin and 6,12-epoxy-6H,12H-dibenzo[b,f][1,5] dioxocin, were identified as weak and moderate sensitisers, resp. (91).

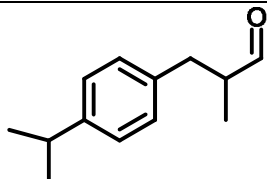
Coumarin is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CUMINALDEHYDE	
CAS # 122-03-2	
EC # 204-516-9	
4-(1-Methylethyl)-benzaldehyde	
4-Isopropylbenzaldehyde; p-Isopropylbenzaldehyde; 4-(Propan-2-yl)benzaldehyde; 4-Isopropylphenylcarboxaldehyde; Cumaldehyde; Cumic aldehyde; Cuminal; Cuminaldehyde; Cuminic aldehyde; Cuminyaldehyde; NSC 4886; p-Cumic aldehyde; p-Isopropylbenzaldehyde; p-Isopropylbenzenecarboxaldehyde	

Current regulation: /

Clinical data:
The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 15% PT preparation of cuminaldehyde (25).

Additional information: ...

CYCLAMEN ALDEHYDE	
CAS # 103-95-7	
EC # 203-161-7	
α-Methyl-4-(1-methylethyl)-benzenepropanal	
p-Isopropyl-α-methyl-hydrocinnamaldehyde; 2-Methyl-3-(4-isopropylphenyl)propionaldehyde; 2-Methyl-3-(p-isopropylphenyl)propionaldehyde; 3-(4-Isopropylphenyl)-2-methylpropanal; 3-(p-Isopropylphenyl)-2-	

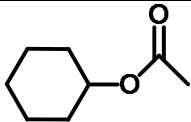
methylpropionaldehyde; methylpropionaldehyde(REACH, EINECS); methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl- α - methylhydrocinnamaldehyde; methylethyl)benzenepropanal; isopropylhydrocinnamaldehyde	3-p-Cumenyl-2- 4-Isopropyl- α - α -Methyl-4-(1- α -Methyl-p-	

Current regulation: ...

Clinical
/

data:

Additional information: It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

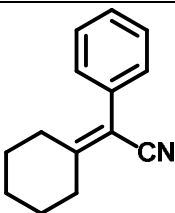
CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.5% positive reactions among 218 patients with know contact allergy to fragrance ingredients were observed (1).

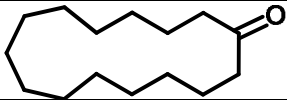
Additional information: A RIFM review is available (92).

<i>alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE</i>	
CAS # 10461-98-0	
EC # 423-740-1	
α-Cyclohexylidenebenzeneacetonitrile	
alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ 1 α -Phenyl- α -Cyclohexaneacetonitrile; 2-Cyclohexylidene-2-phenylacetonitrile; NSC 408284; Peonile (REACH)	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

CYCLOPENTADECANONE	
CAS # 502-72-7	

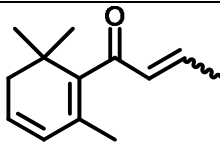
Opinion on fragrance allergens in cosmetic products

EC # 207-951-2	
Cyclopentadecanone	
CPE 218; Exaltone; NSC 63900; Normuscon; Normuscone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, n=3, i.e., 1.7% positive reactions were observed to the compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: ...

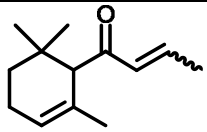
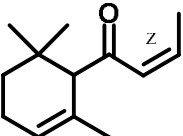
DAMASCENONE	
ROSE KETONE-4 (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-(2-butenoyl)-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-crotonyl-1,3-cyclohexadiene; Rose ketone # 4	

Current regulation: Annex III, part1, n° 160 (max. conc. 0.02%)

Clinical data: /

Additional information:

RIFM reviews are available (93, 94), quoting 1 negative, and 2 positive (2 of 37, 1 of 50 volunteers) HRIPTs with damascenone based on 2 LLNA, the EC3 values were calculated as 1.24% and 1.22%, respectively (94).

<i>alpha-DAMASCONE (TMCHB)</i>	 43052-87-5
CAS # 43052-87-5 / 23726-94-5	
EC # x / 245-845-8	
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-2-enone (43052-87-5); (2Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one (23726-94-5)	
43052-87-5: 2,6,6-Trimethyl-1-crotonyl-2-cyclohexene; α -Damascone	 23726-94-5
23726-94-5: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)- α -Damascone; cis- α -Damascone	

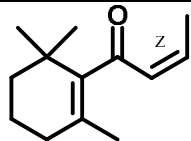
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

Clinical data:
In the Frosch 2002 b study, n=8 (0.5%) mostly strong positive PT reactions to consecutive patients were noted using a mixture of alpha and beta damascene, 0.1% pet. each (17). In human sensitisation experiments, after epicutaneous induction with 30% 1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enone (TMCHB, CAS # 43052-87-5) with adjuvant, to enhance response to this weak sensitiser, 8 of 30 patients were elicited by a challenge with 3% TMCHB 2 weeks later (95).

Additional information:

The former CAS # refers to alpha-Damascone or 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one. The latter CAS # refers to the identified ingredient cis-alpha-Damascone or (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, the content of which is restricted (SCCS-opinion 0392/00).

A RIFM review is available on alpha-damascone (96), quoting a number of partly positive HRIPT and other human studies, as well as different animal experiments. In 1 LLNA reported, an EC3 value of 3.3% was found. Another RIFM review is available for cis-alpha-damascone (97), supplying, however, no data on sensitisation.

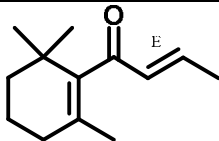
<i>cis-beta-DAMASCONE</i>	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)- β -Damascone	

Current regulation: Annex III, part 1, n° 162 (max. conc. 0.02%)

Clinical data:
Regarding results of the Frosch 2002 b study, see under alpha-damascone.

Additional information:

A RIFM review is available (98), citing several negative and one positive HRIPTs, and a number of – mostly positive – animal experiments.

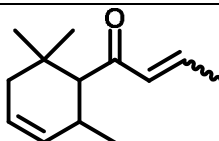
<i>trans-beta-DAMASCONE</i>	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)- β -Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans- β -Damascone; β -Damascone	

Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (99), citing 2 negative HRIPT and 1 negative maximisation test, and a number of positive animal experiments (the EC3 value, based on 1 LLNA, was found to be 2.4%).

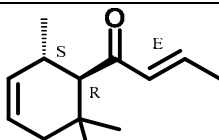
<i>delta-DAMASCONE</i>	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ-Damascone	

Current regulation: Annex III, part 1, n° 161 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (100), citing several positive HRIPT and 1 negative HRIPT. Cross sensitisation to alpha- and beta-damascone was demonstrated in 3 sensitised subjects. 2 LLNA studies are reported on, yielding EC3 values of 5.19% and 9.6%, resp.

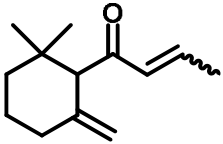
<i>trans-trans-delta-DAMASCONE</i>	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1 α (E),2 β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans- δ -Damascone; δ -Damascone; trans, trans- δ -Damascone	

Current regulation: Annex III, part 1, n° 165 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (101), citing 1 positive HRIPT (2/15 with 1%).

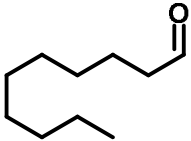
<i>gamma-DAMASCONE</i>	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

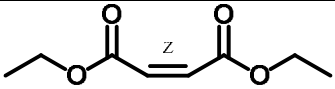
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

<i>DECANAL</i>	
CAS # 112-31-2	
EC # 203-957-4	
n-Decanal	
Capraldehyde; Capric aldehyde; Caprinaldehyde; Caprinic aldehyde; Decaldehyde; Decanaldehyde; Decyl aldehyde; Decylic aldehyde; NSC 6087; n-Decaldehyde; n-Decyl aldehyde	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

<i>DIETHYL MALEATE</i>	
CAS # 141-05-9	
EC # 205-451-9	
(2Z)-Diethyl but-2-enedioate	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

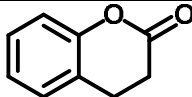
Current regulation: Annex II, n° 426

Clinical

data:

In the Malten 1984 study, 3.2% of 182 patients displayed a positive PT reaction to diethyl maleate 0.1% pet. (24). In this study, it has been noted that “in the max. test and clinically this is a strong sensitiser having caused patch test sensitisation (42%)”

Additional information: /

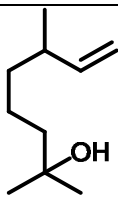
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ -lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

Current regulation: Annex II, n° 427

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dihydrocoumarine 5% pet. (24).

Additional information: /

DIHYDROMYRCENOL	
CAS # 18479-58-8	
EC # 242-362-4	
(±)-2,6-Dimethyloct-7-en-2-ol	
1,1,5-Trimethyl-6-heptenol; 2,6-Dimethyl-7-octen-2-ol; 3,7-Dimethyl-1-octen-7-ol; 2,6-Dimethyl-7-octen-2-ol (INCI)	

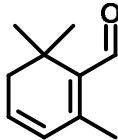
Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (103), listing 2 negative HRIPTs and 1 negative human maximisation test.

It is a “top 100” substance (IFRA, pers. comm.2010).

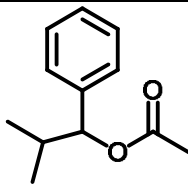
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

Additional information:

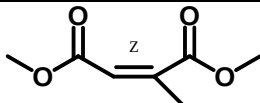
A RIFM review quotes one positive HRIPT (5 of 53) and one negative HRIPT (0 of 54) (93).

DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	
CAS # 151-05-3	
EC # 205-781-3	
2-Methyl-1-phenylpropyl acetate	
Benzeneethanol, α,α-dimethyl-, acetate; Phenethyl alcohol, α,α-dimethyl-, acetate; 1,1-Dimethyl-2-phenylethyl acetate; 2-Methyl-1-phenyl-2-propyl acetate; 2-Methyl-1-phenylpropan-2-yl acetate; Benzyl dimethylcarbinol acetate; Benzyl dimethylcarbinyl acetate; Dimethylbenzylcarbinol acetate; Dimethylbenzylcarbonyl acetate; NSC 46123; α,α-Dimethylphenethyl acetate	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% positive PT reactions to consecutive patients were noted (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and one to 5% DMBCA in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

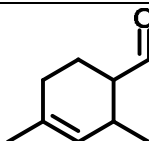
<i>DIMETHYL CITRACONATE</i>	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

Current regulation: Annex II, n° 431

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dimethylcitraconate 12% pet. (24). In this paper, a human maximisation test positive in “4/44” is quoted.

Additional information: ...

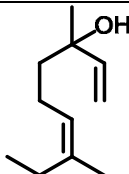
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

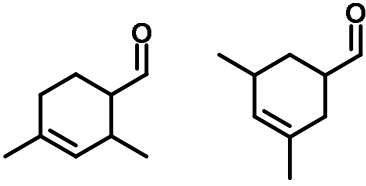
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyllinalool	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (104), citing 1 negative human maximisation test (n=25).

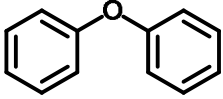
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde 3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
Hivertal; Vertocitral	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 2.3% positive PT reactions were observed with the isomer mixture, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

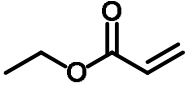
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryn JK-EB; Diphenyl ether; Diphenyl oxide; NSC 19311; Oxybisbenzene; Phenoxybenzene; Phenyl oxide	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

ETHYL ACRYLATE	
CAS # 140-88-5	
EC # 205-438-8	
Ethyl 2-propenoate	
Acrylic acid ethyl ester (6CI,8CI); 2-Propenoic acid ethyl ester; Ethyl 2-propenoate; Ethyl acrylate; Ethyl acrylic ester; Ethyl propenoate; NSC 8263	

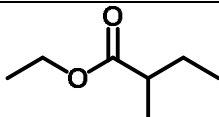
Current regulation: Annex II, n° 435

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In the NACDG 2009 multicentre study, 0.9% of

consecutive patients (n=4428) had a positive PT reaction (21).

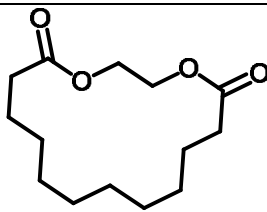
Additional information: /

ETHYL 2-METHYLBUTYRATE	
CAS # 7452-79-1	
EC # 231-225-4	
Ethyl 2-methylbutyrate	
Butyric acid, 2-methyl-, ethyl ester (6CI,7CI,8CI); (±)-Ethyl 2-methylbutanoate; 2-Methylbutanoic acid ethyl ester; 2-Methylbutyric acid ethyl ester; Ethyl 2-methylbutanoate; Ethyl 2-methylbutyrate; Ethyl α-methylbutyrate; NSC 1103	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

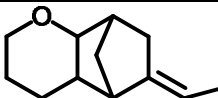
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

Current regulation: /

Clinical data:

In the Larsen 2002 c study on 218 patients with known contact allergy to fragrance ingredients, this compound caused 0.9% positive PT reactions at 5% pet. (1).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	
CAS # 93939-86-7	
EC # 300-376-9	
6-Ethylideneoctahydro-5,8-methano-2H-1-benzopyran	

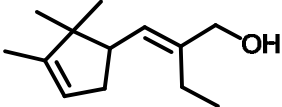
Current regulation: /

Clinical

data:

In the Larsen 2001 study, no positive PT reactions were observed with this compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

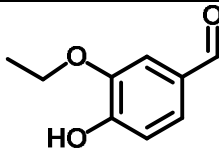
Additional information: /

2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	
CAS # 28219-61-6	
EC # 248-908-8	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol; 2-Ethyl-4-(2',2',3-trimethylcyclopent-3'-enyl)but-2-enol; Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

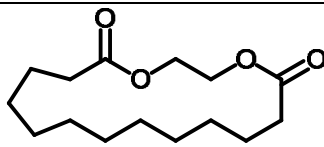
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; 3-Ethoxy-4-hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde ethyl ether; Quantrovanil; Rhodiarome; Vanillal; Vanirom	

Current regulation: /

Clinical data:

The case of a 28-year-old metal grinder with allergic contact dermatitis to a “cutting oil reodorant” has been reported, who tested positively not only to the cutting fluid, the reodorant, but also to several ingredients of the latter product, including “Vanillal S 10026”, 5% pet. (105).

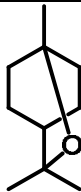
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ETHYLENE BRASSYLATE	
CAS # 105-95-3	
EC # 203-347-8	
1,4-Dioxacycloheptadecane-5,17-dione	
Tridecanedioic acid, cyclic ethylene ester; Ethylene glycol, cyclic tridecanedioate; Astratone; Cyclic ethylene glycol tridecanedioate; Cyclic ethylene tridecanedioate; Emeressence 1150; Ethylene brassylate; Musk T; NSC 46155	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

EUCALYPTOL	
CAS # 470-82-6	
EC # 207-431-5	
1,3,3-Trimethyl-2-Oxabicyclo[2.2.2]octane	
1,8-Epoxy-p-menthane; 1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane; 1,8-Cineol; 1,8-Cineole; 1,8-Epoxy-p-menthane; 2-Oxa-1,3,3-trimethylbicyclo[2.2.2]octane; Cajeputol; Cineol; Cineole; Eucalyptol; Eucalyptole; Eucalytol; Eucapur; Eukalyptol; NSC	

Opinion on fragrance allergens in cosmetic products

6171; Terpan; p-Cineole	

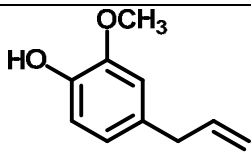
Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010).

See also ***EUCALYPTUS SPP. LEAF OIL***; eucalyptol is the major ingredient there (up to 85%), but found in significant quantities also in a number of other essential oils (see 3.2).

EUGENOL	
CAS # 97-53-0	
EC # 202-589-1	
2-Methoxy-4-(2-propen-1-yl)-phenol	
Other names: 4-Allyl-2-methoxy-phenol; 1-Allyl-4-hydroxy-3-methoxybenzene; 2-Hydroxy-5-allylanisole; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-(2'-propenyl)phenol; 2-Methoxy-4-[2-allyl]phenol; 2-Methoxy-4-allylphenol; 3-(3-Methoxy-4-hydroxyphenyl)propene; 3-(4-Hydroxy-3-methoxyphenyl)-1-propene; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; Allylguaiacol; Bioxeda; Caryophyllic acid; Dentogum; Eugenig acid; Eugenol; NSC 209525; NSC 8895; p-Allylguaiacol; p-Eugenol	

Current regulation: Annex III, part 1, n° 71

Clinical data:

In the “background information” section of the previous opinion (33), eugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.2% in consecutive PT patients and accounting for 4 to 16% of reactions to the FM I. Allergic reactions had been observed in 0.7 – 20% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). F. Giusti et al. examined 1754 consecutive patients tested with eugenol 1% pet. in addition to the baseline series, 09/1998 - 01/2000. 21 patients (1.2%) reacted positively to eugenol (106). In the An 2005 study, 8 of 422 consecutive patients, i.e., 1.9%, had positive reaction (13) (test concentration 2%). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 2.5% positive reactions (22). The IVDK 2010 study, 0.44% (95% CI: 0.04 – 0.84%) of 1214 consecutively tested patients reacted to the compound, while 1.57% (95% CI: 1.19 – 1.95%) of 4801 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to eugenol (48).

Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material was reported from India (108); however, occasional cases are also reported from Europe (109). Occupational exposure to eugenol / zinc oxide type dental restorative material, which is apparently less frequently used nowadays, may lead to occupational sensitisation to eugenol, as illustrated by a case report (110).

Additional information:

Eugenol is the main component (80-95%) of clove oil, but also found in citronella oil, pimento leaf oil and cinnamon bark oil (see section 3.2).

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

FARNESOL

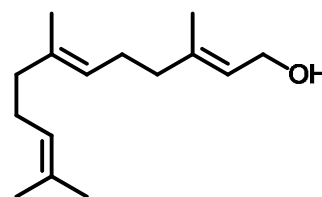
CAS # 4602-84-0

EC # 225-004-1

3,7,11-Trimethyl-2,6,10-Dodecatrien-1-ol

Farnesol; 3,7,11-Trimethyl-2,6,10-dodecen-1-ol; FCI 119a; Farnesyl alcohol; NSC 60597; Nikkosome

Current regulation: Annex III, part 1, n° 82



Clinical data:

In the “background information” section of the 1999 opinion, farnesol is classified as “less frequently reported allergen”; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive PT reactions to MPR (33).

Since the last SCCNFP-opinion of 1999, farnesol is used not only for its scent, but also for its (slight) antimicrobial activity, useful, for instance, in deodorants. Thus, axillary dermatitis is a relatively typical presentation (111). In a multicentre study based on 1997/98 PT data, 0.5% positive reactions in consecutive patients were noted (Frosch 2002 a (16)). Farnesol is included in the FM II. In the original publication on single constituents of the FM II, 6 of 1701 consecutive patients reacted positively to farnesol 5%, ie., 0.35% (95% CI: 0.13 – 0.77%) (10). In a study on consecutive patients tested in 2003, 38 of 4238 patients had positive reactions to farnesol 5% pet. (0.9%, 95% CI: 0.6 – 1.2%) (4)(IVDK 2007). (A paper on farnesol previously published by the IVDK (112) presents results included in this later analysis.) In a series from Nagoya, Japan, 1.1% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at 5% pet.) (14). In the Groningen 2009 study, 0.9% (95% CI: 0.2 – 2.7%) had positive reactions (6).

Additional information:

“Farnesol is an acyclic primary sesquiterpene alcohol found in essential oils such as lemongrass, citronella, tuberose blossom, sandalwood and orange blossom” (23). A RIFM review is available (113).

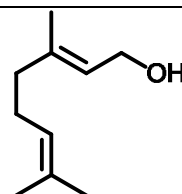
GERANIOL

CAS # 106-24-1

EC # 203-377-1

(2E)-3,7-Dimethyl-2,6-octadien-1-ol

(E)-3,7-Dimethyl-2,6-octadien-1-ol; (E)-Geraniol; (E)-Nerol; 3,7-Dimethyl-trans-2,6-octadien-1-ol; Geraniol; Geranyl alcohol; Lemonol; MosquitoSafe; NSC 9279; trans-3,7-Dimethyl-2,6-octadien-1-ol; trans-Geraniol; β-Geraniol



Current regulation: Annex III, part 1, n° 78

Clinical data:

In the “background information” section of the previous opinion (33), geraniol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.4% in consecutive PT patients and accounting for 3 to 7% of reactions to the FM I. Allergic reactions had been observed in 1.2 – 30% of patients with

eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at 2%, i.e. twice the usual concentration (6). In a series from Nagoya, Japan, 0.3% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at the unusually high concentration of 5% pet.) (14). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=7 (0.9%) positive reactions (22). The IVDK 2010 study, 0.39% (95% CI: 0.10 – 0.69%) of 1214 consecutively tested patients reacted to the compound, while 0.87% (95% CI: 0.63 – 1.10%) of 5695 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=17 reacted positively to geraniol (48).

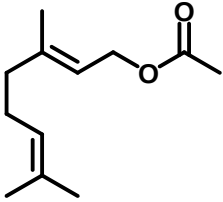
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman with urticaria at the site of application of cosmetics with generalisation (contact urticaria syndrome grade 2), which A. Yamamoto et al. diagnosed as immediate type hypersensitivity to geraniol (without CA) (115).

Additional information:

Geraniol is a component of Palmarosa oil (CYMBOPOGON MARTINI see below), geranium oil (about 40%), citronella oil (30-40%), rose oil, lavender oil, and jasmine oil. It is sensitive to heat which induces autooxidation and isomeric with linalool (53).

Geraniol forms oxidation product with increased sensitizing capacity both via spontaneous autooxidation at air exposure and via metabolic oxidation. Geraniol and neral together with hydroperoxide have been identified as oxidation products when geraniol autoxidizes (84). Geraniol and neral were also identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

A review is available by Hostynek and Maibach (116) and by RIFM (117). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

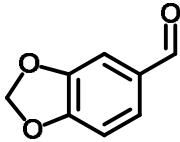
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic acid geraniol ester; Bay pine (oyster) oil; Geranyl acetate; Geranyl ethanoate; NSC 2584; trans-1-Acetoxy-3,7-dimethyl-2,6-octadiene; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-Geranyl acetate; β-Geranyl acetate	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010).

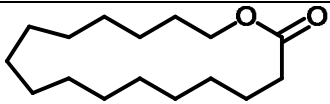
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Heliotropin; Heliotropine; NSC 26826; Piperonaldehyde; Piperonylaldehyde; Protocatechuic aldehyde methylene ether	

Current regulation: /

Clinical data:

In the Frosch 2002 b study, n=2 (0.2%) positive reactions to “piperonal” (1% pet.) and n=6 (0.4%) to “piperonal” (5% pet.), respectively, in 1606 consecutive were observed (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% heliotropine in pet., tested in 106 consecutive patients in Barcelona, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

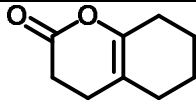
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid; 1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; NSC 33546	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with previously diagnosed contact allergy to fragrance ingredients had a positive PT reaction to this compound, tested 5% pet. (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to 5% “hexadecanolide” (13).

Additional information: /

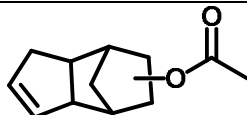
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ -Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ -1,6-2-Oxabicyclo(4.4.0)decen-3-one	

Current regulation: Annex II, n° 1135

Clinical data: /

Additional information:

A RIFM review is available (93), p. S115 ff, citing a number of positive human sensitisation experiments.

3α,4,5,6,7,7α-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxydihydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl acetate; Tricyclodecenyl acetate	

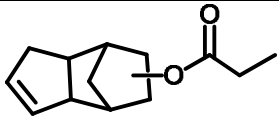
Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 1 to 5% "Cyclacet ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information:

Produced by IFF under the brand name "Cyclacet" (<http://www.iff.com/Ingredients.nsf/0/1C9F2CB39EB1EF6480256993002FBC14>, last accessed 2010-07-08).

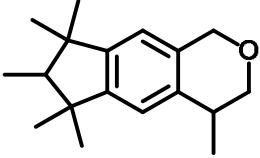
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol propanoate	

3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl propionate; Verdyl propionate	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

HEXAMETHYLINDANOPYRAN	
CAS # 1222-05-5	
EC # 214-946-9	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[γ]-2-benzopyran	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyrane; 1,3,4,6,7,8-Hexahydro-4,6,6,8,8,8-hexamethylcyclopenta-2-benzopyran; Abbalide; Galaxolide; Galaxolide 50; Galaxolide 50BB; Galaxolide 50IPM; Galaxolide White; HHCB; Pearlide	

Current regulation: /

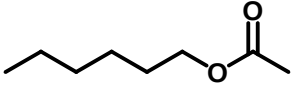
Clinical data:

In the Frosch 2002 a study, n=3 (0.2%) had positive reactions to the compound, tested 10% in isopropyl myristate (with 1 patient reacting positively to the diluent) (16). The Larsen 2001 study, testing with HHCB 7% pet., found 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to "Galaxolide 50", tested at 5% (13) (test concentration 2% pet.). The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 25% PT preparation of HHCB (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Galaxolide 50 ®" in pet., tested in 100 consecutive patients in Stockholm, were observed (15).

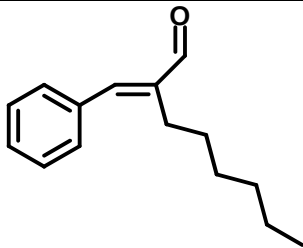
Additional information:

[0403/00 - Opinion concerning Hexahydro-hexamethyl-cyclopenta\(γ\)-2-benzopyran \(HHCB\)](#)

[0610/02 - Opinion on Hexahydro-hexamethyl-Cyclopenta \(γ\)-2-Benzopyran \(HHCB\)](#) (no restrictions) It is a "top 100" substance (IFRA, pers. comm.2010).

HEXYL ACETATE	
CAS # 142-92-7	
EC # 205-572-7	
Hexyl ethanoate	
Acetic acid, hexyl ester, Hexyl alcohol, acetate; 1-Hexyl acetate; Exceed 600; Hexyl acetate; Hexyl ester acetic	

acid;; NSC 7323; n-Hexyl acetate; n-Hexyl ethanoate	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

HEXYL CINNAMAL	
CAS # 101-86-0	
EC # 202-983-3	
α-Hexyl-cinnamaldehyde	
2-(Phenylmethylene)octanal; 2-Hexyl-3-phenyl-2-propenal; 2-Hexylcinnamaldehyde; Hexyl cinnamic aldehyde; NSC 406799; NSC 46150; α-Hexylcinnamaldehyde; α-Hexylcinnamic aldehyde; α-Hexylcinnamyl aldehyde; α-n-Hexyl-β-phenylacrolein; α-n-Hexylcinnamaldehyde	
Current regulation: Annex III, part 1, n° 87	

Clinical data:

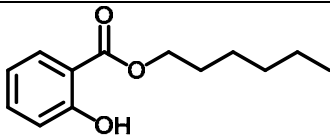
In the “background information” section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as “less frequently reported allergen”; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products were found (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the subsequent EU 2005 study, 2 of 1701 patients had positive reactions to AHCA, and n=16 doubtful or irritant to AHCA at 10% in pet. (10). The IVDK 2007 study yielded n=3, i.e. 0.2% (95% CI: 0.03 – 0.4%) positive reactions in 2019 consecutively PTed patients, using 10% pet. as test concentration (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, using a lower test concentration of 5% pet. (6).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

Hexyl cinnamal is regarded as “a recommended positive control for skin sensitization testing”, e.g., in the context of the LLNA (118).

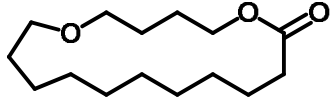
HEXYL SALICYLATE	
CAS # 6259-76-3	
EC # 228-408-6	
Hexyl-2-hydroxybenzoate	
Salicylic acid, hexyl ester; 1-Hexyl salicylate; Hexyl salicylate; n-Hexyl salicylate	

Clinical data:

None of the 218 patients with known contact allergy to fragrance ingredients reacted positively to this compound (tested at 5% in pet.) in the Larsen 2002 c study (1).

Additional information:

In a RIFM review, 2 human sensitisation experiments are mentioned which yielded no evidence of sensitising potential (HRIPT, n=103, maximisation test, n=22) (119). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

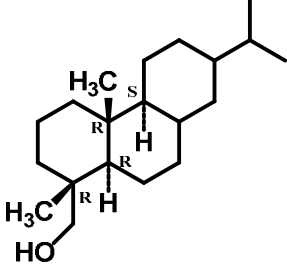
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

Current regulation: /

Clinical data:

None of the 178 patients with known contact allergy to fragrance ingredients reacted positively to "12-oxahexadecanolide" (tested at 5% in pet.) in the Larsen 2001 study (19).

Additional information: /

HYDROABIETYL ALCOHOL, when used as a fragrance ingredient	
CAS # 13393-93-6	
EC # 236-476-3	
(1R,4aR,4βS,10aR)-Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-1-Phenanthrenemethanol	
Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-phenanthrenemethanol; Tetrahydroabietyl alcohol	1-

Current regulation: AnnexII, n° 440

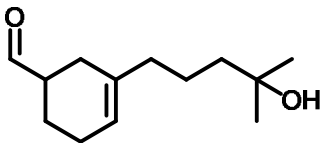
Clinical data:

In the deGroot 2000 study, 17 of 1825 consecutively tested patients had positive reactions to hydroabietyl alcohol (10% pet.) (12).

Additional information:

Commercial hydroabietyl alcohol consists of di- and tetrahydroabietyl alcohol together with non-modified colophony (120)

HYDROXYISOHEXYL	3-CYCLOHEXENE	

CARBOXALDEHYDE (HICC) regioisomers	 51414-25-6
CAS # 31906-04-4 / 51414-25-6	
EC # 250-863-4 / 257-187-9	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (31906-04-4) 3-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (51414-25-6)	

31906-04-4: 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde; 4-(4-Methyl-4-hydroxyamyl)cyclohex-3-ene carboxaldehyde; Lyrall

Current regulation: Annex III, part 1, n° 79

Clinical data:

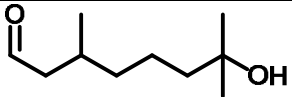
In the “background information” section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 2.7% of the 1855 consecutive patients reacted positively to HICC (5% pet.) (16). In the EU 2005 study, 28 of 1701 patients (1.7%, 95% CI: 1.1 – 2.4%) reacted positively to 5% HICC in pet. (10). In 21325 patients PTed consecutively in the IVDK 2007 study, 2.4% (95% CI: 2.2 – 2.6%) positive reactions were noted to 5% HICC in pet. (4). Similar to other studies, HICC was the most common single fragrance allergen among 320 patients tested in the Groningen 2009 study, with 3.1% (95% CI: 1.5 – 5.7%) positive reactions despite testing with a lower concentration of 2% pet. (6). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded a relatively low prevalence of 0.4% (7 of 1603; exact 95% CI (recalculated): 0.17 – 0.90%) positive reactions with 5% HICC in pet. and even less with lower test concentrations; possible reasons for the much lower prevalence were discussed. The IVDK 2010 study, 2.36% (95% CI: 2.19 - 2.53%) of 37270 consecutively tested patients reacted to HICC (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to HICC (48).

Further clinical data with a focus on quantitative dose-response (see also section 4.3), is discussed in (121).

Among the early case reports, S.A. Hendriks reported the case of a 20 year old patient developing axillary dermatitis after 5 months use of a deodorant containing HICC (122).

Additional information: /

HYDROXYCITRONELLAL	
CAS # 107-75-5	
EC # 203-518-7	
7-Hydroxy-3,7-dimethyl-octanal	

(±)-Hydroxycitronellal; 3,7-Dimethyl-7-hydroxyoctanal; 7-Hydroxy-3,7-dimethyloctanal; 7-Hydroxycitronellal; Citronellal hydrate; Citronellal, hydroxy-; Cyclalial; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguetine principle; NSC

Opinion on fragrance allergens in cosmetic products

406740; Phixia

Current regulation: Annex III, part 1, n° 72

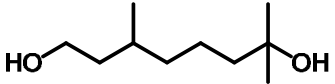
Clinical data:

In the “background information” section of the previous opinion (33), hydroxycitronellal, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.75% in consecutive PT patients and accounting for 6 to 16% of reactions to the FM I. Allergic reactions had been observed in 10 – 45% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.9 – 1.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.2% (95% CI: 0.9 – 4.5%) had positive reactions to this compound, tested at 2% pet., i.e., twice the commonly used concentration (6). The Sugiura 2000 study observed 1% positive PT reactions (test concentration 5% pet.) in 1483 patients tested for suspected cosmetic dermatitis (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 1.17% (95% CI: 0.48 – 1.85%) of 1214 consecutively tested patients reacted to the compound, while 2.95% (95% CI: 2.43 – 3.47%) of 4359 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with hydroxycitronellal, yielding 6 positive reactions (48).

Additional information:

Hydroxycitronellal is a synthetic fragrance, which only recently has been found in a few essential oils, e.g., of a *Narcissus* species and in essential oils of pepper (53)

HYDROXYCITRONELLOL	
CAS # 107-74-4	
EC # 203-517-1	
3,7-Dimethyl-7-octanediol	
2,6-Dimethyl-2,8-octanediol; 3,7-Dimethyl-1,7-octanediol; 3,7-Dimethyloctan-1,7-diol; Citronellol, hydroxy-; Hydroxyciol; Hydroxycitronellol; NSC 406140; NSC 67886	

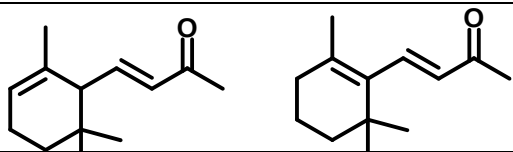
Current regulation: /

Clinical data:

This compound elicited 6.0% positive PT reactions in 218 fragrance sensitive individuals (Larsen 2002 c, (1)).

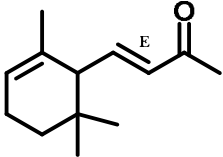
Additional information:

A RIFM review is available, reporting results of a human induction study (maximisation test) in 25 volunteers, yielding no evidence of sensitisation (123).

IONONE isomeric mixture	
CAS # 8013-90-9	
EC # 232-396-8	

Opinion on fragrance allergens in cosmetic products

Ionone	
Irisone, mixture of alpha- and beta ionone	
Current regulation: /	
Clinical data: / (see single isomers)	
Additional information:	
It is a “top 100” substance, further specified with “mixed isomers” (IFRA, pers. comm.2010).	
INCI: “MIXED IONONES”, with CAS # 14901-07-6 / 6901-97-9 / 8013-90-9 (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=35383 , last accessed 2010-07-13).	
A RIFM review is available on “ionone” (124), quoting negative human and experimental results.	

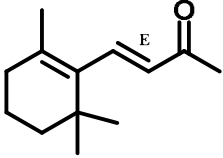
<i>alpha-IONONE</i>	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; 4-(2,6,6-Trimethyl-2-cyclohexenyl)-3-buten-2-one; trans-α-Ionone; α-Cyclocitrylideneacetone; α-Ionone	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% alpha-ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information: A RIFM review is available (125).

<i>beta-IONONE</i>	
CAS # 79-77-6	
EC # 201-224-3	
(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	
(E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; (E)-β-Ionone; Ionone beta; trans-β-Ionone; β-Ionone	

Current regulation: /

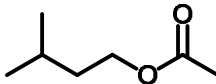
Clinical data:

In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% beta-

ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (126).

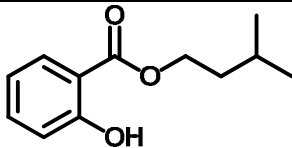
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic acid 3-methylbutyl ester; Acetic acid isopentyl ester; Banana oil; Isoamyl acetate; Isoamyl alcohol acetate; Isoamyl ethanoate; Isopentyl acetate; Isopentyl ethanoate; NSC 9260; Pear oil; i-Amyl acetate; iso-Amyl acetate; iso-Pentyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

In CosIng, it is listed as “solvent” (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=76810>, last accessed 2010-07-13)

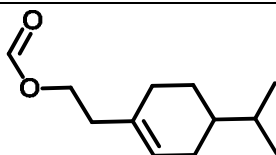
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; NSC 7952	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 50% PT preparation of this compound – this reaction may have been due to an “excited back syndrome” and is thus a limited evidence (25). In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% isoamyl salicylate in pet., tested in 95 consecutive patients, were observed (15).

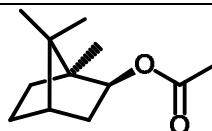
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletely defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

Current regulation: Annex III, part 1, n° 170

Clinical data: /

Additional information: A RIFM review is available (128).

ISOBORNYL ACETATE	
CAS # 125-12-2	
EC # 204-727-6	
(1R,2R,4R)-1,7,7-trimethyl-Bicyclo[2.2.1]hept-2-yl acetate	
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-Trimethyl-, acetate, (1R,2R,4R)-rel- ; Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-; Isoborneol, acetate; (±)-Isobornyl acetate; Isobornyl acetate; NSC 62486; Pichtosin; Pichtosine; exo-Bornyl acetate	

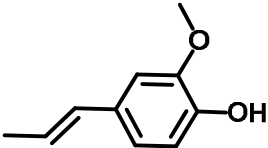
Opinion on fragrance allergens in cosmetic products

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% isobornyl acetate in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ISOEUGENOL	
CAS # 97-54-1	
EC # 202-590-7	
2-Methoxy-4-(1-propen-1-yl)-phenol	
Phenol, 2-methoxy-4-(1-propenyl)- ; Phenol, 2-methoxy-4-propenyl-; 1-(3-Methoxy-4-hydroxyphenyl)-1-propene; 2-Methoxy-4-(1-propenyl)phenol; 2-Methoxy-4-propenylphenol; 3-Methoxy-4-hydroxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-β-methylstyrene; 4-Propenylguaiacol; Isoeugenol; NSC 6769	

Current regulation: Annex III, part 1, n° 73

Clinical data:

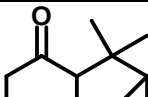
In the “background information” section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been observed in 2 – 25% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.8 – 1.8%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to isoeugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.4% positive reactions (22). At St Johns Institute of Dermatology in London 3636 subjects were patch tested with isoeugenol 2001-2005, 97 of whom were positive. Year-on-year incidence showed an increasing trend, with an overall incidence of 2.67% (129). The IVDK 2010 study, 1.62% (95% CI: 0.87 – 2.38%) of 1214 consecutively tested patients reacted to the compound, while 3.41% (95% CI: 2.90 – 3.92%) of 5747 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=11 reacted positively to isoeugenol (48).

Additional information:

Isoeugenol occurs in a cis- (CAS 5912-86-7) and a trans-isomers (CAS 5932-68-3), the latter dominating in trade products (82-88%) (53).

Isoeugenyl methyl ether caused 7.3% positive reactions in the Larsen 2002 c study (1). A number of derivatives of isoeugenol, such as isoeugenyl acetate, transisoeugenol, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and benzyl isoeugenyl have been examined in 2261 consecutive patients; a varying proportion of positive patch test reactions and a varying proportion of concomitant reactions with isoeugenol have been observed (130). In an earlier study, 5 of 7 patients positive to isoeugenol also displayed positive reactions to isoeugenol acetate (1.2% eth.) (131) (see also section 5 and 6).

ISOLONGIFOLENEKETONE	
CAS # 33407-62-4	

EC # 245-890-3	
1,3,4,6,7,8a-Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	
Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	

Current regulation: /

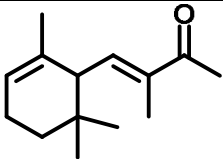
Clinical data:

The Larsen 2001 study identified 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

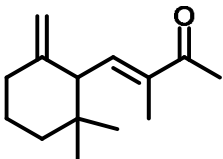
Additional information:

Not listed in CosIng under this CAS #. Other CAS # reported in RIFM review ¹³:

- 29461-14-1 CosIng: INCI name "ISOLONGIFOLENE KETONE EXO";
- 23787-90-8 CosIng: INCI name "ISOLONGIFOLANONE";
- 29461-13-0: CosIng: INCI name "HEXAHYDRO-TETRAMETHYLMETHANONAPHTHALEN-8-ONE".

<i>alpha-ISOMETHYL IONONE</i>	
CAS 127-51-5	
EC 204-846-3	
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	
4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; Cetone Alpha; Isomethyl-α-ionone; NSC 66432; α-Cetone	

Current regulation: Annex III, part 1, n° 90

<i>gamma-Methylionone</i>	
CAS 7388-22-9	
EC /	

According to CosIng, "alpha-ISOMETHYL IONONE" (CAS # 127-51-5) and "gamma-Methylionone" (CAS # 7388-22-99) are synonyms, with one CAS number, and one preferred chemical name. The substance(s) are accordingly treated in the 1999 opinion (33) as one. As this treatment is also found in the literature, both substances are reviewed together.

¹³ Opdyke, D. L. J.; Letizia, C. **Monographs on fragrance raw materials. Isolongifolanone.** Food and Chemical Toxicology (1983), 21(6), 859

Clinical data:

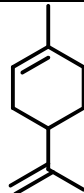
In the “background information” section of the 1999 opinion, “gamma-methylionone” is classified as “less frequently reported allergen”; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

The IVDK 2007 study yielded $n=1$, i.e. 0.1% (95% CI: 0.00 – 0.2%) positive reactions in 2004 consecutively PTed patients (4). In the subsequent period (2005-2008), $n=986$ patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, $n=2$, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In a Korean study with 422 consecutive patients, 2.1% reacted positively to “alpha isomethyl ionone (gamma-methylionone), CAS # 127-51-5”, tested 5% pet. (13)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010) under the label of “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

A RIFM review is available, listing 4 human sensitisation experiments employing different study protocols – all yielding negative results (132). Another review is available by Hostynek and Maibach (133), both referring to “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

(DL)-LIMONENE	
CAS # 138-86-3	
EC # 231-732-0	
1-Methyl-4-(1-methylethenyl)-cyclohexene	
p-Mentha-1,8-diene; (±)-Dipentene; (±)-Limonene; (±)-α-Limonene; 1,8-p-Menthadiene; 1-Methyl-4-(1-methylethenyl)cyclohexene; 1-Methyl-4-isopropenyl-1-cyclohexene; 1-Methyl-4-isopropenylcyclohexene; 1-Methyl-p-isopropenyl-1-cyclohexene; 4-Isopropenyl-1-methyl-1-cyclohexene; 4-Isopropenyl-1-methylcyclohexene; Cajeputen; Cajeputene; Cinen; Cinene; DL-Limonene; Dipenten; Dipentene; Eulimen; Flavor orange; Goldflush II; Kautschin; Limonen; Limonene; NSC 21446; NSC 844; Nesol; Orange X; Orange flavor; PC 560; Roti-Histol; SF 001; dl-Limonene; α-Limonene	

Current regulation: Annex III, part1, n° 88, 167, 168

Clinical data:

In the “background information” section of the 1999 opinion, d-limonene (CAS 5989-27-5) is classified as “less frequently reported allergen in relation to cosmetic exposure”; with contact allergy to oxidised limonene not infrequently reported in the literature (33).

Since 1999, several studies have been performed using limonene where the oxidation state is not given, but intended to be low. In one study, 0.6% positive reactions to limonene (3% pet.) were observed in 1606 consecutive patients (17). The IVDK 2007 study yielded n=3, i.e. 0.1% (95% CI: 0.03 – 0.4%) positive reactions in 2396 patients consecutively PTed with limonene (2% pet.) (4). The IVDK 2010 study, 0.28% (95% CI: 0 – 0.57%; percentages standardised for age and sex) of 1241 patients PTed with dipentene reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed in 320 patients (6).

Regarding selected case reports, a case of a 40 year old citrus fruit picker with work related hand dermatitis and bronchial asthma has been described, who tested extreme positive to DL-limonene (2% pet.), and, less extremely, to citronellol and to the biocide dichlorophene (134). Moreover, limonene is used as a solvent in technical applications and cleaning and can lead to allergic contact dermatitis (e.g., a histopathology technicians (135, 136) or a painter and decorator (137)). In “water-free” hand cleansers it is reported to be used in concentrations around 10 – 20% (137). Wax polishes may contain dipentene and have caused one reported case of occupational ACD in a car mechanic (138). Another case of occupational ACD from dipentene in honing oil has been reported (139). In a case series from Sweden, 2 of 105 car mechanics patch tested for occupational contact dermatitis had positive reactions to oxidised d-limonene (5% pet.) (140).

Additional information:

Limonene is a monocyclic monoterpene existing in two enantiomers: (R)-(+)-limonene (CAS 5989-27-5) and (S)-(-)-limonene (CAS 5989-54-8). Racemic limonene is known as dipentene.

The allergenicity of limonene is closely related to oxidation (71, 72, 141, 142). It has been demonstrated that both enantiomers, R-(+)- and S-(-)-limonene spontaneously autoxidize, and that the primary oxidation products formed, the hydroperoxides, are strong and clinically relevant contact allergens. Among 2411 consecutive patients in a multi-centre European study, 63 (2.6 % [95%CI: 2.0-3.3]) reacted to oxidised (R)-(+)-and/or (S)-(-)-limonene (3.0% pet.) (72). In other multi-studies also, a considerable proportion of patients showed

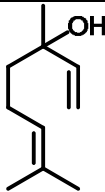
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- between 0.3% and 5.1% of subgroups of 2800 patients in Stockholm and Leuven, depending on test concentration, oxidation state and department(141),
- between 0.3% and 6.5% in 4 different departments in altogether 2273 patients (72, 143).

The primary oxidation products are the major allergens forming specific antigens (Bråred-Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. Contact Dermatitis 2006; 55: 230-237.).

Current IFRA standards emphasise “a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method” (<http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56>, last accessed 2009-11-11). For a more general discussion see section 5.

There is no scientific rationale for the difference in peroxide value allowed for limonene (20 millimoles peroxides per litre) compared to linalool (10 millimoles peroxides per litre). Specific values for hydroperoxides, which are allergens, would be desirable.

LINALOOL	
CAS # 78-70-6 (isomeric mixture)	
EC # 201-134-4; 245-083-6	
See: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=27933	
3,7-Dimethyl-1,6-octadien-3-ol	
(±)-Linalool; 2,6-Dimethyl-2,7-octadien-6-ol; 2-Methyl-1-prenyl-3-buten-2-ol; 3,7-Dimethyl-1,6-octadiene-3-ol; 3,7-Dimethyl-3-hydroxy-1,6-octadiene; L 260-2; Linalol; Linalool; Linalyl alcohol; Linanol; NSC 3789; dl-Linalool; β-Linalool	

Current regulation: Annex III, part 1, n° 84

Clinical data:
In the “background information” section of the 1999 opinion, linalool in non-oxidized form is classified as “less frequently reported allergen”; with 4 cases of contact allergy reported in 2 studies on patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, studies have been performed on contact allergy to linalool, oxidation state not given, but intended to be low. In the Larsen 2002 c study, none of the 218 patients with known contact allergy to fragrance ingredients had a positive reaction to linalool 5% pet., as prepared specially for this study (1). The IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2401 patients consecutively tested with stabilised linalool (10% pet.) (4). The IVDK 2010 study, 1 patient had a weak, and another a ++ reaction among the n=985 patients tested with 10% linalool (stabilised) in pet. (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). The deGroot 2000 study with 1825 consecutively tested patients yielded 3 positive reactions to linalool (12). The DeGroot 1985 study found no positive reactions among 179 patients using a 30 % PT preparation of linalool (25).

Additional information:

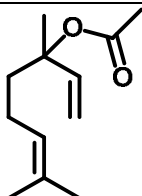
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

2003 in 6 European centres including 1511 consecutive patients, 1.3% showed a positive reaction to oxidized linalool (2.0% pet.) and 1.1% to the hydroperoxide fraction (65). A recent dose-response study in Sweden including 3400 patients in two test centres showed a positive reaction in 5.3% of the 1725 patients tested with oxidized linalool 6% pet. (145).

A review by RIFM is available both regarding linalool (146) and linalool “and related esters” (147). Another review is available by Hostynek and Maibach (148).

It is a “top 100” substance (IFRA, pers. comm.2010).

Additional CAS numbers exist for the single isomers: CAS # 126-90-9 (S-isomer), CAS # 126-91-0 (R-isomer); however, in the studies reviewed the isomeric mixture has been used throughout.

LINALYL ACETATE	
CAS # 115-95-7	
EC # 204-116-4	
3,7-Dimethyl-1,6-octadien-3-yl acetat	
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; Linalool acetate K; (±)-Linaloyl acetate; (±)-Linalyl acetate; 1,5-Dimethyl-1-vinyl-4-hexenyl acetate; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 3-Acetoxy-3,7-dimethyl-1,6-octadiene; Acetic acid linalool ester; Bergamiol; Bergamol; Bergamot mint oil; Linalyl acetate; NSC 2138; dl-Linalool acetate	

Current regulation: /

Clinical data:
In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. (15). In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted (16). Similarly, the RIFM review mentioned quotes a number of studies where no allergic reactions to this compound had been observed, with the exception of one positive reaction in a Dutch study in 1988(149).

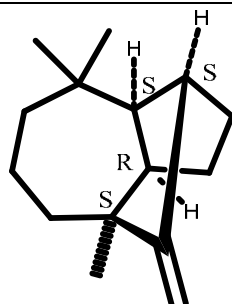
Additional information:

This is the main component of lavender oil (30%), also part of bergamot oil, neroli oil, peppermint oil, lemon oil and jasmine oil (53).

Linalyl acetate autoxidizes spontaneously at air exposure and the major allergens, the hydroperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be metabolically hydrolysed to the corresponding alcohol cross reactions to allergens from oxidized linalool should be possible. This was indicated in a study of lavender oil and oxidised linalyl acetate which elicited positive PT reactions in some patients with known contact allergy to oxidised linalool (n=3) (151).

A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation (152).

It is a “top 100” substance (IFRA, pers. comm.2010).

Longifolene	
CAS # 475-20-7	
EC # 207-491-2	
(1S,3aR,4S,8aS)-Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-; 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1α,3aβ,4α,8aβ)]-; (+)-Longifolene; Junipen; Junipene; Kuromatsuen; Kuromatsuene; Longifolen; NSC 150808; d-Longifolene; α-Longifolene	

Current regulation: /

Clinical data: /

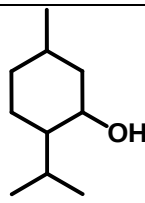
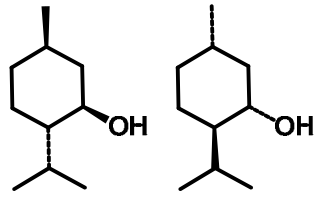
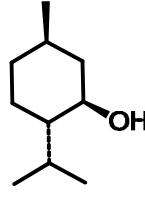
Additional information:

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=77412

This substance is listed in the Register of Flavouring Substances pursuant to Article 3(1) of Regulation EC No. 2232/96 (28 Oct 1996) that lays down a procedure for flavouring substances used or intended for use in or on foodstuffs. Adopted February 23, 1999.

A RIFM review is available citing one negative human maximisation test (n=25) with 10% pet. (153).

MENTHOL	 1490-04-6
CAS # 1490-04-6 / 89-78-1 / 2216-51-5	
EC # 216-074-4 / 239-388-3 / 218-690-9	
5-Methyl-2-(1-methylethyl)-cyclohexanol (1490-04-6) (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol (89-78-1) (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol (2216-51-5)	
Other names: 1490-04-6: Menthol; 1-Methyl-4-isopropyl-3-cyclohexanol; 2-Isopropyl-5-methylcyclohexan-1-ol; 2-Isopropyl-5-methylcyclohexanol; 3-Hydroxy-p-menthane; 5-Methyl-2-(1-methylethyl)cyclohexanol; 5-Methyl-2-isopropylcyclohexanol; Menthyl alcohol; p-Menthan-3-ol 89-78-1: (1a,2β,5a)-5-Methyl-2-(1-methylethyl)-cyclohexanol; cis-1,3,trans-1,4-Menthol; dl-Menthol; (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)cyclohexanol; (±)-Menthol; DL-Menthol; Fisherman's Friend Lozenges; Hexahydrothymol; Menthacamphor; Menthol; Menthomenthol; NSC 2603; Peppermint camphor; Racementhol; Therapeutic Mineral Ice; Thymomenthol; rac-Menthol	 89-78-1  2216-51-5

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1 α ,2 β ,5 α)]-5-Methyl-2-(1-methylethyl)-cyclohexanol; (1R,3R,4S)-(-)-Menthol; (-)-Menthol; (-)-Menthyl alcohol; (-)-trans-p-Methan-cis-3-ol; (1R)-(-)-Menthol; (1R,2S,5R)-(-)-Menthol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexan-1-ol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol; (R)-(-)-Menthol; 1R-Menthol; L-Menthol; L-Mentholum; Levomenthol; NSC 62788; l-(-)-Menthol; l-Menthol

Current regulation: /

Clinical data:

Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 10 patients had positive (+ to +++) PT reactions to menthol 5% pet. at D4, mostly reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). In 63 patients positive to the FM I, 1 had a positive PT reaction to menthol, 5% pet., in the Santucci 1987 study (28). The IVDK 2010 study, 1 of 1147 patients tested with 1% menthol in pet. had a weak positive reaction to menthol (7).

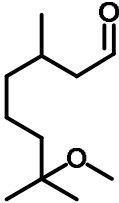
A case of contact allergy to “peppermint and menthol” in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155). Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application of 2% menthol in pet. or 5% peppermint oil in pet., has been reported (156). “A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. During the last two years, the patient had presented dyspnoea, wheezing and nasal symptoms when exposed to mentholated products such as toothpaste and candies. The aetiology was suggested by the history of exposure and diagnosis was established by skin tests and bronchial challenge with menthol. The patient achieved control of symptoms by avoiding menthol and its derivatives.” (157).

Additional information:

Menthol is an ingredient of several essential oils, like peppermint oil, and has been identified as causative allergen in case reports listed above.

Four stereoisomeric forms are known. Natural menthol occurs as L-form (CAS 2216-51-5), trade products are DL-menthol (CAS 1490-04-6). D-form: CAS 89-78-1, racemic: CAS 15356-70-4. Sensitive to light, air and heat (53).

L-menthol and menthol (isomer not specified) are “top 100” substances (IFRA, pers. comm.2010). RIFM reviews are available regarding “menthol” (158), D-menthol (159), L-menthol (160), DL-menthol (161) and menthol, racemic (162). A CIR expert panel review is available (163).

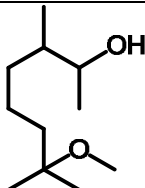
METHOXYCITRONELLAL	
CAS # 3613-30-7	
EC # 222-784-5	
7-Methoxy-3,7-dimethyl-octanal	
7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal	

Current regulation: /

Clinical data:

Nakayama et al. found 1974 (after (29)) 12 “strong positive” and 10 “weak positive” reactions to methoxycitronellal (unknown test concentration), with cross-reactions to hydroxycitronellal (proportion not given), in 183 patients.

Additional information: /

METHOXYTRIMETHYLHEPTANOL	
CAS # 41890-92-0	
EC # 255-574-7	
7-Methoxy-3,7-dimethyl-2-octanol	
3,7-Dimethyl-7-methoxy-2-octanol; Dihydromethoxyelgenol; Elesant; Osyrol	

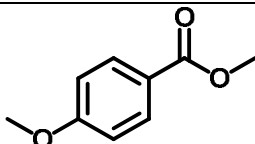
Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.9% of the patients with known contact allergy to fragrance ingredients had a positive PT reaction to this ingredient not reported as allergen previously (1).

Additional information:

A RIFM review is available (128) citing 1 negative maximisation test (n=27).

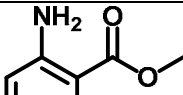
METHYL <i>p</i>-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

Current regulation: /

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to methyl anisate 4% pet. (24).

Additional information: /

METHYL ANTHRANILATE	
CAS # 134-20-3	

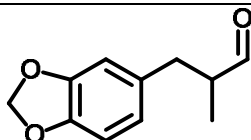
EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; ReJex-iT AP 50; ReJex-iT TP 40; Sunarome UVA; [2-(Methoxycarbonyl)phenyl]amine; o-(Methoxycarbonyl)aniline; o-Aminobenzoic acid methyl ester; o-Carbomethoxyaniline	

Current regulation: /

Clinical data:

In 91 Israeli patients with a positive or doubtful reaction to FM I or MP methyl anthranilate was tested (conc. not given), with a negative result (164).

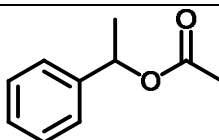
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYLENEDIOXYPHENYL METHYLPROPANAL	
CAS # 1205-17-0	
EC # 214-881-6	
3-(1,3-Benzodioxol-5-yl)-2-methylpropanal	
Hydrocinnamaldehyde, α-methyl-3,4-(methylenedioxy)-; 2-Methyl-3-(3,4-methylenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methylenedioxyphenyl)propionaldehyde; 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal; Heliobouquet; Heliofresh; Heliogan; Helional; Helipropanal; NSC 22282; Tropional; α-Methyl-1,3-benzodioxole-5-propanal; α-Methyl-3,4-(methylenedioxy)hydrocinnamaldehyde	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

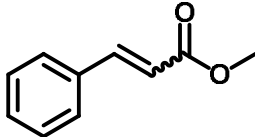
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl phenyl carbinyol acetate; Methylphenylcarbinol acetate; NSC 2397; Styrallyl acetate;	

Styrylallyl acetate; dl-1-Phenylethyl acetate; sec-Phenethyl acetate; sec-Phenylethyl acetate; α-Methylbenzenemethanol acetate; α-Methylbenzyl acetate; α-Methylbenzyl alcohol, acetate; α-Phenethyl acetate; α-Phenylethyl acetate

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL CINNAMATE	
CAS # 103-26-4	
EC # 203-093-8	
Methyl 3-phenylprop-2-enoate	
3-Phenyl-2-propenoic acid methyl ester; Cinnamic acid, methyl ester; 3-Phenyl-2-propenoic acid methyl ester; 3-Phenylacrylic acid methyl ester; Methyl 3-phenyl-2-propenoate; Methyl 3-phenylacrylate; Methyl 3-phenylpropenoate; Methyl cinnamate; Methyl cinnamylate; NSC 9411; SemaSORB 9815	

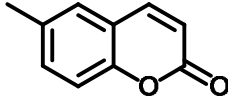
Current regulation: /

Clinical data:

Patch tests with some components of Peru balsam were carried out at 8 worldwide centers in 142 patients who had previously reacted to 25% MP. Reactions to methyl cinnamate (dose and vehicle not reported) were observed in 6 of 142 patients (no further details reported) (165).

Additional information:

A RIFM review is available (166), reviewing, e.g., a number of animal studies with conflicting results. See also under Myroxylon pereirae.

6-METHYL COUMARIN	
CAS # 92-48-8	
EC # 202-158-8	
6-Methylchromen-2-one	
Coumarin, 6-methyl-; 6-MC; 6-Methyl-2H-1-benzopyran-2-one; 6-Methyl-2H-chromen-2-one; 6-Methylbenzopyrone; 6-Methylcoumarin; 6-Methylcoumarinic anhydride; NSC 5870; Toncarine	

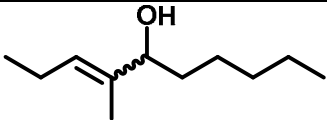
Current regulation: Annex III, part 1, n° 46

Clinical data:

Two of 24 white volunteers developed a photoallergic reaction after single epicutaneous exposure with 5% methyl coumarin in ethanol and UV-A radiation (16 J/cm²). After a photomaximisation test, 6 of 10 subjects developed photocontact allergic reactions

(167). Cardoso et al. report on 2 photoallergic patch test reactions to this substance, which were apparently clinically relevant, in 83 Portuguese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

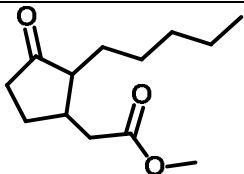
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (170), reporting 1 negative HRIPT (n=50). It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL DIHYDROJASMONATE	
CAS # 24851-98-7	
EC # 246-495-9	
Methyl 2-(3-oxo-2-pentyl cyclopentyl) acetate	

Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester; Kharismal; MDJ; Methyl (3-oxo-2-pentylcyclopentyl)acetate; Methyl 3-oxo-2-pentylcyclopentane ethanoate; Hedione

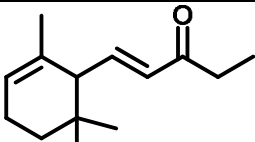
Current regulation: /

Clinical data:

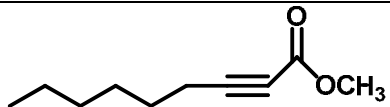
In the Frosch 2002 b study, 3 of 1606 consecutive patients (0.2%) showed positive reactions to hedione (5% pet.) (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% hedione in pet., tested in 100 consecutive patients in Belfast, were observed (15).

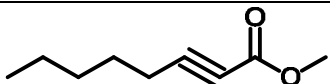
Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). An older RIFM review exists (128) citing 1 negative human maximisation test (n=25).

METHYL IONONE (mixture of isomers)	
CAS # 1335-46-2	
EC # 215-635-0	

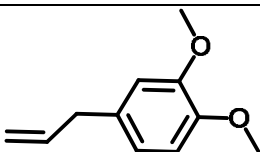
1-(2,6,6-Trimethyl-1-cyclohex-2-enyl)pent-1-en-3-one	
6-Methylionone	
Current regulation: /	
Clinical data:	
See METHYLIONANTHEME for one clinical case report. Regarding methyl ionone gamma, the Frosch 1995 dose-finding pilot study found no positive reaction to 1% and 5% of this substance in pet., tested in 100 consecutive patients in Belfast (15).	
Additional information:	
It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (171).	

METHYL OCTINE CARBONATE	
CAS # 111-80-8	
EC #	
Methyl 2-octynoate	
Methyl 2-Nonynoate, MOC	
Current regulation: Annex III, part 1, n°173	
Clinical data:	
English and Rycroft reported a case of a 19-year-old laboratory technician working in the fragrance industry, who developed hand dermatitis after contact with methyl heptine and methyl octane carbonates; patch testing was strongly positive to both compounds at 1% in MEK (172).	
Additional information: /	

METHYL 2-OCTYNOATE	
CAS # 111-12-6	
EC # 203-836-6	
Methyl oct-2-ynoate	
M2O; Methyl heptin carbonate; Folione; Methyl hept-1-yne-1-carboxylate; Methyl pentylacetylenecarboxylate; NSC 72098; Vert de violette artificiel	
Current regulation: Annex III, part 1, n° 89	

had positive reactions to this allergen, tested at only 2% pet. (6). In a previous case report of a fragrance laboratory assistant with work-related ACD both methyl heptin and methyl octin carbonate had been found sensitisers – probably due to their very similar chemical structure (172). In a recent bi-centric study with 350 eczema patients who were consecutively tested with 1% and 2% M2O in pet.; 0.8% positive reactions were observed. However, in 3 additional cases active sensitisation, with first reactions appearing 2 to 4 weeks after the patch test, and prompt reactions in the 2 cases repeat-patch tested, was observed (174).

Additional information: /

METHYL EUGENOL	
CAS # 93-15-2	
EC # 202-223-0	
1,2-Dimethoxy-4-(prop-2-enyl)benzene	
4-Allylveratrole; Eugenyl methyl ether extra; 1,2-Dimethoxy-4-allylbenzene; 1,3,4-Eugenol methyl ether; 1-(3,4-Dimethoxyphenyl)-2-propene; 1-Allyl-3,4-dimethoxybenzene; 3,4-Dimethoxy-1-(2-propenyl)benzene; 3,4-Dimethoxyallylbenzene; 3-(3,4-Dimethoxyphenyl)propene; 4-Allyl-1,2-dimethoxybenzene; Benzene, 4-allyl-1,2-dimethoxy-; Chavibetol methyl ether; Ent 21040; Eugenol methyl ether; Eugenyl methyl ether; Methyl eugenol ether; Methyl eugenyl ether; Methylchavibetol; NSC 209528; NSC 8900; O-Methyleugenol; Veratrole methyl ether; Veratrole, 4-allyl-	

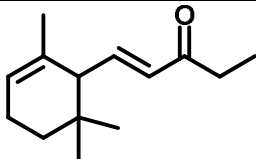
Current regulation: Annex II, 451

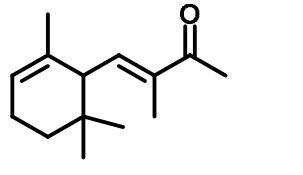
Clinical data:

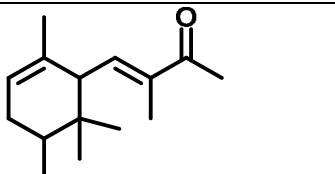
In a previous study by Larsen et al (2002 c), 1.8% of patients with contact allergy to fragrance ingredients reacted positively to this compound (1).

Additional information:

Quote from the SCCS-opinion [0373/00](#): “Methyleugenol should not be intentionally added as a cosmetic ingredient. However, when fragrance compounds containing methyleugenol naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01 % in fine fragrance, 0.004 % in eau de toilette, 0.002 % in a fragrance cream, 0.0002 % in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.” (The reason is genotoxicity and carcinogenicity).

METHYLIONANTHEME	
CAS # 55599-63-8	
EC #	
(1E)-2-Methyl-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-penten-3-one mixt. with (3E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	

8-Methyl- α -ionone-10-methyl- α -ionone mixt.; Iralia Mixture	
Current regulation: ...	
Clinical	data:
One case of ACD has been reported, caused by an E.d.C. (175).	
Additional information:	
Patented by GIVAUDAN SA 1933, is composed of isomeric n-methylionones and iso-methylionones. Methylionone has CAS # 1335-94-0 (not in CosIng) and 1335-46-2 (METHYL α -IONONE ISOMERS); other names: Methyl- α -cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.detail&id=41456 , last accessed 2010-07-14).	

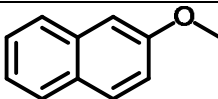
5-METHYL-<i>alpha</i>-IONONE	
CAS # 79-69-6	
EC # 201-219-6	
4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	
Methyl- α -Ionone; 6-Methyl- α -ionone; α -Irone	
Current regulation: /	

Clinical data:

In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to α -irone (10% pet.) (17).

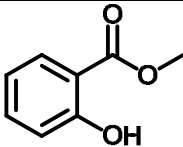
Additional information:

A RIFM review is available (176), citing a (negative) human maximisation test and the study results quoted.

METHYL beta-NAPHTHYL ETHER	
CAS # 93-04-9	
EC # 202-213-6	
2-Methoxynaphthalene	
beta-Naphthyl methyl ether; methyl 2-naphthyl ether; Nerolin (old); NSC 4171; Yara yara; β-Methoxynaphthalene; β-Naphthol methyl ether; β-Naphthyl methyl ether; 2-Methoxynaphthalene; Methyl β-naphthyl ether; 2-Naphthol methyl ether; 2-Naphthyl methyl ether; 6-Methoxy-2-naphthalene	

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

METHYL SALICYLATE	
CAS # 119-36-8	
EC # 204-317-7	
Methyl 2-hydroxybenzoate	
Other names: Salicylic acid, methyl ester; 2-(Methoxycarbonyl)phenol; 2-Carbomethoxyphenol; 2-Hydroxybenzoic acid methyl ester; Analgit; Anthrapole ND; Ben Gay; Exagien; Flucarmit; Methyl ester of 2-hydroxy benzoic acid; Methyl o-hydroxybenzoate; Methyl salicylate; NSC 8204; Wintergreen oil; o-Hydroxybenzoic acid methyl ester; "Oil of wintergreen"	

Current regulation: /

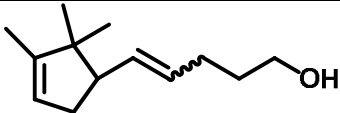
Clinical data:

The deGroot 2000 study yielded 7 positive reactions to methyl salicylate (2% pet.) in 1825 consecutive patients (12).

A case of ACD following the application of a compress bandage containing methyl salicylate has been reported, using 2% "o.o." as PT concentration; the dose per area of methyl salicylate in the occlusive bandage was not reported (177). A similar case was reported in 1977, positive to 2% methyl salicylate in olive oil, with elicitation of pruritus and erythema after oral ingestion of acetyl salicylic acid (178).

Additional information:

A RIFM review is available (179) providing an overview on 3 human sensitisation experiments (e.g., the HRIPT) which were all negative, and clinical data. In a number of older PT studies, positive test results were seen in 6 of 4600, 3 of 183, 3 of 241, 17 of 585, 1 of 70, all employing a test concentration of 2%, usually in pet., according to above review. Methyl salicylate may occur in topical analgesic (OTC) medications, in Germany, for instance, in "Camphopin® Salbe" („Rote Liste 2010").

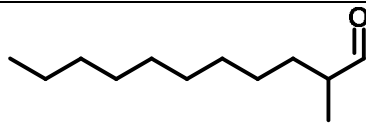
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "MTCP", tested 5% pet. (19). In the An 2005 study, 12 of 422 consecutive patients, i.e., 2.8%, had positive reactions to "ebanol", tested at 5% (13).

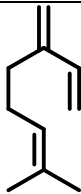
Additional information: /

METHYLUNDECANAL	
CAS # 110-41-8	
EC # 203-765-0	
2-Methylundecanal	
Aldehyde c-12 mna; undecenal, 2-methyl-; 2-Methyl-1-undecanal; Aldehyde M.N.A.; Methyl n-nonyl acetaldehyde; Methylnonylacetaldehyde; NSC 46127	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

MYRCENE	
CAS # 123-35-3	
EC # 204-622-5	
7-Methyl-3-methylideneocta-1,6-diene	
2-Methyl-6-methylene-2,7-octadiene; 7-Methyl-3-methylene-1,6-octadiene; NSC 406264; β-Geraniolene; β-Myrcene	

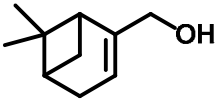
Current regulation: /

Clinical data:

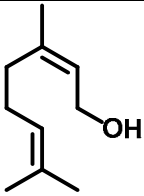
In a clinical study in 6 European centres, including 1511 consecutive patients, 1 patient had a positive reaction to oxidized myrcene (65).

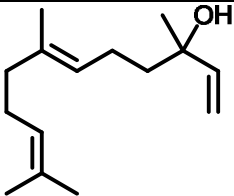
Additional information:

Myrcene autoxidizes spontaneously and rapidly at air exposure. In experimental studies on beta-myrcene an EC3 value of 4.3% was seen for a sample air-exposed 10 weeks (Sköld M. Contact allergy to autoxidized fragrance terpenes (180).

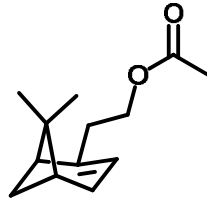
MYRTENOL	
CAS # 515-00-4	
EC # 208-193-5	

(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol
Current regulation: /
Clinical data: /
Additional information:
A RIFM review exists (181), citing 2 of 3 HRIPT studies with 1 case of sensitisation to myrtenol each.

NEROL	
CAS # 106-25-2	
EC # 203-378-7	
(2Z)-3,7-Dimethylocta-2,6-dien-1-ol	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; (Z)-3,7-Dimethyl-2,6-octadien-1-ol; (Z)-Geraniol; (Z)-Nerol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 3,7-Dimethyl-cis-2,6-octadien-1-ol; Nerol 900; Neryl alcohol; cis-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; β-Nerol; cis-geraniol – i.e., isomeric to geraniol	
Current regulation: /	
Clinical data:	
In the Larsen 2002 c study, 6.0% of the fragrance sensitive patients reacted positively to 5% in pet. (1).	
Additional information:	
A RIFM review is available (182) citing (negative) human sensitisation experiments, an older study from Japan and the Larsen 2002 c study (see above).	
Regarding autoxidation studies – see geraniol.	

Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	
Current regulation: /	
Clinical data: /	
Additional information:	

RIFM review is available (183) citing the occurrence of “3 positive reactions in 2273 patients”. Another RIFM review is available on cis-nerolidol (184), mentioning that no data on this compound are available.

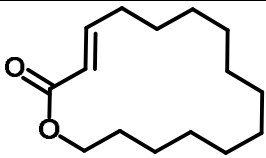
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol acetate; Citroviol; NSC 1286; NSC 404963; Nopol acetate; Nopyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 25% PT preparation of this compound – reactions may have at least partly been due to an “excited back syndrome” and thus a limited evidence (25).

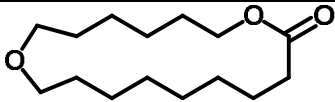
Additional information: /

OXACYCLOHEXADECENONE	
CAS # 34902-57-3	
EC # 609-040-9	
(3E)-Oxacyclohexadec-3-en-2-one	
Globalide; Oxacyclohexadecen-2-one	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

OXALIDE	
CAS # 1725-01-5	
EC # 217-033-3	
1,8-Dioxacycloheptadecan-9-one	
Nonanoic acid, 9-[(6-hydroxyhexyl)oxy]-, o-lactone; 10-Oxa-16-hexadecanolide; Oxalide; Oxalide T	

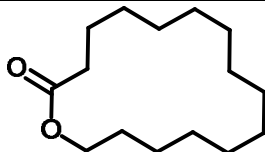
Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "10-oxahexadecanolide", tested 5% pet. (19).

Additional information:

A RIFM review is available (128), citing a negative maximisation test (n=29).

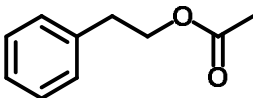
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ -lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; Muskalactone; NSC 36763; Pentadecalactone; Pentadecanolactone; Pentadecanolide; Pentalide; Thibetolide; cpd Supra; ω -Pentadecalactone; angelica lactone; hexaltolide	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). The substance has been used for clinical olfactory testing in the 60ies under the name of exaltolide.

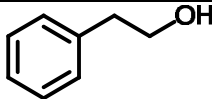
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbiny acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl acetate; β-Phenylethanol acetate; β-Phenylethyl acetate	

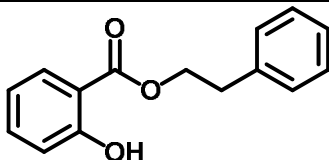
Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). Exposure via plants (*Tanacetum parthenium*) is possible (185).

PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol; β -(Hydroxyethyl)benzene; β -PEA; β -Phenethanol; β -Phenethyl alcohol; β -Phenethylol; β -Phenylethanol; β -Phenylethyl alcohol	
Current regulation: /	
Clinical data:	
The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 25% PT preparation of phenylethyl alcohol (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to this compound, tested 1% pet. in 100 consecutive patients in Odense, DK, was observed (15).	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

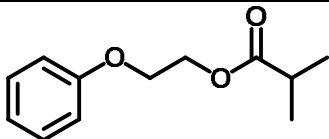
PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201-732-5	
2-Phenylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	

Current regulation: /

Clinical data: /

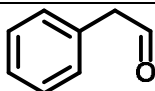
Additional information:

A RIFM review exists (186), quoting a negative human maximisation test and a number of animal experiments, including cross-sensitisation experiments with benzyl salicylate. One LLNA study is reported yielding an EC3 value of 2.1%.

PHENOXYETHYL ISOBUTYRATE	
CAS # 103-60-6	
EC # 203-127-1	
2-Phenoxyethyl 2-methylpropanoate	
Isobutyric acid, 2-phenoxyethyl ester; Ethanol, 2-phenoxy-, isobutyrate; 2-Phenoxyethyl isobutyrate; NSC 227210; NSC	

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406209; Phenoxyethyl isobutyrate; β -Phenoxyethyl isobutyrate	
Current regulation: /	
Clinical data: /	
Additional information:	
It is a "top 100" substance (IFRA, pers. comm.2010).	

PHENYLACETALDEHYDE	
CAS # 122-78-1	
EC # 204-574-5	
2-Phenylacetaldehyde	
Benzylcarboxaldehyde; Hyacinthin; NSC 406309; Phenacetaldehyde; Phenylacetaldehyde; Phenylacetic aldehyde; Phenylethanal; α-Phenylacetaldehyde; α-Tolualdehyde; α-Toluic aldehyde	

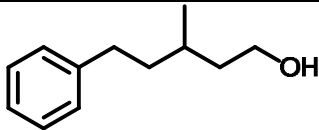
Current regulation: /

Clinical data:

In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to phenylacetaldehyde 2% pet. (24). In a case report, Sanchez-Politta et al. describe a 26-year-old worker in a perfume factory, who suffered from a spill of pure phenylacetaldehyde and became sensitised, as proven by positive patch tests with 0.5%, 1% and 2% (10 healthy controls negative) (187).

Additional information:

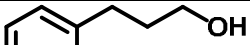
SCCS opinion: [1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assessment" \(QRA: Citral, farnesol and phenylacetaldehyde\)](#)

PHENYLISOHEXANOL	
CAS # 55066-48-3	
EC # 259-461-3	
3-Methyl-5-phenylpentan-1-ol	
3-Methyl-5-phenyl-1-pentanol; 3-Methyl-5-phenylpentanol; 5-Phenyl-3-methylpentanol; Mefrosol; Phenoxanol	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

PHENYLPROPANOL	

Opinion on fragrance allergens in cosmetic products

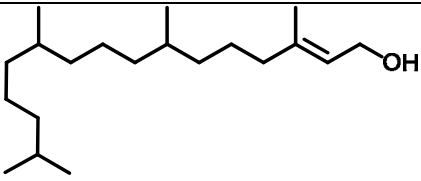
CAS # 122-97-4	
EC # 204-587-6	
3-Phenylpropan-1-ol	
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; NSC 16942; γ-Phenylpropanol; γ-Phenylpropyl alcohol; Phenethyl Carbinol	

Current regulation: /

Clinical data:

The Larsen 2002 c study yielded 0.9% positive reactions in 218 patients with contact allergy to fragrance ingredients (1).

Additional information: ...

PHYTOL	
CAS # 150-86-7	
EC # 205-776-6	
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
Phytol; (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol	

Current regulation: /

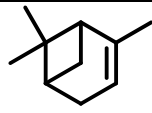
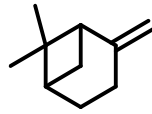
Clinical

data:

/

Additional information:

Phytol is a main constituent of Jasmin abs. with 7.4% reported content (17). In a human maximization study involving 25 subjects, there was one case of contact sensitization to 10% phytol (6900 µg/cm²), applied in petrolatum, as reported in a RIFM review (188).

alpha-PINENE and beta-PINENE	
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	 80-56-8
EC # 201-291-9 (alpha-Pinene; according to CAS service: 219-445-9); EC # 204-872-5 (beta-Pinene; according to CAS service: 245-424-9)	 127-91-3
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (80-56-8)	
6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane (127-91-3)	

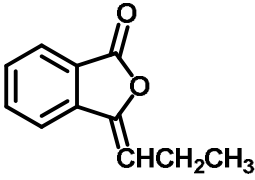
80-56-8: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene 127-91-3: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190; Nopinen; Nopinene; PC 600; PC 600 (pesticide); Pseudopinen; Pseudopinene; Terebenthene; β-Pinene	
Current regulation: Annex III, part 1, n° 130 (Peroxide value less than 10 mmoles/L in substance)	

Clinical data:

In 63 patients positive to the FM I, 2 had a positive PT reaction to beta-pinene (and none to alpha-pinene 5% pet.), 1% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 17 of 30 patients reacted positively to alpha-pinene, and 2 to beta-pinene (189). In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear et al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190).

A case report from Zacher and Ippen on 2 patients with allergic contact dermatitis due to bergamot oil (191) describes positive patch test reactions to alpha-pinene and beta-pinene in one, a worker in a perfume factory.

Additional information: /

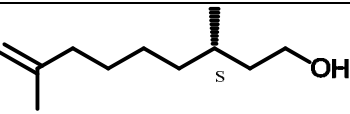
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; Propylidenephthalide; Celeriax; Propylidenephthalide	3-

Current regulation: Annex III, part 1, n° 175

Clinical data:

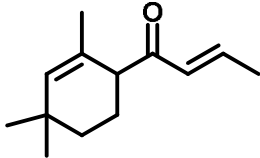
In the Malten 1984 study, 2.6% of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In this paper, "3/25" positive results in human maximisation tests are listed.

Additional information: /

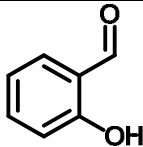
RHODINOL	
CAS # 6812-78-8	
EC # 229-887-4	
(3S)-3,7-Dimethyloct-7-en-1-ol	
Rhodinol; (-)-Rhodinol; α-citronellol; (-)-α-Citronellol; (S)-	

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α -Citronellol	
Current regulation: /	
Clinical data: / (see below)	
Additional information:	
A RIFM review exists citing a positive HRIPT with several cases of sensitisation, 5 of these proven upon re-challenge, and a negative human maximisation test (192). In a previous RIFM review (128), a Japanese clinical study (source not accessible) is cited: "In patch tests using cosmetics ingredients and fragrance materials on patients with eczema and dermatitis, 5% rhodinol (vehicle not specified) produced one sensitization reaction in 202 patients (Itoh et al., 1988 ¹⁴)"	

trans-ROSE KETONE-5	
CAS # 39872-57-6	
EC # 254-663-8	
(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one	
alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	
Current regulation: Annex III, part 1, n° 159 (max. conc. 0.02%)	

Clinical data: /
Additional information:
A RIFM review is available (193) quoting 2 HRIPT studies: one with 0.2% concentration in DEP in 103 volunteers, and negative result, one with 2% concentration, sensitising 2 of 22 volunteers.

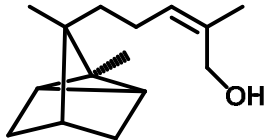
SALICYLALDEHYDE	
CAS # 90-02-8	
EC # 201-961-0	
2-Hydroxybenzaldehyde	
Salicylaldehyde; 2-Formylphenol; NSC 112278; NSC 49178; NSC 83559; NSC 83560; NSC 83561; NSC 83562; NSC 97202; Salicylal; Salicylic aldehyde; o-Formylphenol; o-Hydroxybenzaldehyde	
Current regulation: /	

Clinical data:

¹⁴ Itoh M., Hosono K., Kantoh H., Kinoshita M., Yamada K., Kurosaka R. and Nishimura M. (1988) Patch test results with cosmetic ingredients conducted between 1978-1986. *Nippon Koshohen Kagakkaishi* 12 (1), 27-41.

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to salicylaldehyde was observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to salicylaldehyde 2% pet. (22). The IVDK 2010 study, 0.48% (95% CI: 0.18 – 0.79%; percentages standardised for age and sex) of 2729 patients PTed reacted to the compound (7). An earlier study by Bruze and Zimerson points to possible cross-reactivity between salicylaldehyde and “simple methylol phenols” occurring in synthetic resins based on phenol and formaldehyde (194). Among 24 patients sensitised to resorcinol by application of a wart remover, 2 positive reactions to salicylaldehyde were observed (195).

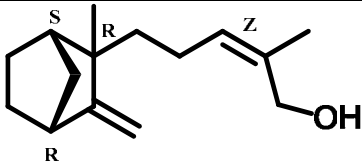
Additional information: Along with other derivatives of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

<i>alpha-SANTALOL</i>	
CAS # 115-71-9	
EC # 204-102-8	
(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.0^{2,6}]hept-3-yl)-2-methylPent-2-en-1-ol	
2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.0 ^{2,6}]heptane, 2-penten-1-ol deriv.; (+)-(Z)-α-Santalol; (+)-α-Santalol; (Z)-α-Santalol; Sandal; Santalol a; cis-α-Santalol; d-α-Santalol	

Current regulation: /

Clinical data: / (see beta-santalol)

Additional information:
Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans.

<i>beta-SANTALOL</i>	
CAS # 77-42-9	
EC # 201-027-2	
(2Z)-2-Methyl-5-[(1S,2R,4R)-2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl]pent-2-en-1-ol	
2-Methyl-5-(2-methyl-3-methylene-2-norbornyl)-2-penten-1-ol; [1S-[1α,2α(Z),4α]]-2-Methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)-2-penten-1-ol; β-Santalol; (-)-(Z)-β-Santalol; (-)-β-Santalol; Santalol b; cis-β-Santalol	

Current regulation: /

Clinical

data:

A RIFM review is available for alpha-santalol (197) and on “santalol” (CAS # 11031-45-1 (198)). The former review cites a Japanese study: “Between April 1979 and August 1990, a total of 3123 male and female patients were patch tested to 2% santalol (.alpha. or .beta. not specified) in petrolatum. Reactions were observed in 47/3123 (1.5%) of the patients. The incidence of positive reactions from 1979 to 1990 was 1.5%. The rate of reactions observed was higher during the earlier period of the patch testing than the later stage (Utsumi et al., 1992)¹⁵.” In another Japanese study cited by the RIFM review “... patch tests were conducted with 0.05–0.5% santalol (specified as santalol 1) in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm² lint with a 2 cm² cellophane disc placed on the lint and then covered with a 4 cm² plaster. Patches were applied to the back, the forearm, and the inside of the upper arm for 24–48 h. Reactions were observed in 15 patients and questionable reactions were observed in 10 patients out of the total 427 participating. A second sample of santalol (specified as santalol 2) was tested on 214 patients. Reactions were observed in three patients and questionable reactions were observed in six patients (Takenaka et al., 1986)¹⁶.” Moreover, “The Mid-Japan Contact Dermatitis Research group (MJDCRG) conducted a 6-year (1976–1981) patch test study on facial dermatoses patients with various fragrance materials. During the year 1979, a total of 327 patients were tested with a mixture of .alpha. and .beta. santalol at concentrations of 10%, 2%, and 1% in white petrolatum. Reactions were observed in 1.5%, 0.6% and 0.6% of the 327 patients tested at concentrations 10%, 2%, and 1%, respectively (MJDCRG, 1984)¹⁷.”

The Goossens 1997 study found 5 of 111 patients positive to “santalol 10% pet.” (isoform not specified) – all sensitised to other fragrance allergens as well (23). In the Larsen 2001 study, patch testing with “2-methyl-5-(2,3-dimethyltricyclo[2.2.1.0(2,6)]hept-3-yl-2-pentenol(.alpha.-form) and 2-methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-3-yl-2-penten-1-ol(beta-form) 5% pet.” (no CAS numbers given) yielded a total of 2 positive reactions among the 178 patients with known contact allergy to fragrance ingredients (19).

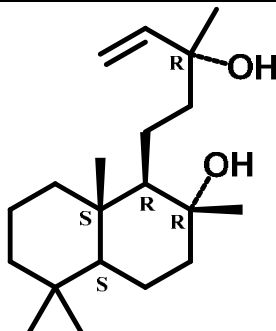
Additional information: “There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)” (A.M. Api, pers. comm., 2010).

Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans

¹⁵ Utsumi, M., Sugai, T., Shoji, A., Watanabe, K., Asoh, S., Hashimoto, Y., 1992. Incidence of positive reactions to sandalwood oil and its related fragrance materials in patch tests and a case of contact allergy to natural and synthetic sandalwood oil in a museum worker. *Skin Research* 34, 209–213

¹⁶ Takenaka, T., Hasegawa, E., Takenaka, U., Saito, F., Odaka, T., 1986. Fundamental studies of safe compound perfumes for cosmetics Part 1. The primary irritation of compound materials to the skin. *Unknown Source*, 313–329.

¹⁷ Mid-Japan Contact Dermatitis Research Group, 1984. Determination of suitable concentrations for patch testing of various fragrance materials. A summary of group study conducted over a 6-year period. *Journal of Dermatology*, 11(1), 31–35.

SCLAREOL	
CAS # 515-03-7	
EC # 208-194-0	
(1R,2R,8aS)-1-[(3R)-3-Hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-2-ol	
(αR,1R,2R,4aS,8aS)-α-Ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1-naphthalenepropanol; [1R-[1α(R*),2β,4αβ,8α]] - α-ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1 Naphthalenepropanol; (13R)-Labd-14-ene-8,13-diol; Sclareol; (-)-Sclareol; [1R-[1.alpha.(R*),2.beta.,4α.beta.,8α.alpha.]]-2-hydroxy-α,2,5,5,8a-pentamethyl-α-vinyldecahydronaphthalene-1-propan-1-ol	

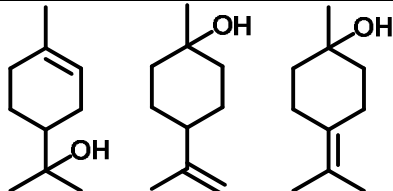
Current regulation: /

Clinical data: /

Additional information:

An older RIFM review exists (128), reporting several human maximisation tests with different samples of sclareol, yielding partly positive, partly negative results. A more recent RIFM review is available (199), citing no clinical data, but several maximisation studies, one of which was positive in a few volunteers, which was apparently due to an impurity.

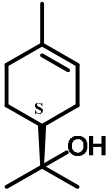
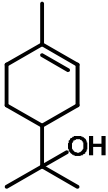
[0986/06 - Opinion on Sclareol \(sensitisation only\)](http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)
(http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

TERPINEOL	
CAS # 8000-41-7	
EC # 232-268-1	
Mixtures of isomers	
Terpineol 318, mixture of terpineol isomers alfa, beta, gamma	alfa gamma beta

Current regulation: /

Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study "Takenaka 1986", finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpineol in pet., tested in 100 consecutive patients in Belfast, were observed (15).

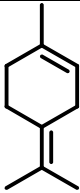
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

<i>alpha-TERPINEOL</i>	 10482-56-1
CAS # 10482-56-1 / 98-55-5	
EC # 233-986-8 / 202-680-6	
2-[(1S)-4-Methyl-1-cyclohex-3-enyl]propan-2-ol (10482-56-1)	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol (98-55-5)	
10482-56-1: (S)-(-)-p-Menth-1-en-8-ol; (-)-α-Terpineol; (S)-(-)-Terpineol; (S)-(-)-α-Terpineol; (S)-α-Terpineol; l-α-Terpineol 98-55-5: p-Menth-1-en-8-ol; (±)-α-Terpineol; 1,1-Dimethyl-1-(4-methylcyclohex-3-enyl)methanol; 1-p-Menthen-8-ol; 2-(4-Methyl-3-cyclohexenyl)-2-propanol; 4-(2-Hydroxy-2-propyl)-1-methylcyclohexene; 8-Hydroxy-p-menth-1-ene; NSC 21449; NSC 403665; PC 593; Pine Oil 593; Terpeneol 350; dl-α-Terpineol; α,α,4-Trimethyl-3-cyclohexene-1-methanol; α-Terpineol	 98-55-5
Current regulation: /	

Clinical data:

A RIFM review is available (201) specifically on (-)-alpha-terpineol stating that “no data is available” regarding skin sensitisation. Another RIFM review is available on alpha-terpineol (202). In the Frosch 2002 b study, 1 of 1606 consecutive patients showed a positive reaction, but 11 patients doubtful reactions to alpha-terpineol (5% pet.) (17). The DeGroot 1985 study identified no positive reactions among 179 patients using a 15% PT preparation of terpeneol (mixed isomers) (25). In 63 patients positive to the FM I, 2 had a positive PT reaction to alpha terpeneol, 5% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 3 of 30 patients reacted positively to alpha-terpineol (189)

Additional information: see also terpeneol (mixture of isomers). Comments on turpentine under pinene.

<i>Terpinolene</i>	
CAS # 586-62-9	
EC # 209-578-0	
1-Methyl-4-propan-2-ylidenecyclohexene	
p-Mentha-1,4(8)-diene; 1-Methyl-4-(1-methylethylidene)-cyclohexene; 4-Isopropylidene-1-methylcyclohexene; Isoterpinene; Nofmer TP; Terpinolen; Terpinolene; α-Terpinolene; δ-Terpinene	

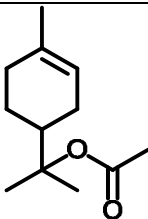
Current regulation: Annex III, part 1, n° 133 (Peroxide value less than 10 mmoles/L in substance)

Clinical data:

A 49-year-old machine cleaner developed occupational contact dermatitis due to the cleaner, which gave a positive patch test result at 1:10 000 in water. Of the ingredients identified by chromatography, only .delta.-3-carene and terpinolene, tested 5% pet.,

gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).

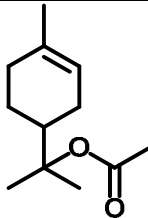
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

TERPINEOL ACETATE (Isomer mixture)	
CAS # 8007-35-0	
EC # 232-357-5	
4-Methyl-1-propan-2-yl-1-cyclohex-2-enyl acetate	
Terpinyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpinyl acetate in pet., tested in 106 consecutive patients in Barcelona, were observed (15)

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

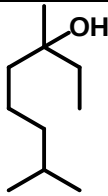
alpha-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate; α-Terpineol acetate; p-Menth-1-en-8-yl acetate; 1-Methyl-1-(4-methylcyclohex-3-enyl)ethyl ethanoate; (±)-.alpha.,.alpha.,4-trimethylcyclohex-3-ene-1-methyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified no positive reactions among 179 patients using a 10% PT preparation of “terpinyl acetate” (25).

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

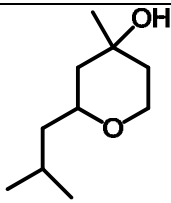
Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	

Current regulation: /

Clinical data:
/

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM

review is available (205) quoting 1 negative human maximisation test.

TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	
CAS # 63500-71-0	
EC # 405-040-6	
4- Methyl-2-(2-methylpropyl)tetrahydro-2H-4-pyranol	
2-(2-Methylpropyl)-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-methyltetrahydropyran-4-ol; 4-Hydroxy-4-methyl-2-(2-methylpropyl)tetrahydropyran; Florosa; Rozanol	

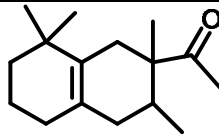
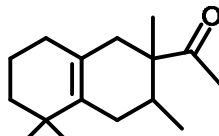
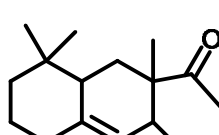
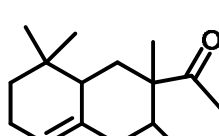
Current regulation: /

Clinical

data:

/

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES		54464-57-2
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9		54464-59-4
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9		68155-66-8
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2)		68155-67-9
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone (54464-59-4)		
1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-66-8)		
1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-67-9)		
54464-57-2: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone; 1',2',3',4',5',6',7',8'-Octahydro-2',3',8',8'-tetramethyl-2'-acetoneaphthone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemon E; OTNE; Orbitone		

Current regulation: /

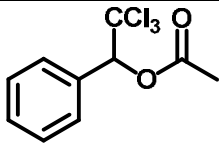
Clinical

data:

In the Frosch 2002 a study, 0.2% of 1855 consecutive patients reacted to the compound (brand name mentioned: „Iso E. Super“, 5% pet.) (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction both to 1% and 5% “Iso E Super ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15). The Larsen 2001 study yielded 1.7% positive reactions (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: According to CosIng: "Mixture of isomers: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-67-9); 1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-66-8) ", (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40504>, last accessed 2009-11-11).

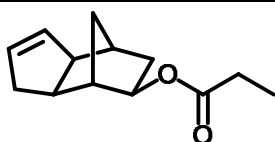
It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICHLOROMETHYL PHENYL CARBINYL ACETATE	
CAS # 90-17-5	
EC # 201-972-0	
2,2,2-Trichloro-1-phenylethyl acetate	
Benzenemethanol, α-(trichloromethyl)-, acetate; Benzyl alcohol, α-(trichloromethyl)-, acetate (Trichloromethyl)phenylcarbinyl acetate; (±)-α-(Trichloromethyl)benzyl acetate; 2-Acetoxy-1,1,1-trichloro-2-phenylethane; Crystal rose; NSC 165582; Rosacetol; Rosephenone; Rosetone; Rosone; α-(Trichloromethyl)benzyl acetate	

Current regulation: /

Clinical data: /

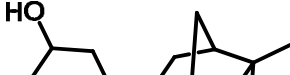
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICYCLODECENYL PROPIONATE	
CAS # 17511-60-3	
EC # 241-514-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-6-yl propionate	
4,7-Methano-1H-inden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propionate; Cyclaprop; Florocyclene; Greenyl propionate; Tricyclo(5.2.1.02,6)dec-3-en-8-yl propionate.	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL	

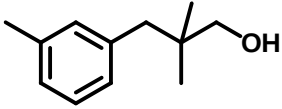
Opinion on fragrance allergens in cosmetic products

CAS # 3407-42-9	
EC # 222-294-1	
3-(5,5,6-Trimethyl-6-bicyclo[2.2.1]heptanyl)cyclohexan-1-ol	
3-(5,5,6-Trimethyl-2-norbornyl)-cyclohexanol; 3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexan-1-ol; 3-Hydroxy-1-(5-isocampyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of “synthetic sandalwood oil”.

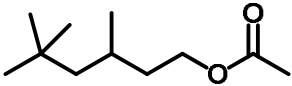
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, majantol (conc. not given, elsewhere reported as 5% pet.) caused positive PT reactions in 3.2% of patients with known contact allergy to fragrance ingredients. In a later study by the IVDK, 0.5% (95% CI: 0.3 – 0.7%) consecutive patients displayed a positive reaction to majantol 5% pet. (206). In the IVDK 2010 study, majantol was tested both in n=2189 consecutive patients, yielding 0.36 % (95% CI: 0.12—0.60%) positive reactions, and in the context in a special series, applied in an aimed fashion to n=4972 patients, yielding 0.76% (95% CI: 0.49—1.03%) (standardised) positive reactions (7). In a recent study from Copenhagen, DK, 6 of 722 patients tested with this compound were found positive, 2 of these to material used earlier provided by Symrise, 4 to material by Allmiral/Hermal/Trolab used later instead. There was no significant difference between these proportions obtained with batches of majantol from different production processes (207).

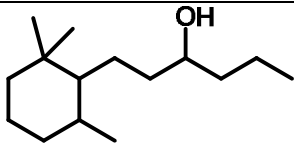
Additional information: /

TRIMETHYLHEXYL ACETATE	
CAS # 58430-94-7	
EC # 261-245-9	
3,5,5-Trimethylhexyl acetate	
1-Hexanol, 3,5,5-trimethyl-, acetate; Vanoris; neononyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

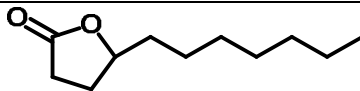
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethylcyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; Finotimber; Timberol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

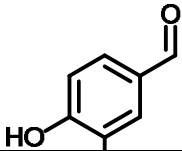
<i>gamma</i>-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ -lactone; (RS)- γ -Undecalactone; ($\pm$)- γ -Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 350120-1; Peach lactone; Peche Pure; Persicol; γ -(n-Heptyl)- γ -butyrolactone; γ -Heptyl- γ -butyrolactone; γ -Heptylbutyrolactone; γ -Undecalactone; γ -Undecanolactone; γ -Undecanolide; γ -n-Heptylbutyrolactone	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm. 2010)

VANILLIN	
CAS # 121-33-5	
EC # 204-465-2	

4-Hydroxy-3-methoxybenzaldehyde	
2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; 4-Formyl-2-methoxyphenol; 4-Hydroxy-5-methoxybenzaldehyde; 4-Hydroxy-m-anisaldehyde; H 0264; Lioxin; NSC 15351; NSC 403658; NSC 48383; Rhovanil; Vanillaldehyde; Vanillic aldehyde; Vanillum; m-Methoxy-p-hydroxybenzaldehyde; p-Hydroxy-m-methoxybenzaldehyde; p-Vanillin	

Current regulation: /

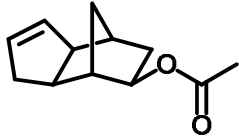
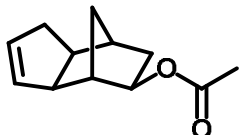
Clinical data:

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to vanillin was observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to vanillin 10 % pet. (22). The IVDK 2010 study, n=10, i.e., 0.19% (95% CI: 0.07 – 0.32%; percentages standardised for age and sex) of 4377 patients PTed reacted to the compound, tested 10% pet. (7). In n=102 patients with a positive reaction to MPR, 19 compounds of this natural mixture were tested, among these, vanillin, to which none reacted positively (208). In 21 patients with contact allergy to propolis, 2 also reacted to vanillin (10% pet.) (209).

A 13-year-old girl with recurrent (peri-)cheilitis after application of a vanilla lip salve tested strongly positive to this salve (as is), “Vanilla 10% pet.” (unclear, whether natural extract or vanillin) and MPR (210). Trattner/David identified 1 / 641 consecutive patients with positive reaction to vanillin (31).

Additional information:

Naturally occurring in the fruit of *Vanilla planifolia* after a fermentation process, in styrax, clove oil, potatoes, wood, including Myroxylon pereirae resin, and other material (53). Nowadays, vanillin is synthesised from eugenol, guajakol and lignin residues from paper production, however, not fully achieving the subtle scent and taste of the natural material (53). It is a “top 100” substance and classified as R43 (IFRA, pers. comm. 2010).

VERDYL ACETATE	
CAS # 2500-83-6/ 5413-60-5	
EC # 219-700-4 / 226-501-6	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6)	2500-83-6
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat (5413-60-5)	
2500-83-6: 4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; NSC 142428; NSC 94573	
5413-60-5: 4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 8-Acetoxytricyclo[5.2.1.0 ^{2,6}]dec-3-ene; Greenyl acetate;	5413-60-5

Opinion on fragrance allergens in cosmetic products

Herbaflorat; Jasmacyclene; NSC 6598	

Current regulation: /

Clinical data: /

Additional information:

In CosIng, both above CAS numbers are listed under “verdyl acetate” (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41289>, last accessed 2010-07-19).

In the CAS, there are 2 separate entries; moreover, there are 2 separate RIFM reviews:

- # 2500-83-6: Other names: Tricyclo[5.2.1.0^{2,6}]dec-4-en-8-yl acetate (REACH, EINECS, INCI Name according to CAS); 3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl Acetate; Tricyclodecen-4-yl 8-Acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available, stating that “no data is available” regarding the skin sensitising properties of the substance (211).
- # 5413-60-5: Other names: 3a,4,5,6,7,7a-hexahydro-4,7-methanoinden-6-yl acetate (REACH, EINECS, INCI Name according to CAS), 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 8-Acetoxytricyclo[5.2.1.0^{2,6}]dec-3-ene; Tricyclodecenyl acetate; Greenyl acetate; Herbaflorat; Jasmacyclene; NSC 6598; Verdyl acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available (212), citing 2 negative human maximisation tests and 1 negative HRIPT.

Natural extracts / essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic material. Moreover, several compounds cannot be synthesised at a competitive price, and the demand for perfumes based on natural materials is considerable (34).

The three main methods used to concentrate plant fragrance substances as essential oils comprise steam distillation, mechanical processes from the epicarp of Citrus fruits ("pressing") and dry distillation. An Essential oil is „obtained by steam distillation with addition of water in the still (hydrodistillation) or without addition of water in the still (directly by steam“)(213). Essential oil of fruit juice is „obtained by from a fruit juice during its concentration or during UHT (flash pasteurization) treatment“ (213). Cold pressed essential oil is „obtained by mechanical processes from the epicarp of the fruit of a Citrus, at ambient temperature“(213). Citrus peel oils, apart from distilled Citrus oils, are produced with various methods (214). The oil consists of a high volume of volatile terpenes, mostly monoterpenes but also contains small amounts of non-volatile compounds such as dyes, waxes and furocoumarines.

The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concrete: an extract „obtained from a fresh plant natural raw material by extraction with a solvent“¹⁸, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolute: „product, obtained by extraction with ethanol from a concrete, a floral pomade, a resinoid or a supercritical fluid extract. The ethanolic solution is generally cooled down and filtered in order to eliminate the «waxes»; the ethanol is then eliminated by distillation“¹⁹. Resinoids, used for their fixative properties, are „obtained from a dry plant natural raw material by extraction with a solvent“²⁰. The products are usually highly viscous and thus might sometimes be diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are extracts „of spice or aromatic herb“ by „treating a natural raw material with a solvent, then, after filtration if necessary, the solvent is eliminated“²¹.

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case report or clinical studies regarding essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g., masseurs,

¹⁸ ISO/DIS 9235

¹⁹ ISO/DIS 9235

²⁰ ISO/DIS 9235

²¹ ISO/DIS 9235

physiotherapists (215, 216), aromatherapists (217-221), beauticians doing massages (222); for further details, e.g., PT results with various essential oils, see original case reports. "Current Regulation" refers to the EU Cosmetics Directive only.

Catalogue of natural extracts / essential oils evaluated

ACORUS CALAMUS ROOT OIL

CAS 84775-39-3; EC 283-869-0

Calamus Oil; "Sweet Flag Oil"

(*Acorus calamus*, ext. = INCI name)

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "calamus" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to "calamus" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Acorus calamus* L. (sweet flag calamus). *Acorus Calamus* Root Oil is an essential oil obtained from the rhizomes of the calamus, *Acorus calamus* L., Araceae. It contains beta-asarone (up to 96%, depending on ploidy, and with this, origin (34)), calamene (about 4%), calamol (about 3%) alpha-asarone (about 1%), camphene (about 1%) and some beta-pinene and asaronaldehyde (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41330>, last accessed 2010-01-29. Use is restricted due to potential toxicity of beta-asarone (34).

CANANGA ODORATA and Ylang-ylang oil

Ylang-ylang and cananga oils are essential oils that are obtained from two subspecies of the cananga tree (34). In the INCI nomenclature, both are not differentiated.

CANANGA ODORATA FLOWER EXTRACT

CAS 83863-30-3; EC 281-092-1
(ylang-ylang, ext.) INCI name:
CANANGA ODORATA EXTRACT

CANANGA ODORATA FLOWER OIL

CAS 8006-81-3, 68606-83-7; EC / (oils,
ylang-ylang) INCI name: CANANGA
ODORATA OIL

Current regulation: ...

Clinical data:

Ylang-ylang oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma*

genuina)

In the Larsen 2002 c study, “synthetic ylang-ylang oil” caused 6.4% positive reactions in 218 patients with known contact allergy to fragrance ingredients (1). In a Japanese study, M. Sugawara et al. noted a significant decline of the proportion of patients reacting positively to “ylang-ylang oil 5% pet.” from 1971 to 1989, the overall number in patients with cosmetic dermatitis amounting to 176 of 1438 (12.2%, 95% CI: 10.6 – 14.0%) (223). In the Frosch 2002 b study, two fractions of Ylang-Ylang oil (I and II) were separately tested, each at 10% pet. Fraction I yielded 2.6%, fraction II 2.5% positive test reactions (no data on concomitant reactivity given) (17). The deGroot 2000 study, with 1825 consecutively tested patients, found 18 positive PT reactions to “ylang-ylang oil”, tested at 4% in pet. (12). The Sugiura 2000 study with 1483 patients with suspected cosmetic dermatitis observed 0.8% positive PT reactions with ylang-ylang oil (5% pet.) (14). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with ylang-ylang oil (2% pet.) 13.4% positive reactions (9). The Belsito 2006 study (20) yielded 0.6% positive reactions to ylang-ylang oil. The subsequent NACDG 2009 study identified 1.5% positive reactions in 4434 patients PTed with 2% “ylang-ylang oil” (21). The IVDK 2010c study found 2.5% positive reactions in 3175 consecutively tested patients, and 3.9% in 2155 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to ylang-ylang oil and 3 to “cananga oil” (48).

Cananga oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma macrophylla*. For Oil of cananga (*Cananga odorata* (Lam.) Hook. f. et Thomson, *forma macrophylla*) an ISO standard exists: ISO 3523:2002. Cananga oil is produced by steam distillation of the flowers of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *macrophylla* (*Annonaceae*). The composition resembles that of “ylang-ylang III”, but with a higher content of caryophyllene (30-40%). Cananga oil originates almost exclusively in Java; annual production about 50 t. The oil is used mainly in perfuming soaps where it is more stable than ylang-ylang oils due to its lower ester content (34).

Sugiura et al. (2000) found 1.1% positive reactions to “cananga oil”, tested 5% pet. (14). Cananga oil (2% pet.) mentioned in the same Portuguese study already cited (9) yielded 10.4% positive reactions. In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to cananga odorata oil tested at 2% concentration (13).

Studies with both oils

The Goossens 1997 study found 3 of 111 patients positive to “ylang-ylang oil 5% pet.”, and 4 to “cananga oil 15% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 1 positive reaction in 200 patients to “cananga” and 4 to “ylang-ylang” essential oil, both tested 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=10 (11.6%) positive reactions to “cananga” and n=8 (9.3%) to “ylang-ylang” essential oils, each tested at 2% pet. (27). Nakayama et al. found 1974 (after (29)) 11 “strong positive” and 15 “weak positive” reactions to “Cananga oil” and 9 and 16, resp., to “Ylang-ylang oil” (unknown test concentration) in 183 patients.

A number of case reports highlight the possibility of occupational contact and sensitisation, e.g. (222, 224).

Additional information:

Ylang-ylang oil

The composition of this essential oil is defined by a standard: ISO 3063:2004. Ylang-ylang oils are obtained by steam distillation of freshly picked blossoms of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *genuina* (*Annonaceae*). The oil is produced mainly in Madagascar and the Comoro islands. Four fractions are collected at progressively longer distillation times and are known as “extra”, “I”, “II” and “III”. The composition of the various oil fractions depends on the duration of distillation. The first fraction has the highest content of strongly odiferous constituents such as p-cresyl methyl ether (5-16%), methyl benzoate (4-9%), (-)-linalool (7-24%), benzyl acetate (5.5-17.5%), and geranyl acetate (2.5-14%). The other fractions contain increasing amounts of sesquiterpene hydrocarbons such as caryophyllene, germacrene-D, and (E,E)-alpha-farnesene (> 70% in “ylang-ylang III”). Components such as p-cresol, eugenol and isoeugenol are important for odour, although they are present only in low concentration (34). According to (30) the maximum observed concentration in ylang-ylang I and II are (in %): germacrene-D (28); (E,E)-alpha-farnesene (21); caryophyllene (17); linalool (I: 19.0; II: 9.5); benzyl benzoate (8.0); farnesol (4.0); benzyl salicylate (4.0); (E,E)-farnesyl acetate (3.5); geraniol (2.5); isoeugenol (0.8); benzyl alcohol (0.5); eugenol (0.5); p-cresyl methyl ether (I: 5.0; II 3.5); methyl benzoate (I: 5.5; II: 3.5); benzyl acetate (I: 10.0; II: 5.0); geranyl acetate (I: 15.0; II: 12.0).

CEDRUS ATLANTICA BARK OIL

CAS 92201-55-3; EC 295-985-9
(*Cedrus atlantica*, ext. = INCI) /
8000-27-9; EC / (Oils,
cedarwood) INCI name: CEDRUS
ATLANTICA OIL

Cedarwood oil

Current regulation: /

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=5 (0.7%) positive reactions to cedarwood oil 10% pet. (22). (The exact origin of “cedarwood oil” in this study is not clear.) The IVDK 2010 c study identified 0.8% positive reactions in 6223 patients tested in the context of a special series with a cedarwood oil tagged with CAS # 8000-27-9 (30).

Additional information:

Cedrus Atlantica Bark Oil is the volatile oil obtained from the bark of *Cedrus atlantica*, *Pinaceae*

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55309>, last accessed 2010-01-05). The main odiferous component is alpha-atlantone [32207-08-2] (39)

Nomenclature also used: *Cedrus atlantica* wood oil (*Cedrus atlantica* (Endl.) G.Manetti ex Carrière)²²

See also *Juniperus virginiana*.

²² ISO 4720:2009 nomenclature

CEDRUS DEODARA WOOD OILCAS 91771-47-0; EC 294-939-5 (*Cedrus deodara*, ext.)*Cedarwood oil*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “cedarwood” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “Himalayan cedarwood” essential oil 2% pet. (27). (The labelling in the latter report points to *Cedrus deodara* as source of “cedarwood oil” in these 2 Polish studies.)

Additional information:

Cedrus Deodara Wood Oil, Himalayan cedarwood oil (*Cedrus deodara* (Roxb. ex D.Don) G. Don)²³, is the volatile oil obtained by steam distillation of the stumps of the Deodar Cedar, *Cedrus deodara*, *Pinaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55311>, last accessed 2010-01-29).

Several other conifer species are called cedars, and the corresponding oils vary considerably in composition. These include Cedar leaf oil (*Thuja* oil) produced by steam distillation of fresh leaves and branch ends of *Thuja occidentalis* L. (*Cupressaceae*) from North America, containing a minimum of 60% thujone [8007-20-3] [90131-58-1] (34). Texas cedarwood oil is produced by steam distillation of chopped wood of *Juniperus mexicana* Schiede (*Cupressaceae*), containing alpha-cedrene (15-25%), thujopsene (25-35%), cedrol 20% minimum [8000-27-9] [91722-61-1] (34). Chinese cedarwood oil is similar to Texas cedarwood oil, obtained by steam distillation of *Cupressus funebris* Endl., *Cupressaceae* (*Chamaecyparis funebirs* (Endl.) France), which is a weeping cypress [8000-27-9] [85085-29-6] (34).

CINNAMOMUM CASSIA LEAF OIL

94961-46-6 [invalid] / 8007-80-5; EC / (Oils, cassia) INCI name: CINNAMONUM CASSIA OIL

*Cassia Oil; Cassia leaf Oil; Cinnamon Oil Chinense***CINNAMOMUM ZEYLANICUM BARK OIL**CAS 84649-98-9; EC 284-635-0 (*Cinnamomum zeylanicum*, ext. = INCI)*Cimmamon Bark Oil Ceylon; Cinnamon Oil Ceylon*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “cassia” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=24

²³ ISO 4720:2009 nomenclature

(27.9%) positive reactions to "cassia" essential oil 2% pet. (27).

A 32 year old Spanish physiotherapist developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: "cinnamon oil" (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Cinnamomum tsumu* Helms, syn. *Cinnamomum cassia* auct. and *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* J. Presl, respectively. Cassia oil (Chinese cinnamon oil) is obtained by steam distillation of the leaves, twigs, and bark of *Cinnamomum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamon bark oil (see below), cassia oil contains a considerable amount of 2-methoxycinnamal (3-15%), in addition to its main constituent, cinnamal (70-88%). Cassia oil is predominantly used in flavouring soft drinks, with an annual production of a few hundred tons (34). For Oil of cassia, Chinese type (*Cinnamomum aromaticum* Nees, syn. *Cinnamomum cassia* Nees ex Blume) an ISO standard exists: ISO 3216:1997

Cinnamomum Zeylanicum Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, *Cinnamomum zeylanicum*, *Lauraceae*. It contains mainly cinnamaldehyde (34), e.g. 50-60%, and lesser quantities of eugenol (4-8%), phellandrene

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75370>, last accessed 2009-11-16). For Oil of cinnamon leaf, Sri Lanka type (*Cinnamomum zeylanicum* Blume) an ISO standard exists: ISO 3524:2003

Cinnamomum Cassia Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamon, *Cinnamomum cassia* (L.), *Lauraceae*. It contains 80% eugenol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75368>, last accessed 2009-11-16). The cinnamon leaf oil produced by steam distillation of the leaves of *Cinnamomum zeylanicum* Blume (*C. verum* J.S. Presl) similarly has a content of 70-83% eugenol (34).

Considering the content of well-known allergenic compounds, the essential oil is considered an Established contact allergen in humans,

CITRUS AURANTIUM AMARA FLOWER OIL

CAS 8016-38-4, 68916-04-1; EC / (Oils, neroli) /

Neroli oil

CITRUS AURANTIUM AMARA PEEL OIL EXPRESSED

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

"Bitter Orange Oil"

INCI names: CITRUS AURANTIUM AMARA ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "neroli oil" (2% pet.) 6.6% positive reactions (9). The Rudzki 1976 study found 3 positive reactions in 200 patients to "bitter orange" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%)

positive reactions to “bitter orange” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.7% positive reactions in 6220 patients tested in the context of a special series (30)

Additional information:

ISO 4720:2009 nomenclature: *Citrus aurantium* L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso. For Oil of neroli (*Citrus aurantium* L. spp. *aurantium*, syn. *Citrus aurantium* L. spp. *amara* var. *pumilia*) an ISO standard exists: ISO 3517:2002. Citrus Aurantium Peel Oil Expressed is an essential oil expressed from the fresh epicarps of the Sour Orange, *Citrus aurantium*, Rutaceae. It contains D-limonene (about 90%), citral, decanaldehyde, methyl anthranilate, linalool, terpineol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41394>, last accessed 2010-01-29). The aldehyde content is lower and the ester content (e.g., linalyl and geranyl acetate) is higher than in sweet orange oil (34). It is predominantly used for flavouring alcoholic beverages. According to (30) the maximum observed concentration in neroli oil are (in %): linalool (44); limonene (18); β -pinene (17); linalyl acetate (15); *trans*- β -ocimene (8); geranyl acetate (5); *trans*-nerolidol (5); (*E,E*)-farnesol (4); myrcene (4); farnesol (4,0); geraniol (3,5); citral (0,3) (30).

CITRUS AURANTIUM AMARA LEAF OIL

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

Petitgrain oil Paraguay / ... bigarade

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Petitgrain bigarade” and “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “Petitgrain bigarade” and n=4 (4.6%) to “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* L. Pers. X *Citrus aurantium* L. ssp. *amara* var. *pumilia*. Petitgrain oils in general are steam distilled from the leaves of citrus trees. Citrus Aurantium Leaf Oil is an essential oil obtained from the leaves of the Sour Orange, *Citrus aurantium*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41392>, last accessed 2010-02-10). Petitgrain oil Paraguay is obtained from an acclimatised variety of the bitter orange tree. Main constituents are linalool (15-30%) and linalyl acetate (40-60%). A number of trace constituents contribute essentially to the odour (34). Petitgrain oil bigarade is derived from the same species of tree grown in France, Italy, Spain and North Africa (34). For Oil of bitter orange petitgrain, cultivated (*Citrus aurantium* L.) an ISO standard exists: ISO 8901:2003.

Considering the content of well-known allergenic compounds, the essential oil is regarded as an established contact allergen in humans

CITRUS BERGAMIA PEEL OIL EXPRESSED

CAS 89957-91-5, 8007-75-8; EC

	289-612-9 (<i>Bergamot, ext.</i>)
<i>Bergamot Oil, Bergamot Orange Oil</i>	INCI: CITRUS AURANTIUM BERGAMIA EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “Bergamot” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found no positive reaction to “Bergamot” essential oil 2% pet. (27). In 63 patients positive to the FM I, 2 had a positive PT reaction to bergamot oil, 2% pet., in the Santucci 1987 study (28). A case report from Zacher and Ippen describes 2 patients with allergic contact dermatitis due to bergamot oil (191), one a worker in a perfume factory, the other sensitised by non-occupational use of cosmetics.

Additional information:

ISO 4720:2009 nomenclature: *Citrus bergamia* (Risso et Poit.), syn. *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler. Citrus Bergamia Peel Oil Expressed is an essential oil expressed from the epicarps of the Bergamot, *Citrus bergamia* risso, Rutaceae. It contains 35-45% L-linalyl acetate, about 6% linalool, D-limonene, DL-limonene and bergaptene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=se_arch.details&id=41398, last accessed 2009-11-27). According to Surburg/Panten: linalyl acetate 22-36%, linalool 3-15%, geranial 0.25-0.5%, citral 1%, with a relatively low terpene content of 25-50% (34, 39). Bergaptene content by HPLC is 0.18-0.38% (34). Annual production from Italy, Brazil, Spain and Ivory Coast is 100 to 150 t. For Oil of bergamot [*Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler], Italian type an ISO standard exists: ISO 3520:1998.

CITRUS LIMONUM PEEL OIL EXPRESSED

CAS 84929-31-7, 8008-56-8; EC 284-515-8 (*Lemon, ext.*)

Lemon oil

INCI names: CITRUS MEDICA
LIMONUM ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with “lemon oil” (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to “lemon oil” 2% pet. (22).

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lemon” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “lemon” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus limon* (L.) Burm. f. According to (30) the maximum observed concentration in lemon oil are (in %): limonene (80); β -pinene (16.5); γ -terpinene (12); citral (3.0); geranial (2.0); neral (1.2); β -bisabolene (0.9); geranyl

acetate (0.7); neryl acetate (0.6); linalool (0,3); geraniol (0,2) (30). An ISO standard exists for Oil of lemon [Citrus limon (L.) Burm. f.], obtained by expression: ISO 855:2003. The composition of lemon oil depends on the variety of lemon and the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

Parameter	Type	Mediterranean		Equatorial	
	American Origin				
	Coast	Desert	Italy	Spain	Ivory coast, Brazil
d_{20}^{20}	0.851–0.857	0.849–0.854	0.850–0.858	0.849–0.858	0.845–0.854
n_D^{20}	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4790
α_D^{20}	+57° to +65°6'	+67° to +78°	+57° to +66°	+57° to +66°	+57° to +70°
Composition by GC [area %]					
β -Pinene	9–14	10–13	10–16.5	10–16.5	7–16
Limonene	63–70	70–80	60–68	60–70	59–75
γ -Terpinene	8.3–9.5	6.5–8	8–12	8–12.8–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geraniol	1.0–2	0.5–0.9	0.8–2	0.6–2	0.5–2
Evaporation residue [weight %]					
	1.75–3.9		1.5–3.9	1.5–3.9	1.5–4
Carbonyl value					
	8–14	6.25–12	11–17	11–17	6–17
CD value	min. 0.2	min. 0.2	0.45–0.9	0.4–0.9	0.2–0.96

CITRUS PARADISI PEEL OIL

CAS 8016-20-4 ; EC /

Grapefruit oil, expressed

INCI: CITRUS GRANDIS OIL

Current regulation: II/358 R1

Clinical data: /

Additional information:

Citrus Paradisi Peel Oil is the volatile oil expressed from the peel of the Grapefruit, Citrus paradisi, Rutaceae
http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=55434

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

CITRUS SINENSIS (syn.: AURANTIUM DULCIS) CAS 97766-30-8, 8008-57-9, EC
 PEEL OIL EXPRESSED 307-891-8 (Orange, sweet,

(Sweet) Orange oil

Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 (Orange, sweet, ext.)

INCI names: CITRUS AURANTIUM DULCIS ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "orange oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to orange oil 2% pet. (22). The Rudzki 1976 study found 1 positive reaction in 200 patients to "sweet orange" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "sweet orange" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, neither positive nor irritant reaction to 1% and 5% "orange oil Bras." in pet., tested in 205 consecutive patients in Dortmund and Göttingen, were observed (15). The IVDK 2010 c study identified 0.2% positive reactions in 6246 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* (L.) Osbeck. For Oil of sweet orange (*Citrus sinensis* (L.) Osbeck), CAS 8008-57-9, obtained by mechanical treatment, an ISO norm exists: ISO 3140:2005. The oils have a high terpene hydrocarbon content (> 90%, mainly (+)-limonene. Important for aroma are aldehydes, mainly decanal and citral, and aliphatic and terpenoid esters. The sesquiterpene aldehydes alpha-sinensal [17909-77-2] and beta-sinensal [6066-88-8] contribute particularly to the special sweet aroma (34). According to (30) the maximum observed concentration in sweet orange oil are (in %): *limonene* (95.0); *linalool* (0.7); n-decanal (0.7); *citral* (0.3); alpha-sinensal (0.05); beta-sinensal (0.06) (30). Worldwide production is more than 30000 tons / year. Main uses comprise the flavouring of beverages and confectioneries and perfuming E.d.C, soaps and household products.

For the latter uses relevant here, both "Orange peel oil, sweet (*Citrus sinensis* (L.) Osbeck) (8008-57-9)", "Orange peel, sweet, extract (*Citrus sinensis* L. Osbeck) (8028-48-6)" and "Orange, sweet, Valencia, ext. (97766-30-8)" are among the top 100 used fragrance materials and classified as R43 (IFRA, pers. comm. 2010).

ORANGE OIL TERPENES (CAS # 68647-72-3) are a "top 100 mixture of substances and classified as R43 (IFRA, pers. comm.2010). Other names: ORANGE, SWEET, TERPENES (REACH); Terpenes and Terpenoids, sweet orange-oil (REACH). The CAS entry refers to a group of substances "Terpenes and Terpenoids, sweet orange-oil" (REACH).

CITRUS TANGERINA ...

Oil of tangerine

CAS 223748-44-5; EC /

[no info in CAS database]

Current regulation: /

Clinical data:

In a 17 year old girl, the perfume used for 3 months caused ACD due to the ingredient "oil of tangerine", with a strong positive PT reaction (to 2% or 10% in pet.; 50 controls

negative) (226).

Additional information:

Citrus Tangerina Peel Oil is the volatile oil expressed from the peel of the ripe fruit the Tangerine, *Citrus Tangerina*, *Rutaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55441>, last accessed 2010-01-29); (*Citrus tangerina* Tanaka).

CORIANDRUM SATIVUM HERB OIL

CAS 84775-50-8; EC 283-880-0
(Coriander, ext.)

Coriander oil

INCI: CORIANDRUM SATIVUM
EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “coriander” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “coriander” essential oil 2% pet. (27).

Additional information:

Coriander Sativum Herb Oil is an essential oil obtained from the herbs of the Coriander, *Coriandrum sativum* L., *Umbelliferae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39388>, last accessed 2010-01-29). The main component of coriander oil is linalool (by GC: 65-78%) and mono- and polyunsaturated fatty aldehydes contributing to the particular aroma. In contrast to the seed oil, coriander leaf oil contains these aldehydes as main constituents, e.g. 2-deccanal and 2-dodecanal (34). For Oil of coriander fruits (*Coriandrum sativum* L.) an ISO standard exists: ISO 3516:1997.

CYMBOPOGON OILS

Cymbopogon oils are produced from several aromatic grasses that belong to the genus *Cymbopogon* Speng. (*Poaceae*). The oils are obtained by steam distillation of the aerial parts of the plants (34).

The composition of the essential oil derived from *Cymbopogon flexuosus* (Nees ex Steudel) J.F. Watson is defined by a standard: ISO 4718:2004, as is the oil derived from *Cymbopogon citratus*: 3217:1974.

CYMBOPOGON CITRATUS LEAF OIL

Cymbopogon citratus (DC.) Stapf.²⁴

CAS 89998-14-1; EC 289-752-0
(*Cymbopogon citratus*, ext. =
INCI)

²⁴ ISO 4720:2009 nomenclature

Lemon Grass Oil; Indian Verbena Oil; Indian Melissa Oil

CYMBOPOGON SCHOENANTHUS OIL

Cymbopogon flexuosus (Nees ex Steudel) J.F. Watson²⁵

CAS 8007-02-1; EC 289-754-1 (oils, lemongrass) / 89998-16-3; EC 289-752-0 (Cymbopogon Schoenanthus, ext. = INCI)

Lemon Grass Oil

Current regulation: /

Clinical data:

The Frosch 2002 b study on 1606 consecutive patients reported 1.6% positive reactions to "lemongrass oil (East India), CAS 8007-02-1", PTed at 2% pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 3 positive reactions to lemongrass oil were observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=6 (0.8%) positive reactions to lemongrass oil 2% pet. (22). The IVDK 2010 c study identified 0.6% positive reactions in 2435 consecutively tested patients and 2.3% positive reactions in 8445 patients tested in the context of a special series (30).

Additional information:

Cymbopogon Citratus Leaf Oil is an essential oil obtained from the leaves of the Lemon Grass, *Cymbopogon citratus* (DC., ex Nees), *Poaceae*. It contains citral (75-85%), methylheptenone, citronellal, geraniol, limonene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39457>, last accessed 2009-11-12). According to Surburg/Panten, by GC: neral (31-40%), geranial (40-50%) (34).

Indian lemongrass oil is obtained by the so-called Indian variety of lemongrass, *Cymbopogon flexuosus* (Nees ex Steud.) Stapf. Content by GC: 25-35% neral, 35-47% geranial (34).

Cymbopogon Schoenanthus Oil is the volatile oil obtained by the steam distillation of fresh Lemon Grass, *Cymbopogon schoenanthus* (L.), *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75419>, last accessed 2009-11-12). According to (30) the maximum observed concentration in lemongrass oil are (in %): citral (85.0); geraniol (7.0); limonene (4.0); geranyl acetate (2.2); caryophyllene (1.6); trans-isocitral (1.4); 6-methyl 5-hepten-2-one (1.3); caryophyllene oxide (1.2); 4-nonanone (1); citronellol (0.8); eugenol (0.3); linalool (0.2) (also according to (227))

In a LLNA study by RIFM, the lemongrass oil as used was reported to contain 68.8% citral, 6.7% limonene, 6.1% geraniol, 2.2% geranyl acetate, 1.6% caryophyllene, 1.4% trans-isocitral, 1.3% 6-methyl 5-hepten-2-one, 1.2% caryophyllene oxide and 1% 4-nonanone, according to analyses of the supplier. The EC3 value was calculated to be 6.5% (227).

CYMBOPOGON MARTINI HERB EXTRACT

CAS 84649-81-0; EC 283-461-2 (Cymbopogon Martini, ext)

²⁵ ISO 4720:2009 nomenclature

INCI: CYMBOPOGON MARTINI OIL*Palmarosa oil*

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon martini* (Roxb.) Will. Watson var. *motia* and var. *sofia*. Cymbopogon Martini Herb Extract is an extract obtained from the herbs of the plant, *Cymbopogon martini*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39460>, last accessed 2009-11-24), namely, by steam distillation of wild or cultivated *Cymbopogon martini* (Roxb.) J.F. Wats., collected when in blossom (34). The main constituent is geraniol (72-94%) (34).

In a LLNA study by RIFM, the palmarosa oil as used was reported to contain 79.4% geraniol, 9.4% geranyl acetate and 1.9% caryophyllene, according to analyses of the supplier. The EC3 value was calculated to be 9.6% (227).

CYMBOPOGON NARDUS HERB OIL

CAS 89998-15-2; EC 289-753-6 (*Cymbopogon nardus*, ext. = INCI)

*Citronella Oil (Sri Lanka)***CYMBOPOGON WINTERIANUS HERB OIL**

CAS 91771-61-8; EC 294-954-7 (*Cymbopogon Winterianus*, ext. = INCI)

Citronella Oil (Java)

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 5 positive reactions in 200 patients to "citronella" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "citronella" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf. and *Cymbopogon winterianus* Jowitt, respectively. Cymbopogon Nardus Herb Oil is an essential oil obtained from the herbs of the plant, *Cymbopogon* (syn: *Andropogon*) *nardus* (L.), Gramineae. The Ceylon citronella oil contains geraniol (about 60%), citronellal (about 15%), camphene, limonene, linalool, borneol. According to Surburg/Panten, the Sri Lankan oil contains citronellal (3-6%), borneol (4-7%), citronellol (3-8.5%), geraniol 15-23%) and methyl isoeugenol (7.11%) (34).

The Java citronella oil contains 25-50% citronellal, 25-45% geraniol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=39469>, last accessed 2009-11-24). Cymbopogon Winterianus Herb Oil as a synonym for Java citronella oil is obtained from the herbs of the plant, *Cymbopogon winterianus*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=sea>

[rch.details&id=39472](#), last accessed 2009-11-24). This oil, produced in Taiwan and Java, contains citronellal (31-40%), geraniol (20-25%), citronellol (8.5-14%), geranyl acetate (2.5-5.5%), citronellyl acetate (2-4%) and many minor components. Annual worldwide production is currently at around 1000 t (34). For Oil of citronella, Sri Lankan type (*Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf.) an ISO standard exists: ISO 3849:2003, for Oil of citronella, Java type the ISO 3848:2001.

In a LLNA study by RIFM, the citronella oil as used was reported to contain 36.6% citronellal, 20.6% geraniol, 4.1% limonene, 3.7% geranyl acetate, 3.0% citronellyl acetate, 2.6% elemol, 2.2% beta-bourbonene, 1.9% delta-cadiene, 1.6% isopugenol I, 1.4% germacrene D and eugenol and linalol at < 1%, according to analyses of the supplier. The EC3 value was calculated as > 50 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

EUCALYPTUS SPP. LEAF OIL

CAS 92502-70-0; EC 296-357-7
(*Eucalyptus*, ext. = INCI)

Eucalyptus Oil

CAS 8000-48-4; EC / (Oils,
eucalyptus) INCI: *EUCALYPTUS*
GLOBULUS OIL

Current regulation: /

Clinical data:

In a study with 218 fragrance sensitive patients, 1.8% reacted positively to 10% eucalyptus oil (pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to "eucalyptus oil" was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to eucalyptus oil 2% pet. (22). The Rudzki 1976 study found 3 positive reactions in 200 patients to "eucalyptus" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "Eucalyptus" essential oil 2% pet. (27). The IVDK 2010 c study identified 0.2% positive reactions in 6680 patients tested in the context of a special series (30).

In a professional athlete, the use of an "analgesic and anti-inflammatory cream" over 2 years lead to ACD, which was attributed to eucalyptol (eucalyptus oil, 1% pet., 25 controls negative), the sole ingredient of the cream eliciting a positive PT reaction (228)

Additional information:

ISO 4720:2009 nomenclature: *Eucalyptus globulus* Labill. Eucalyptus oils are produced from plants belonging to the genus *Eucalyptus* (*Myrtaceae*), which includes about 500 species in Australia, the country of origin, alone. At present, few of the oils, which are used to characterise species, are commercially important (34). Some species are rich in 1,8-cineole (80-85% content). Other species contain less cineole, but 10-22% alpha-pinene. *E. citriodora* predominantly contains citronellal (min. 75% by GC), with some citronellol and isopulegol (5-10% each) (34). *E. dives* contains (-)-piperitone and 15-25% alpha-phellandrene (34). According to (30) the maximum observed concentration in eucalyptus oil are (in %): 1,8-cineole (58; 70-80 after rectification); α -pinene (22); limonene (8); para-cymene (5); trans-pinocarveol (5); aromadendrene (10); globulol (2.5) [the latter 2 components only traces after rectification] (30).

For Crude or rectified oils of *Eucalyptus globulus* (*Eucalyptus globulus* Labill.) an ISO standard exists: ISO 770:2002.

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL

CAS 8000-34-8; EC / (Oils, clove)

Clove oil

INCI: EUGENIA CARYOPHYLLUS OIL

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 19.3% of patients with known contact allergy to fragrance ingredients reacted positively to "clove bud oil" (10 % pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "clove oil" were observed (3). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with clove oil (2% pet.) 13.4% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.6% positive reactions 2% pet. (22). The Rudzki 1976 study found 2 positive reactions in 200 patients to "clove" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=12 (13.3%) positive reactions to "clove" essential oil 2% pet. (27). The IVDK 2010 c study identified 1.5% positive reactions 6893 patients tested in the context of a special series (30).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: cinnamon oil (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Syzygium aromaticum* (L.) Merr. & L. M. Perry syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison. Standards regarding the composition of clove oil are available: ISO 3141:1997, ISO 3142:1997, ISO 3143:1997. Clove oils are produced from the clove tree *Syzygium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Spreng.) Bullock ex S.G. Harrison]. The content of clove bud, clove leaf and clove stem oil has, with little variation, been determined by GC as 75-92% eugenol, 2-17% caryophyllene and 0.2-15% eugenyl acetate – the latter compound found in particularly high concentration in bud oil (34). According to another source, the following maximum content (%) has been observed regarding the constituents listed: eugenol (92,0);

caryophyllene (17); eugenyl acetate (15); isoeugenol (0.5) (30).

In a LLNA study by RIFM, the clove leaf oil as used was reported to contain 85.3% eugenol, 9.9% caryophyllene and 2.2% alpha humulene, according to analyses of the supplier. The EC3 value was calculated to be 7.1% (227).

EVERNIA FURFURACEA LICHEN EXTRACT

CAS 90028-67-4; EC 289-860-8
(*Evernia furfuracea*, ext. = INCI)

Tree moss extract

Current regulation: /

Clinical data:

The Larsen 1977 study in 20 “perfume-sensitive patients” yielded n=6 positive reactions with “treemoss abs. in benzyl benzoate, 5% petrolatum” (18). In the IVDK 2007 study, 2.7% (95% CI: 2.0 – 3.6%) of 1658 consecutive patients had a positive reaction to “tree moss absolute” (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to the allergen, tested at 2%, i.e., twice the commonly used concentration, and not in pet., but in diethylphthalate (6). The IVDK 2010 study, 6.02% (95% CI: 4.90 – 7.14%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7).

Additional information:

Syn.: *Pseudoevernia furfuracea* (L.) Zopf (53). The lichen grows on the bark of pine and fir trees. The extraction process with carbohydrate solvents yields a “concrete” (2-5% yield) which, in a next step eliminating waxy compounds, is extracted with warm alcohol and subsequent cooling, yielding an “absolute” (40-60% yield) (53).

EVERNIA PRUNASTRI

CAS 90028-68-5; EC 289-861-3
(*Evernia prunastri*, ext. = INCI)

Oak moss abs.

Current regulation: Annex III, part 1, n° 91

Clinical data:
In the “background information” section of the 1999 opinion, oak moss extract is classified as “most frequently reported allergen”; in consecutive PT patients, about 2.8% positive reactions had been reported (33). The German MAK commission has labelled oak moss extract as ‘sensitising to the skin’ (229).

Since the last SCCNFP-opinion of 1999, a “polymer based method” was developed to reduce the natural content of these two compounds from around 1 - several percent to < 75 ppm for atranol and < 25 ppm for chloratranol. However, PTing 14 subjects with previous positive PT reactions to the “oak moss” allergen preparation with the modified *Evernia prunastri* material still elicited positive reactions in 8/14 subjects; thus, the reduction in allergen content was deemed unsafe for the consumer (230). In a study of 885 consecutive eczema patients tested in Gentofte, Denmark, 3.2% had a positive or follicular patch test response to oak moss absolute. Two types of oak moss absolute were tested, one contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types of oak moss absolute

(231). The IVDK 2007 study yielded 2.2% (95% CI: 1.6 – 3.0%) positive reactions in 2063 consecutively tested patients (4). In the Groningen 2009 study, 1.9% (95% CI: 0.7 – 4.0%) had positive reactions to oak moss, tested at 2% pet., i.e., twice the commonly used concentration (6). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reaction (13) (test concentration 2% pet.). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.0% positive reactions (22). The IVDK 2010 study, 1.81% (95% CI: 1.07 – 2.56%) of 1213 consecutively tested patients reacted to the compound, while 5.59% (95% CI: 4.90 – 6.27%) of 4482 of patients tested in a more aimed manner, partly as breakdown testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with *E. prunastri* extract, yielding 2 positive reactions (48).

L. Kanerva et al. report on a 41 year old female hairdresser in whom oak moss abs. contained in a perming solution (concentration in the product unknown) was unequivocally identified as allergen causing (i) occupational hand dermatitis and (ii) scalp dermatitis after application to the own hair (232). Another case of occupational hand dermatitis in a grinding engineer was, at least partly, attributable to contact sensitisation to "oak moss resin" contained in a soluble oil (233).

Additional information:

Source: *Evernia prunastri* (Oak moss) (*Evernia prunastri* var. *prunastri* L. Ach). Oak moss is extracted as described above. Chloratranol and atranol are the degradation products of chloratranorin and atranorin, resp., which are recognised as the main sensitisers in *Evernia prunastri* extracts.

ILLICIUM VERUM FRUIT OIL

CAS 84650-59-9, 8007-70-3; EC 283-518-1

"Anise Oil", Star anise oil

(Star anise, *Illicium verum*, ext. = INCI)

Current regulation: /

Clinical data:

In a study involving 100 consecutive patients, Rudzki and Grzywa found (i) a relatively high frequency of active sensitisation to star anise oil (n=5) tested with 0.5, 1 and 2% concentration (most likely in yellow petrolatum, as the other allergens in this series). Later patch testing with constituents of this essential oil (1%) in 3 patients yielded positive results to anethole in 3 cases, and to alpha-pinene and safrole in the 1 case tested to these substances. 34% of the consecutive patients reacted positively to star anise oil at 1%, which was considered as (marginally) non-irritating PT concentration (234).

Additional information:

ISO 4720:2009 nomenclature: *Illicium verum* Hook. f. *Illicium Verum* Fruit Oil is an essential oil distilled from the fruits of the Star Anise, *Illicium verum*, Illiciaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40297>, last accessed 2010-01-29). The main component is trans-anethole (86-93%), which can be purified from star anise oil. Main uses are alcoholic beverages, food flavouring and oral care products (34, 39). For Oil of star anise, Chinese type (*Illicium verum* Hook. f.) an ISO standard exists: ISO 11016:1999.

JASMINUM GRANDIFLORUM FLOWER EXTRACT	CAS 84776-64-7; EC 283-993-5 (Jasmine, <i>Jasminum grandiflorum</i> , ext. = INCI)
<i>Jasmine abs.</i>	
JASMINUM OFFICINALE FLOWER OIL	CAS 90045-94-6; EC 289-960-1 (Jasmine, <i>Jasminum officinale</i> , ext. = INCI)
JASMINUM OFFICINALE OIL	CAS 8022-96-6; EC / (Oils, jasmine) INCI: JASMINUM OFFICINALE OIL

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 1.2% of 1606 consecutive patients had a positive PT to "jasmine absolute", tested 5% in pet. (17). The deGroot 2000 study yielded 13 positive reactions to "jasmine, synthetic" in 1825 consecutively tested patients (12). In the early Larsen 1977 study, 18 of 20 "perfume sensitive patients" reacted to "Jasmin synthetic" 10% pet. (18), while 7 reacted to "Jasmin absolute" (10% pet.) – all of these also positive to the synthetic fragrance. The Sugiura 2000 study set in Nagoya, Japan, yielded 1% positive PT reactions in 1483 patients PTed for suspected cosmetic dermatitis, using 5% pet. as test concentration (14). The Larsen 2001 study in 178 patients with known contact allergy to fragrance ingredients found 16.9% positive reactions to jasmine absolute (10% pet.) (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to Jasmine officinale oil (Jasmine absolute, Egyptian), tested at 2% (13). In the NACDG 2009 study, 1.1% of 4447 patients tested with "Jasmine absolute 2% pet." were found PT-positive (21). The Belsito 2006 study (20) yielded 0.4% positive reactions to "jasmine absolute". The Goossens 1997 study found 5 of 111 patients positive to "jasmine absolute" (10% pet.) – all sensitised to other fragrance allergens (23). In 63 patients positive to the FM I, 13 had positive PT reactions to "jasmine absolute", 2% pet., and 12 to "jasmine synthetic", 2% pet. in the Santucci 1987 study – the amount of concomitant reactivity was not examined (28). Nakayama et al. found 1974 (after (29)) 19 "strong positive" and 25 "weak positive" reactions to "jasmin oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.5% positive reactions in 3668 consecutively tested patients and 1.2% positive reactions in 982 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with jasmine absolute, yielding 3 positive reactions, and with "Jasmine synthetic", also resulting in 3 positive reactions (48).

Additional information:

Jasminum Grandiflorum Flower Extract is an extract obtained from the flowers of the Spanish Jasmine, *Jasminum grandiflorum* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39752>, last accessed 2009-11-12).

Jasminum Officinale Flower Oil is an essential oil obtained by molecular distillation of the flowers from the Jasmine, *Jasminum officinale* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39754>, last accessed 2009-11-25).

Jasminum Officinale Oil is the volatile oil obtained from the flowers of the Jasmine, *Jasminum officinale* L., Oleaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=34776>, last accessed 2010-01-05); this latter extract is used by Almirall/Hermal/Trolab for the preparation of a PT allergen.

Jasmine absolute is obtained by solvent extraction, via concrete, from the flowers of *J. grandiflorum* (L.) Aiton from China and India. The main volatile compound is benzyl acetate, however, minor compounds such as indole [120-72-9], cis-jasmone [488-10-8] and methyl jasmonate [1211-29-6] contribute to the typical jasmine fragrance (34). Reported compounds include the following (maximum observed concentration given in parentheses): benzyl acetate (28); benzyl benzoate (24.0); phytol acetate (9); isophytol (8.5); phytol (7.4); linalool (7.0); eugenol (4.0); squalene (4); indole (3.5); benzyl alcohol (2.5); cis-jasmone (2.5); methyl linolenate (2.0); methyl palmitate (1.4); p-cresol (1.0); cis-3-hexenyl benzoate (1.0); benzyl salicylate (0.4); jasmin lactone (0.9); methyl jasmonate (0.7); isoeugenol (0.4) ((30), also according to (17))

JUNIPERUS VIRGINIANA OIL

CAS 8000-27-9; EC / (Oils, cedarwood) [this also refers to *Cedrus atlantica* ...] / 85085-41-2; EC 285-370-3 (Juniper, *Juniperus virginiana*, ext. = INCI)

JUNIPERUS VIRGINIANA WOOD OIL

CAS 85085-41-2; EC 285-370-3

Cedar Wood Oil (Virginian)

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 0.6% of 1606 consecutive patients had a positive PT to "cedarwood oil (Moroccan and Chinese 1:1)", tested 10% in pet. (17). After application of Penaten-baby™ oil as immersion oil for dermatoscopy a patient developed multiple patches of eczema at the application sites. Investigation revealed that the oil was kept in a bottle previously used for *Juniperus virginiana* oil, to which contact sensitisation was verified by patch testing (235).

Additional information:

ISO 4720:2009 nomenclature: *Juniperus virginiana* L.. *Juniperus Virginiana* Oil is the volatile oil obtained from the fruits and leaves of the Red Cedar, *Juniperus virginiana* L., Cupressaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=78070>, last accessed 2010-01-05)

Juniperus Virginiana Wood Oil is an essential oil obtained from the wood and twigs of the Red Cedar, *Juniperus virginiana* L., Cupressaceae. It contains chiefly (alpha and beta) cedrene and cedral (cedar camphor), cuparene, thujopsene, widdrol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39767>, last accessed 2009-11-12)(235). According to Surburg/Panten by GC: alpha-cedrene 22-35%, thujopsene 10-25%, cedrol 16-25% (34).

See also *Cedrus atlantica*. According to (30) the maximum observed concentration in cedar wood oil are (in %): α-cedrene (32); thujopsene (25); cedrol (25); β-cedrene (6); widdrol (5) and cuparene (traces) (30).

For Oil of cedarwood, Virginian (*Juniperus virginiana* L.) an ISO standard is available: ISO 4724:2004. For Oil of cedarwood, Texas (*Juniperus mexicana* Schiede) an ISO standard exists: ISO 4725:2004.

LAURUS NOBILIS OIL

CAS 8002-41-3; EC / (Oils, laurel)
 INCI: LAURUS NOBILIS OIL /
 8007-48-5; EC / (Oils, sweet
 bay)/ 84603-73-6; EC 283-272-5
 (Laurus nobilis, ext.) INCI:
 LAURUS NOBILIS EXTRACT

Laurel oil

Current regulation: Annex II, n° 359 (seed oil)

Clinical data:

In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to "laurel oil" 2% pet. (22).

After sensitisation by a one-time occlusive application a 36 year old Turkish patient developed widespread allergic contact dermatitis 3 days after massage with olive oil containing Laurus nobilis oil; sensitisation was proven by a strong positive reaction to the commercial test preparation and the massage oil previously used (236). Topical application of laurel oil for knee arthropathy led to an erythema exudativum multiforme-like rash on the legs of a 63 year old patient; interestingly, laurel oil yielded a "target like" strongly positive PT reaction in this case (237). In an earlier Turkish case with a similar history, the EEM-like appearance was lacking; however, a very intense, edematous reaction was noted (238). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "laurel oil" were observed (3). The IVDK 2010 c study identified 1.0% positive reactions in 6297 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Laurus nobilis* L. Laurel leaf oil is obtained by steam distillation of leaves from *Laurus nobilis* L. (Lauraceae), an evergreen cultivated primarily in the Mediterranean countries. The main components are 1,8-cineole (30-70%), linalool (about 10%) and eugenol (34). According to (30) the maximum observed concentration in laurel oil are (in %): 1,8-cineole (70); β -caryophyllene (11); linalool (11); limonene (5.0); eugenol (2.0); geraniol (0.3) (30).

LAVANDULA HYBRIDA HERB OIL

CAS 91722-69-9; EC 294-470-6
 (Lavender, *Lavandula hybrida*,
 ext. = INCI)

Lavandin Oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "lavandin" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "lavandin" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% lavandin oil in pet., tested in 205 consecutive patients in Dortmund and Göttingen, and just 1 irritant reaction to

the higher concentration, were observed (15).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. *Lavandula* *hybrida*, *Labiatae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39789>, last accessed 2010-01-29. Nomenclature according to Surburg/Panten: *Lavandula* x *intermedia* Lois, which is a hybrid of lavender and spike (see below) (34). The oils from the most important variants, abrial and grosso, contain linalool (24-38%), linalyl acetate (20-38%) as well as 1,8-cineole (4-11%), and camphor (6-11%) (34). A third variant is called super because of its high concentration of linalyl acetate (35-47%), more closely resembling lavender oil (34). For Oil of lavandin Grosso (*Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik.), French type an ISO standard exists: ISO 8902:2009, for Oil of lavandin Abrial (*Lavandula angustifolia* Miller x *Lavandula latifolia* Medikus), French type a different ISO standard: ISO 3054:2001.

It is a "top 100" substance (IFRA, pers. comm.2010)

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LAVANDULA OFFICINALIS FLOWER OIL

CAS 84776-65-8, 8000-28-0; EC 283-994-0 (*Lavender*, *Lavandula angustifolia angustifolia*, ext. = *INCI*)

Lavender oil

Current regulation: /

Clinical data:

In a large series from Nagoya, Japan, 1483 patients were tested with lavender oil 20% in pet., with overall 3.7% positive reactions from 1990 to 1998. However, within this period, a sharp increase was noted in 1997 and 1998, which as attributed to changed exposure by M. Sugiura et al. (14). On the individual level, relevance of positive reactions remained unclear in about half of the cases. The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lavender absolute" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Lavandula augustifolia oil" (Lavender absolute) 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "lavender oil 20% pet." – all of them sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "lavender" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "lavender" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 6 "strong positive" reactions to "Lavender oil" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with "lavender absolute", yielding 2 positive reactions (48).

R. Goiriz et al. report on a case of photo contact allergy (10 controls negative) in a 45 year old woman developing after application of a ketoprofen-containing topical gel ("Fastum") (239). A physiotherapist developed acute, recurrent dermatitis after use of "Difflam® gel", scented with lavender oil. Both the gel and lavender oil (2% pet.) tested positive; avoidance resulted in clearing (240). In a study on 218 patients with known

contact allergy to fragrance ingredients, Larsen (2002 c) found positive reactions to 10% lavender oil (pet.) in 2.8% of these (1). A case of vulvovaginitis with spread and affecting the dominant hand applying various tea tree and lavender oil creams was reported by S. Varma; the PT with 10% lavender oil abs. in pet. (50 controls negative) was positive (241). In two cases, facial "pillow dermatitis" due to lavender oil, applied to the pillows, developed, confirmed by positive PT to lavender abs. (2% pet.) (242).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. *Lavandula officinalis* Flower Oil is an essential oil obtained from the fresh flowering tops of the Lavender, *Lavandula officinalis* (syn: *L. vera*), *Labiatae*. It contains 30-40% esters calculated as linalyl acetate, linalool, pinene, limonene, geraniol, some eucalyptol (cineol) (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40370>, last accessed 2009-11-09). According to Surburg/Panten, lavender oil is obtained by steam distillation of freshly cut flowering tops of *Lavandula angustifolia* Mill. (Lamiaceae). Main constituents according to GC are linalyl acetate (25-45%), cis-ocimene (4-10%), trans-ocimene (1.5-6%), 1,8-cineole ($\leq 1\%$) camphor ($\leq 0.5\%$), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] ($\geq 2\%$) (34).

In addition to distillation, both *Lavandula officinalis* and Lavandin are also solvent extracted, yielding concretes and, after ethanol extraction, absolutes, which are said to have a longer-lasting odour (34).

For Oil of lavender (*Lavandula angustifolia* Mill.) an ISO standard exists: ISO 3515:2002.

LAVANDULA SPICA HERB OIL

CAS 97722-12-8; EC 307-762-6
(Lavender, *Lavandula spica*, ext.
= INCI

"Spike Oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "spike" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=8 (9.3%) positive reactions to "spike" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula latifolia* Medik. *Lavandula Spica* Herb Oil is an essential oil distilled from the flowering herbs of the Spikenard, *Lavandula spica* (syn: *Lavandula latifolia*), *Labiatae*. It contains eucalyptol (35%), camphor, linalool, borneol, terpineol, D-camphene and sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40372>, last accessed 2010-01-29). According to Surburg/Panten, Spanish spike lavender oil is steam distilled from the flowering tops of *Lavandula latifolia* Medik.. The main components are linalool (34-50%), 1,8-cineole (16-39%) and camphor (8-16%) (34). For Oil of spike lavender (*Lavandula latifolia* (L.f.) Medikus), Spanish type an ISO standard exists: ISO 4719:1999

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LITSEA CUBEBA FRUIT EXTRACT

CAS 90063-59-5, 68855-99-2; EC 290-018-7 (*Litsea cubeba*, ext.)
INCI: LITSEA CUBEBA OIL

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 3 positive reaction in 200 patients to “*Litsea cubeba*” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to this essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Litsea cubeba* (Lour) Pers. *Litsea Cubeba* Fruit Extract is an extract obtained from the fruits of the plant, *Litsea cubeba*, Lauraceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40036>, last accessed 2009-11-24. The content by GC is: neral (25-33%), geranial (38-45%) – i.e. about ¾ citral, for which the extract had previously served as a raw material (34); direct use for perfuming is limited to household products (39). For Oil of *Litsea cubeba* (*Litsea cubeba* Pers.) an ISO standard exists: ISO 3214:2000.

In a LLNA study by RIFM, the “*Litsea cubeba* oil” as used was reported to contain 85.7% citral, 2.9% limonene, 1.7% linalool, 1.4% citronellal and < 1% caryophyllene and methyl heptanone, according to analyses of the supplier. The EC3 value was calculated as 8.4 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

MENTHA ARVENSIS LEAF OIL

CAS 68917-18-0 ; EC /

Cornmint oil

INCI: MENTHA ARVENSIS OIL

Current regulation: /

Clinical data: /

Additional information:

Mentha Arvensis Leaf Oil is the oil derived from the leaves of the Horse Mint, *Mentha arvensis* L., Labiatae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=57860)

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

MENTHA PIPERITA OIL

CAS 8006-90-4; EC / (Oils, peppermint) INCI: MENTHA

*PIPERITA OIL / 84082-70-2; EC
282-015-4 (Peppermint, ext.) INCI
names: MENTHA PIPERITA ...*

Peppermint oil

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% of 1606 consecutive patients reacted positively to "peppermint oil (American)", tested 2% in pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "peppermint oil" were observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to peppermint oil 2% pet. (22). Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 6 patients had positive (+ to +++) PT reactions to "peppermint oil" 1% pet. at D4, mostly combined with positive reactions to menthol (see above) and reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). The Rudzki 1976 study found 1 positive reaction in 200 patients to "Peppermint" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=6 (6.9%) positive reactions to "peppermint" essential oil 2% pet. (27). In 63 patients positive to the FM I, 3 had positive PT reactions to peppermint oil, 2% pet., in the Santucci 1987 study (28). The IVDK 2010 c study identified 0.6% positive reactions in 6546 patients tested in the context of a special series (30).

An unusual case of "baboon-like" allergic contact dermatitis of the vulva after drinking excessive amounts of a herbal tea containing, among other ingredients, peppermint. While the PT reaction to peppermint oil was only weak to doubtful, dramatic improvement after cessation and prompt relapse after repeat ingestion proved the diagnosis (243). Recurrent foot and lower leg dermatitis after the application of a "foot spray" (containing peppermint oil) was diagnosed as allergic contact dermatitis due to this ingredient in a 59 year old golf player (244). In another case, ACD after application of a transdermal system for the treatment of lumbar pain was attributed to CA to peppermint oil (2% pet.) and its main ingredient menthol (1% pet.) (155). In a patient with toothpaste-induced cheilitis, not only *M. piperita*, but also *M. arvensis*, but not *M. spicata* or *cardica* extracts (all tested 1% pet.), as well as natural and synthetic menthol caused positive PT reactions (245).

Additional information:

ISO 4720:2009 nomenclature: *Mentha x piperita* L. A standard by ISO exists for Oil of peppermint (*Mentha x piperita* L.): ISO 856:2006. A review by the Cosmetic Ingredient Review Expert Panel, Washington, DC on the "Final report on the safety assessment of *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water" is available (163), stating that "Peppermint Oil is used at a concentration of < or = 3% in rinse-off formulations and < or = 0.2% in leave-on formulations. Peppermint Oil is composed primarily of menthol and menthone. Other possible constituents include pulegone, menthofuran, and limone. According to Surburg/Panten: (-)-menthol (34-46%), (-)-menthone (15-27%), (-)-menthyl acetate (2.5-7%) and menthofuran [17957-94-7] (0.5-6%) (34). According to (30) the maximum observed concentration in peppermint oil are (in %): (-)-menthol (49); (-)-menthone (28); (-)-menthyl acetate (8); mentofuran (8); isomenthone (8); neo menthol (6); pulegone (3.5); limonene (3.0); linalool (0.4) (30). Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. ... Repeated

intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits” concluding that “with the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations”.

MENTHA SPICATA HERB OIL

CAS 84696-51-5, 8008-79-5; EC 283-656-2 (Spearment, ext.)

Spearment oil

INCI: MENTHA VIRIDIS EXTRACT

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to “spearment oil (American)”, tested 2% in pet. (17). The CAS # quoted (8008-79-5) refers, according to CosIng, to MENTHA VIRIDIS LEAF OIL, the volatile oil obtained from the dried tops and leaves of the Garden Mint, *Mentha viridis* L., Labiatae. The Larsen 2001 study diagnosed 5.0% positive reactions in 178 patients with known contact allergy to fragrance ingredients, using this oil at 5% pet. test concentration (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to “*Mentha viridis* oil” 5% (13). PT results with toothpaste ingredients were positive in 7 patients, of whom 4 had strong positive reactions to spearment (246).

Additional information:

ISO 4720:2009 nomenclature: *Mentha spicata* L. *Mentha Spicata* Oil is an essential oil obtained from the herbs of the Spearment, *Mentha spicata* L., Labiatae (syn: *Mentha viridis* L., Labiatae). It contains carvone (more than 50%), limonene, pinene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40394>, last accessed 2009-11-11). According to Surburg/Panten, the content is limonene (9-16.5%), (-)-carvone (60-70%), menthone (0-0.2%) and viridiflorol (0-0.5%) (34). Exposure by toothpastes, and subsequent contact allergic reaction of the lips or the oral mucosa, have been reported (e.g., (247, 248)). L-Carvone is a component of the oil from *Mentha spicata* (spearment) (53) and had been tested with positive results in “toothpaste cases”, even at a concentration as low as 0.067% (68).

For Oil of spearment -- Part 1: Native type (*Mentha spicata* L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearment -- Part 2: Chinese type (80 % and 60 %) (*Mentha viridis* L. var. *crispa* Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearment -- Part 3: Indian type (*Mentha spicata* L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearment -- Part 4: Scotch variety (*Mentha x gracilis* Sole): ISO 3033-4:2005.

MYROXYLON PEREIRAE RESIN

CAS 8007-00-9; EC 232-352-8 (Balsams, Peru)

Balsam of Peru

INCI: MYROXYLON PEREIRAE / Balsams, Peru

Current regulation: Annex III, part1, n° 154

Clinical data:

This natural mixture has been employed as screening agent in Baseline series worldwide for many decades. Hence, a wealth of data is available; table 3.2 – 1 summarises results of the past 10 years.

Additional information:

ISO 4720:2009 nomenclature: *Myroxylon pereirae* (Royle) Klotzsch, syn. *Myroxylon balsamum* var. *pereirae* (Royle) Harms. *Myroxylon pereirae* resin (MPR, Balsamum peruvianum) is harvested from the balsam of Peru tree, *Myroxylon balsamicum* (L.) HARMS var. *pereirae* (ROYLE) HARMS, synonymous *Myroxylon pereirae* (ROYLE) KLOTZSCH (249) after thermal stress, almost exclusively in El Salvador. Main constituents of the pleasantly, vanilla-like smelling dark brown liquid are benzyl esters of cinnamic and benzoic acid (35 – 75%), up to 30% cinnamic acid, up to about 10% benzoic acid, approximately 5% alpha- and beta-nerolidol, benzyl alcohol and mostly less than 1% cinnamyl alcohol, benzyl ferulate and -isoferulate, cinnamic acid amyl ester, coniferyl alcohol, coniferyl benzoate, eugenol, isoeugenol, farnesol, vanillin, and several trace constituents (250-253). The composition of MPR varies with the origin and other factors; moreover, MPR is sometimes blended with other natural mixtures such as turpentine, styrax or colophonium (249).

MPR can be used to improve taste or smell in gargling solutions, cosmetic products such as soaps, shampoo or lipsticks, as well as sweets, tobacco and beverages (249, 254). According to EU legislation and IFRA guidelines MPR should not be used in products intended for skin contact; however, extracts and distillates of MPR may be used in a concentration of < 0,4% (IFRA-Guidelines, www.ifraorg.org (255)). E. Temesvári et al. report on the interesting case of severe ACD with subsequent hypopigmentation after a “temporary henna tattoo”, which was, unexpectedly, not due to p-phenylene diamine, but to the oil used to disperse the pigment, which presumably contained allergens also included in the FM I and MPR, both of which were extreme positive on a later PT (256).

In addition to delayed type hypersensitivity reactions, MPR (and some of his constituents such as benzoic acid (257)) are capable of eliciting (non-immunological) urticarial immediate reactions (258-260). In one case, the immediate reaction to MPR (and to FM I) at the test site spread systemically in terms of a generalised urticaria, while no delayed type reactions were observed to the PT (261). Generally, there is apparently no association of immediate reactions to MPR (and cinnamal or cinnamyl alcohol) and contact sensitisation to these compounds (262). In animal experiments the sensitising potency of MPR was clearly established (250), with coniferyl benzoate identified as single compound with the most marked potency (252). However, due to the limited chemical stability of this compound it is unclear whether other, more stable compounds are, in fact, more important allergens, such as cinnamic acid and (iso-) ferulic acid esters or oxidised constituents of the resin fraction (263).

Table 3.2.2 – 1: Results with contact allergy to fragrance ingredients screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Myroxylon pereirae resin** (Balsam of Peru) 1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the reviewers.

Country	Population	Years	No. tested	Crude % positive (95% CI) §
Tel Aviv, Israel (264) #	Consecutive patients	1999-2000	943	6.6 % (5.1 – 8.4) §
South Korea (13)	Consecutive patients	04/2002 – 06/2003	422	7.3% (5.1 – 10.3%) §

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Tel Aviv, Israel (265)	Consecutive patients	1998-2004	2156	3.6 (2.9 – 4.5) § %
Manipal, India (266)	Dermatitis patients	1989-1998	1780	n=17
Tehran, Iran (267)	Consecutive patients	2002-2004	250	2.4 (0.9 – 5.2) § %
Sevilla, Spain (268)	Consecutive patients	2002-2004	863	5.8 (4.3 – 7.6) § %
Ankara, Turkey (269)	Consecutive patients	1992-2004	1038	2.1 (1.3 – 3.2) § %
Vienna, Austria (22)	Consecutive patients of one clinic	1997-2000	2660	5.4% (4.6 – 6.3%) §
Czech Republic (270)	Consecutive patients	1997-2001	12058	7.3% (6.8 – 7.8) §
Copenhagen, Denmark (271)	Consecutive patients	1985-2007	16173	3.9 (3.6 – 4.2) § %
Sweden (272)	Consecutive patients	2000	3790	6.5%
9 European countries (273) §	Consecutive patients	2002-2003	9672	6.1 %
Germany, 3 Swiss + 1 Austrian Dept. (7)	Consecutive patients	2005-2008	36919	8.0% (7.7 – 8.3%)
10 depts. From 7 EU countries (274) *	Consecutive patients	1996-2000	26210	6.0 %
USA (Canada) (20)	Probably consecutive patients	2003	1603	6.6%
NACDG 2009 (21)	Consecutive patients	2005-2006	4449	11.9%

§ Calculated by reviewers, where possible (if actual numbers were given)

Probably included in (265)

\$ > 5-fold difference between departments

* About 4-fold difference between departments

NARCISSUS SPP. EXTRACT / OIL

CAS: diverse

Narcissus abs.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 1.3% positive reactions to “narcissus absolute” (2% pet.) were observed in 1606 consecutive (17). The extract used by the PT allergen provider Almirall/Hermal/Trolab has the CAS number 90064-25-8. The IVDK 2010 c study identified 0.5% positive reactions in 2445 consecutively tested patients and 0.6% positive reactions in 809 patients tested in the context of a special series (30).

Additional information:

Commonly used: *Narcissus poeticus* L. According to (30) the maximum observed concentration in Narcissus abs. are (in %): α -terpineol (23.7); trans-Isoeugenol methyl ether (20); benzyl benzoate (20); coumarin (5.7); benzyl alcohol (4.0); Δ^3 -carene (3.4); cinnamyl alcohol (2.5); phenylethyl alcohol (2.2); ethyl palmitate (2.2); phenylpropyl acetate (1.7); 1,8-cineole (1.5); caryophyllene (1.0); benzyl acetate (0.7); isoeugenol (0.5); farnesol (0.3) (also according to (17)) (30).

OCIMUM BASILICUM HERB OIL

CAS 84775-71-3; EC 283-900-8 (*Ocimum basilicum*, ext. = INCI)

Basil Oil (sweet)

Current regulation: /

Clinical data:

/

Additional information:

ISO 4720:2009 nomenclature: *Ocimum basilicum* L. For Oil of basil, methyl chavicol type (*Ocimum basilicum* L.) an ISO standard exists: ISO 11043:1998. *Ocimum Basilicum* Herb Oil is an essential oil obtained from the herbs of the Sweet Basil, *Ocimum basilicum* L., *Labiatae*. (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40474>, last accessed 2009-11-24). The chemical composition varies greatly with the origin (34):

- Basil oil of the methylchavicol type (Réunion type) is extracted from flowering tops or whole plants from Réunion, Comores, Madagascar, but also other countries such as Egypt. Mainly used for seasoning food. Content by GC: methylchavicol 75-87%, linalool 0.5-3%
- Basil oil, linalool type is produced mainly in the Mediterranean area. Content by GC: Linalool 45-62%, methylchavicol trace to 30%, eugenol 2-15%
- Indian Basil oil is produced exclusively in India. Content by GC: methylchavicol trace to 70%, linalool 25%.

In a LLNA study by RIFM, the basil oil as used was reported to contain 51% linalool, 10.4% eugenol, 7.7% cineol, 3.7% bergamotene, 2.7% germacrene D, 2.7% cadinol and 1.3% cadinene, according to analyses of the supplier. The EC3 value was calculated to be < 2.5% (227).

PELARGONIUM GRAVEOLENS FLOWER OIL

CAS 90082-51-2; EC 290-140-0 (*Pelargonium graveolens*, ext. = INCI) / 8000-46-2; EC / (Oils, geranium) INCI: GERANIUM

Geranium Oil Bourbon

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "geranium oil Bourbon" (2% pet.) 7.4% positive reactions (9). In the Larsen 2001 study, 8.4% positive reactions were observed in 178 patients with known contact allergy to fragrance ingredients ("geranium oil Bourbon", 10% pet.) (19). The Goossens 1997 study found 3 of 111 patients positive to "geranium oil 20% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 3 positive reactions in 200 patients to "geranium" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "geranium" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 3 "strong positive" reactions to "Geranium oil" (unknown test concentration) in 183 patients, Trattner/David 1 / 641 consecutive patients positive to "Geranium oil" (31). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to geranium oil bourbon (48).

Additional information:

ISO 4720:2009 nomenclature: *Pelargonium x ssp.* For Oil of geranium (*Pelargonium X ssp.*) an ISO standard exists: ISO 4731:2006 *Pelargonium Graveolens* Flower Oil is the volatile oil obtained from the flowers of the Bourbon Geranium, *Pelargonium graveolens* L. Hér. Ex Aiton, *P. roseum* Willdenow (and other nondefined hybrids that have developed in different regions of the world) Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=57527>, last accessed 2009-11-16)(34). The Bourbon type (Réunion, Madagascar) is more valuable than the North African and Chinese products, and differs in characteristic components: (-)-6,9-guaiadiene [36577-33-0] 5-9% in the Bourbon type, and 10-epi-gamma-eudesmol [15051-81-7] 3-6% in the African type, in addition to the main components (-)-citronellol, isomenthone, formates and tiglates. Chinese oil is similar to Bourbon oil, however, it contains more citronellol (32-43%) and lower amounts of linalool (2-4.5%) and geraniol (5-12%) (34).

In a LLNA study by RIFM, the geranium oil as used was reported to contain 41.1% citronellol, 9.8% 2,6-guiadine, 6.2% isomethone, 4.9% geraniol, 2.2% cis-rose oxide, 2.1% linalool, 1.5% geranyl formate, 1.3% phenyl ethyl tiglate, 1.0% trans-rose oxide, and geranyl tiglate and alpha-pinene at < 1%, according to analyses of the supplier. The EC3 value was calculated to be > 50% (227).

PELARGONIUM ROSEUM LEAF OIL

CAS 90082-55-6; EC 290-144-2
(*Pelargonium roseum*, ext. =
INCI)

Geranium Oil; Rose Geranium Oil

Current regulation: /

Clinical data:

In the Sugiura 2000 study, among 1483 patients with suspected cosmetic dermatitis, 2.1% positive PT reactions to "geranium oil" (tested 20% in pet.) were observed (14).

Additional information:

Pelargonium Roseum Leaf Oil is an essential oil obtained from the leaves of the plant, Pelargonium roseum, Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40565>, last accessed 2009-11-16).

PIMENTA RACEMOSA LEAF/FRUIT OIL

CAS 85085-61-6; EC 285-385-5

Bay oil (34)

Current regulation: /

Clinical data:
/

Additional information:

ISO 4720:2009 nomenclature: *Pimenta racemosa* (Mill.) J.W. Moore. For Oil of bay [*Pimenta racemosa* (Mill.) J.W. Moore] an ISO standard exists: ISO 3045:2004 Pimenta Racemosa Leaf/Fruit Oil is an essential oil obtained from the fruits of the plant, Pimenta racemosa, Myrtaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41014>, last accessed 2010-02-10).

Steam distillation of the leaves of Pimenta racemosa (Mill.) J.W. Moore (Myrtaceae) yields bay oil, which consists of myrcene (20-30%), eugenol (42-56%) and chavicol (8-13%) (34).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

Pinus mugo leaf and twig oil and extract

CAS 90082-72-7, 8000-26-8; EC 290-163-6

Dwarf pine needle oil
(German: Latschenkiefernöl)

Current regulation: Annex III, part 1, 109

Clinical data:

In the Frosch 2002 b study, 0.7% positive reactions to dwarf pine needle oil (2% pet.) were observed in 1606 consecutive (17). The Rudzki 1976 study found 4 positive reactions in 200 patients to "Pine needle" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "pine needle" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Pinus mugo* Turra syn. *Pinus montana* Mill.) Pinus Mugo Twig Oil is an essential obtained from the twigs of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09). Pinus Mugo Twig Leaf Extract is an extract obtained from the twigs leaves of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09).

[h.details&id=41473&back=1](#), last accessed 2010-03-09).

Dwarf pine needle oil is obtained from *Pinus mugo* Turra subsp. *mugo* and subsp. *pumilio* (Haenke) Franco (34). For Oil of dwarf pine (*Pinus mugo* Turra) an ISO standard exists: ISO 21093:2003. American pine oils contain almost no 3-carene or camphene (34).

PINUS PUMILA TWIG LEAF EXTRACT / OIL

CAS 97676-05-6; EC 307-681-6
(Pine, *Pinus pumila*, ext. = INCI)

Dwarf pine needle oil

Current regulation: Annex III, part 1, 114

Clinical data: /

Additional information:

Pinus Pumila Twig Leaf Extract obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41483&back=1>, last accessed 2009-11-12), *Pinus Pumila* Twig Leaf Oil is the essential oil obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41484&back=1>, last accessed 2009-11-12). Main constituents are alpha-pinene (60-70%) and beta-pinene (20-25%). (34) Occurrence from Siberia to Japan, classified as Endangered Species

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

POGOSTEMON CABLIN OIL

CAS 8014-09-3; EC / (Oils, patchouli) / 84238-39-1; EC 282-493-4 (Patchouli, ext.)

Patchouli oil

INCI: POGOSTEMON CABLIN / Patchouli, ext.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% positive reactions to patchouli oil (10% pet.) in 1606 consecutive were observed (17). Nakayama et al. found 1974 (after (29)) 3 “strong positive” and 8 “weak positive” reactions to “Patchouli oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 0.6% positive reactions in 2446 consecutively tested patients and 1.4% positive reactions in 828 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Pogostemon cablin* (Blanco) Benth. syn. *Mentha cablin* Blanco. An ISO standard is available for Oil of patchouli (*Pogostemon cablin* (Blanco) Benth.): ISO 3757:2002. *Pogostemon Cablin* Leaf Oil is an essential oil obtained from the fermented leaves of the Patchouli, *Pogostemon cablin* (syn: *Pogostemon patchouli*),

Labiatae (Lamiaceae (34)). It contains patchouli alcohol, beta-patchoulene, azulene, eugenol, sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40927>, last accessed 2009-11-12). Although the sesquiterpene alcohol (-)-patchoulol [5986-55-0] is the main component of patchouli oil (27-35%), the compound largely contributing to the characteristic odour is norpatchoulol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-β-patchoulene [514-51-2] (1.8-3.5%) and (-)-seychellene [20085-93-2] (1-3%) (34). According to (30) the maximum observed concentration in patchouli oil are (in %): (-)-patchoulol (35); (+)-alpha-lulnesene (21); (-)-alpha-guajene (16); β-pinene (6); (-)-β-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulol (1) (30).

It is a "top 100" substance (IFRA, pers. comm.2010).

ROSE FLOWER OIL (ROSA SPP.)

CAS 8007-01-0; EC / (Oils, rose)

ROSA ALBA FLOWER EXTRACT

CAS 93334-48-6; EC 297-122-1
(Rose, *Rosa alba*, ext. = INCI)

ROSA CANINA FLOWER OIL

CAS 84696-47-9; EC 283-652-0
(Rose, *Rosa canina*, ext.) INCI:
ROSA CANINA

ROSA CENTIFOLIA FLOWER OIL

CAS 84604-12-6, EC 283-289-8
(Rose, *Rosa centifolia*, ext.) INCI:
ROSA CENTIFOLIA / Rose, *Rosa centifolia*, ext.

ROSA DAMASCENA FLOWER OIL

CAS 90106-38-0; EC 290-260-3
(Rose, *Rosa Damascena*, ext. = INCI)

ROSA GALLICA FLOWER OIL

CAS 84604-13-7; EC 283-290-3
(Rose, *Rosa Gallica*, ext.) INCI:
ROSA GALLICA

ROSA MOSCHATA OIL

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ROSA RUGOSA FLOWER OIL

CAS 92347-25-6; EC 296-213-3
(Rose, *Rosa rugosa*, ext.)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "rose oil Bulgaria" (2% pet.), yielding 0.4% positive reactions (14); Trattner/David found 2 / 641 consecutive patients positive to "Rose oil (Bulgarian)" (31). The Bulgarian rose oil usually corresponds to *Rosa Damascena* Flower Oil (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-16). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "rose Bulgarian oil" (2% pet.) 4.5% positive reactions (9). One case of contact allergy to "Bulgarian rose oil (2 % pet.)" – and geraniol – in a 48-year-old female with ACD after application of "Eau de Rochas" E.d.C. was diagnosed, among 326 patients with suspected contact allergy to fragrance ingredients had tested negative (275). However, other rose oils are also used (and capable of eliciting ACD) as illustrated by the case of a 27 year old woman who developed ACD after using "Rose Absolute Eau ® eau de

parfum", a "non-scented" body lotion and a number of other topicals. PTing revealed a number of (previously) relevant reaction, including "Rose centifolia" (5% alc.) and "Rose oil Bulgarian" (2% pet.) essential oil preparations (276). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Rose oil Bulgarian", tested at 2% concentration (13). Nakayama et al. found 1974 (after (29)) 4 "strong positive" reactions to "Rose oil Bulgarian" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with rose oil absolute, yielding 6 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Rosa x damascena* Mill. and *Rosa sertata* X *Rosa rugosa*. Rose Flower Oil is the volatile oil obtained from the flowers of *Rosa* spp. , rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59362>, last accessed 2009-11-16). "Rose oil, meaning either rose otto (attar of rose, attar of roses) or rose absolute, is the essential oil extracted from the petals of various types of rose. Rose ottos are extracted through steam distillation, while rose absolutes are obtained through solvent extraction or supercritical carbon dioxide extraction, with the absolute being used more commonly in perfumery" (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-17). There are several more specifically named flower extracts used for masking or perfuming:

- Rosa Alba Flower Extract is an extract obtained from the flowers of the Rose, *Rosa alba* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40969>, last accessed 2009-11-16).
- Rosa Canina Flower Oil is the volatile oil obtained from the flowers of the Hip Rose, *Rosa canina* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59263>, last accessed 2009-11-16).
- Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of the Cabbage Rose, *Rosa centifolia* (L.), Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79757>, last accessed 2009-11-16).
- Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of the Damask Rose, *Rosa damascena*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79760>, last accessed 2009-11-16).
- Rosa Gallica Flower Oil is the volatile oil obtained from the flowers of the French Rose, *Rosa gallica* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59346>, last accessed 2009-11-16).
- Rosa Moschata Oil is the oil obtained from the Musk Rose, *Rosa moschata*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79761>, last accessed 2009-11-16).
- Rosa Rugosa Flower Oil is the volatile oil obtained from the flowers of the Rose, *Rosa rubiginosa* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=83588>, last accessed 2009-11-16).

Apparently, the *Rosa Damascena* and the *Rosa centifolia* are the species most commonly used for extraction of essential rose oils, the former mostly grown in Bulgaria, Turkey, Russia, India and China, the latter more commonly in Morocco, France and Egypt (276). Main constituents by GC are: citronellol (20-49%), geraniol (6-23%), nerol (3-12%) and phenylethyl alcohol (up to 3.5%) (34).

For Oil of rose (*Rosa x damascena* Miller) an ISO standard exists: ISO 9842:2003.

ROSMARINUS OFFICINALIS FLOWER OILCAS 84604-14-8; EC 283-291-9
(Rosemary, ext.)

"Rosemary Oil"

INCI: ROSMARINUM OFFICINALIS
/ Rosemary, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "rosemary" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "rosemary" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Rosmarinus officinalis* L. Rosmarinus Officinalis Flower Oil is an essential oil obtained from the leaves and fresh flowering tops of the Rosemary, *Rosmarinus officinalis* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40978>, last accessed 2010-01-29). Major constituents are: 1,8-cineole (17-55%), alpha-pinene (9-26%), camphor (5-22%) and verbenone [18309-32-5] as traces in North African oils, but between 0.7 and 2.5% in Spanish oils (34). For Oil of rosemary (*Rosmarinus officinalis* L.) an ISO standard exists: ISO 1342:2000.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SALVIA spp. HERB OIL

Sage oil

SALVIA OFFICINALIS LAVANDULIFOLIA HERB OILCAS 97952-71-1; EC 308-365-0
(Sage, *Salvia officinalis* *lavandulifolia*, ext. = INCI)**SALVIA LAVANDULIFOLIA HERB OIL**CAS 90106-49-3; EC 290-272-9
(Sage, *Salvia lavandulifolia*, ext. = INCI)**SALVIA SCLAREA FLOWER OIL**CAS 84775-83-7; EC 283-911-8
(Sage, *Salvia sclarea*, ext.) INCI: SALVIA SCLAREA / Sage, *Salvia sclarea*, ext.**SALVIA HISPANICA HERB OIL**CAS 93384-40-8; EC 297-250-8
(Sage, *Salvia hispanica*, ext. = INCI)

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "Clary sage", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "clary sage" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Salvia officinalis* L. *Salvia Officinalis Lavandulifolia* Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia officinalis* L. spp. *lavandulifolia*, Lamiaceae, Syn. Dalmatian sage (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41084>, last accessed 2010-01-29).

Salvia Lavandulifolia Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia lavandulifolia*, Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40987>, last accessed 2010-01-29).

Salvia Sclarea Flower Oil is an essential oil obtained from the flowers and foliage of the Clary Sage, *Salvia sclarea* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41086>, last accessed 2010-01-29).

Salvia Hispanica Herb Oil is an essential oil obtained from the herbs of the Spanish Sage, *Salvia hispanica* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40985>, last accessed 2010-01-29).

Clary sage oil is obtained by steam distillation of flowering tops and foliage of cultivated *Salvia sclarea* L. (Lamiaceae). Main constituents are linalyl acetate (56-78%) and linalool (6.5-24%) (34). Dalmatian sage oil is steam distilled from partially dried leaves of *S. officinalis* L. (Lamiaceae). The content by GC is: alpha-thujone (18-43%), beta-thujone (3-8.5%), 1,8-cineole (5.5-13%), camphor (3-8.5%) as main constituents (34). Spanish sage oil does not contain thujone, but mainly camphor (15-36%) and 1,8-cineole (11-30%), and is used mainly in pharmaceutical preparations and technical perfumery (34). For Oil of sage, Spanish (*Salvia lavandulifolia* Vahl) an ISO standard exists: ISO 3526:2005, for Oil of Dalmatian sage (*Salvia officinalis* L.): ISO 9909:1997.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SANTALUM ALBUM WOOD OIL

CAS 84787-70-2; EC 284-111-1
(Sandalwood, ext.) INCI:
SANTALUM ALBUM / Sandalwood,
ext.

Sandalwood oil ([East] India)

SANTALUM ALBUM OIL

CAS 8006-87-9; EC / (Oils,
sandalwood)

Sandalwood oil ([East] India)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "sandalwood oil" (2% pet.), yielding 0.8% positive reactions (14). In the Frosch 2002 b study, "sandalwood oil (East India)" is mentioned with a CAS # 8015-65-4, which, however, is attributed to AMYRIS BALSAMIFERA BARK OIL, see above. Assuming that this CAS # is erroneous, study results are considered to be valid for *S. album* wood oil, tested at 2% and 10% concentration, yielding 0.4% and 0.9% positive reactions,

respectively (17). Out of 6 of 15 patients with a positive reaction to the higher concentration no clinical relevance was found, compared to 2 of 7 patients positive to the lower concentration (17). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "sandalwood oil" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 10 of 422 consecutive patients, i.e., 2.4%, had positive reactions to "Santalum album oil" 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "sandalwood oil 10% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "sandalwood", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "sandalwood" essential oil 2% pet. (27). In 63 patients positive to the FM I, 1 had a positive PT reaction to sandalwood oil, 2% pet., in the Santucci 1987 study (28). Nakayama et al. found 1974 (after (29)) 6 "strong positive" and 8 "weak positive" reactions to "Sandalwood oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.3% positive reactions in 3671 consecutively tested patients and 1.8% positive reactions in 1002 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with sandalwood oil, yielding 2 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Santalum album* L. *Santalum Album* Oil is the volatile oil obtained from the heartwood of the Sandalwood, *Santalum album* L., Santalaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=80209>, last accessed 2009-11-26).

Santalum Album Wood Oil is an essential oil obtained from the wood of the Sandalwood, *Santalum album* L., Santalaceae. It contains 75% santalol isomers (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41092>, last accessed 2009-11-12), typically up to 55% .alpha.-santalol and up to 24% .beta.-santalol (30). East Indian sandalwood oil consists almost exclusively of closely related sesquiterpenoids; by far the main constituents are the alcohols alpha-santalol [115-71-9] (41-55%) and cis-beta-santalol [77-42-9] (16-24%), the latter being mainly responsible for the specific odour (34, 39).

An ISO standard regarding the composition of "*Santalum album* oil" is available: ISO 3518:2002. "Sandalwoods" are labelled as *Amyris balsamifera*, *Eremophila mitchelli*, *Fusanus acuminatus* (= *Santalum acuminatum*), *Santalum album*, *S. austrocaledonicum*, *S. latifolium*, *S. spicatum* and *S. yasi*. The majority of currently available trade oils, reportedly from *S. album*, contained approximately 50-70% santalols (Z-alpha and Z-beta), as analysed with gas chromatography-mass spectrometry (GC-MS) (277). A review on the toxicological properties of "*Santalum album* oil" is available (278).

AMYRIS BALSAMIFERA BARK OIL (*Sandalwood oil (Caribbean)*), CAS 8015-65-4; EC / (Oils, amyris) / 90320-49-3; EC 90320-49-3 (*Amyris balsamifera*, ext. = INCI name) is used as a cheap substitute for East Indian Sandalwood in perfumes and cosmetics. Originally cultivated primarily in Haiti where it was known as 'candle wood' and used as a torch by locals due to the tree's high oil content (<http://www.amphora-retail.com/sandalwood-amyris-essential-10ml-p-107.html>, last accessed 2009-11-12). The major components are sesquiterpenoids such as valerianol, elemol, β -eudesmol and epi-gamma-eudesmol (39). For Oil of amyris (*Amyris balsamifera* L.) an ISO standard exists: ISO 3525:2008. *Amyris Balsamifera* Bark Oil is the volatile oil distilled from the bark of the tree, *Amyris balsamifera*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=74455>, last accessed 2009-11-12).

SANTALUM SPICATA WOOD OILCAS 8024-35-9; EC 296-618-5
(Sandalwood oil, Western
Australia)*Sandalwood oil (Australia)*

Current regulation: /

Clinical data:

In clinical studies, mostly *S. album* wood oil had been used (see above); in a number of studies this is not clear.

Additional information:

ISO 4720:2009 nomenclature: *Santalum spicatum* (R.Br.) A. DC, syn. *Eucarya spicata* (R.Br.) Sprag & Summ. For Oil of Australian sandalwood (*Santalum spicatum* (R.Br.) A.DC.) an ISO standard exists: ISO 22769:2009. Santalum Spicata Wood Oil is an essential oil obtained from the wood of the Australian Sandalwood, *Santalum spicata*, Santalaceae. It contains 75% santalols and 10% farnesol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41093>, last accessed 2009-11-12). This oil also contains santalols as main constituents but differs somewhat in the remaining composition. Today, it makes up a considerable part of the sandalwood oil market (34).

Considering the content of well-known allergenic compounds (santalols), this essential oil is regarded as established contact allergen in humans.

TAGETES PATULA FLOWER OILCAS 91722-29-1; EC 294-431-3
(*Tagetes patula*, ext. = INCI)*"Marigold Oil; Tagetes Oil"*

Current regulation: /

Clinical data:

In an aromatherapist, an essential oil solvent-extracted from *Tagetes patula*, patch tested at 1.5% in grapeseed oil (vehicle negative, 7 controls negative to essential oils) resulted in a +++ reaction, in accordance with a work-related bilateral hand dermatitis (217).

Additional information:

Tagetes Patula Flower Oil is an essential oil obtained by hydrodistillation of the flowers of the *Tagetes*, *Tagetes patula* L., *Compositae*. It contains mainly D-limonene, ocimene, 2,6-dimethyloct-7-en-4-one (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41506>, last accessed 2010-01-28). According to Surburg/Panten, tagetes oil is steam distilled from the flowering plants of *Tagetes minuta* L. (*T. glandulifera* Schrank., *Asteraceae*). Main components comprise cis-ocimene, dihydrotagetone, tagetone, and cis- and trans-ocimenone (34, 39).

THYMUS spp. HERB OIL

THYMUS VULGARIS HERB OIL

CAS 84929-51-1, 8007-46-3; EC 284-535-7 (Thyme, Thymus vulgaris, ext.)

"Thyme oil"

INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "thyme" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "thyme" essential oil 2% pet. (27). In 63 patients positive to the FM I, none had a positive PT reaction to thymol, 1% pet., in the Santucci 1987 study (28).

Additional information:

ISO 4720:2009 nomenclature: *Thymus vulgaris* L. *Thymus vulgaris* Herb Oil is an essential oil obtained from the herbs of the Thyme, *Thymus vulgaris* L., Lamiaceae. It contains 20-40% thymol and carvacrol, cymene, pinene, linalool, bornyl acetate (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41133>, last accessed 2010-01-29).

Other species are used for extraction, e.g., *Thymus Mastichina* (CAS 84837-14-9), *Thymus Serpillum* (CAS 84776-98-7), *Thymus Zygis* (CAS 85085-75-2), according to CosIng. The main constituent is thymol (37-56%) (34). For Oil of thyme containing thymol, Spanish type [*Thymus zygis* (Loefl.) L.] an ISO standard exists: ISO 14715:2010, for Oil of Spanish wild marjoram (*Thymus mastichina* L.): ISO 4728:2003.

TURPENTINE (oil)

CAS 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / -

Current regulation: III/124 ; III/125 ; III/126

Clinical data:

Oil of turpentine has been patch tested in a number of baseline series, i.e., in consecutive patients, although not included in the European Baseline series.

In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190)

Table 3.2.2 – 2: Overview of results with **Oil of turpentine** in patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS.

Country	Population	Years	No. tested	Crude % positive (95% CI) §

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Lisbon, Portugal (189); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 % (1.9 – 2.8) §
Birmingham, UK (190)	Potters with occup. dermatitis hand	6 months; prior to 1996	24	14 / 24 pos. to “Indonesian turpentine”
Austria/Germany (IVDK) (279)	Consecutive patients	1992-1995	27658	0.47 % (0.39 – 0.55) §
Austria/Germany (IVDK) (280)	Consecutive patients	1996-2002	59478	Annual prevalences 1.6 to 4.4 %
Augsburg/Germany (281)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (273)	Consecutive patients	2002/03	3767	1.6 %
Austria/Germany/Switzerland (IVDK) (7)	Consecutive patients	2005-2008	37163	1.8 %

Additional information:

ISO 4720:2009 nomenclature: *Pinus pinaster* Aiton and *Pinus massoniana* Lamb. Turpentine, oil: Any of the volatile predominately terpenic fractions or distillates resulting from the solvent extraction of, gum collection from, or pulping of softwoods. Turpentine is a mixture of terpene hydrocarbons obtained from various species of *Pinus* http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=41521

The composition of oil of turpentine varies with its origin, in particular, the content of .delta.-3-carene, one of its main allergenic compounds (189, 279). Similarly, the peroxide degree may vary. The main constituents are .alpha.-pinene (50-72%), .beta.-pinene (6-15%), carenes (< 0.1-17%), camphene (up to 1%), dipentene (0.5-5%), along with a number of other substances (279).

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

Verbena absolute (Lippia citriodora Kunth.)

CAS 8024-12-2, 84961-67-1; EC /)

Current regulation: Annex III, part 1, n° 206

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Aloysia citriodora* Palau syn. *Lippia citriodora* Kunth syn. *Aloysia triphylla* (L' Hér.) Kuntze. An older RIFM review is available citing several positive human maximisation studies both with “Verbena absolute” and “Verbena oil” (128).

VETIVERIA ZIZANOIDES ROOT OIL

CAS 8016-96-4; EC / (Oils, vetiver) / 84238-29-9; EC 282-490-8 (Vetiveria zizanioides, ext. = INCI)

"Vetiver oil; khas khas oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "vetiver" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=9 (10.4%) positive reactions to "vetiver" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Vetiveria zizanioides* (L.) Nash. *Vetiveria Zizanioides* Root Oil is an essential oil distilled from the dried roots of the grass *Vetiveria zizanioides* (L.) Nash *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41293>, last accessed 2010-01-29). Vetiver oil has a high sesquiterpene content. The ketones alpha-vetivone [15764-04-2] (6-12%) and beta-vetivone [18444-79-6] (4-10%), which usually form more than 10% of the oil, as well as khusimol [16223-63-5] (24-36%) and isovelencenol [22387-74-2] (12-24%) are the main constituents (in Bourbon oil, i.e., from Réunion) (34). For Oil of vetiver (*Vetiveria zizanioides* (L.) Nash) an ISO standard exists: ISO 4716:2002.

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References

1. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis - a worldwide multicenter investigation (Part III). *Contact Dermatitis* 2002; 46: 141-144.
2. Hendriks S A, van Ginkel C J. Evaluation of the fragrance mix in the European standard series. *Contact Dermatitis* 1999; 41: 161-162.
3. Katsarma G, Gawkrödger D J. Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. *Contact Dermatitis* 1999; 41: 193-197.
4. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Dermatitis* 2007; 57: 1-10.
5. Temesvari E, Nemeth I, Baló-Banga M J, Husz S, Kohanka V, Somos Z, Judak R, Remenyik E V, Szegedi A, Nebenfuhrer L, Meszaros C, Horvath A. Multicentre study of fragrance allergy in Hungary. Immediate and late type reactions. *Contact Dermatitis* 2002; 46: 325-330.
6. van Oosten E J, Schuttelaar M L, Coenraads P J. Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. *Contact Dermatitis* 2009; 61: 217-223.
7. Uter W, Geier J, Frosch P J, Schnuch A. Contact allergy to fragrances: current patch test results (2005 to 2008) from the IVDK network. *Contact Dermatitis* 2010; 63: 254-261.
8. Schnuch A, Geier J, Uter W, Frosch P J. Another look on allergies to fragrances: frequencies of sensitisation to the fragrance mix and its constituents. Results from the IVDK. *Exog Dermatol* 2002; 1: 231-237.
9. Brites M M, Goncalo M, Figueiredo A. Contact allergy to fragrance mix--a 10-year study. *Contact Dermatitis* 2000; 43: 181-182.
10. Frosch P J, Rastogi S C, Pirker C, Brinkmeier T, Andersen K E, Bruze M, Svedman C, Goossens A, White I R, Uter W, Arnau E G, Lepoittevin J P, Johansen J D, Menne T. Patch testing with a new fragrance mix - reactivity to the individual constituents and chemical detection in relevant cosmetic products. *Contact Dermatitis* 2005; 52: 216-225.
11. Krauthaim A, Uter W, Frosch P, Schnuch A, Geier J. Patch testing with fragrance mix II: results of the IVDK 2005-2008. *Contact Dermatitis* 2010; 63: 262-269.
12. deGroot A C, Coenraads P J, Bruynzeel D P, Jagtman B A, van Ginkel C J W, Noz K, van der Valk P G M, Pavel S, Vink J, Weyland J W. Routine patch testing with fragrance chemicals in The Netherlands. *Contact Dermatitis* 2000; 42: 184-185.
13. An S, Lee A Y, Lee C H, Kim D W, Hahm J H, Kim K J, Moon K C, Won Y H, Ro Y S, Eun H C. Fragrance contact dermatitis in Korea: a joint study. *Contact Dermatitis* 2005; 53: 320-323.
14. Sugiura M, Hayakawa R, Kato Y, Sugiura K, Hashimoto R. Results of patch testing with lavender oil in Japan. *Contact Dermatitis* 2000; 43: 157-160.
15. Frosch P J, Pilz B, Andersen K E, Burrows D, Camarasa J G, et al. Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 1995; 33: 333-342.

16. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. I. Reactivity to 14 frequently used chemicals. *Contact Dermatitis* 2002; 47: 78-85.
17. Frosch P J, Johansen J D, Menne T, Pirker C, Rastogi S C, Andersen K E, Bruze M, Goossens A, Lepoittevin J P, White I R. Further important sensitizers in patients sensitive to fragrances. II. Reactivity to essential oils. *Contact Dermatitis* 2002; 47: 279-287.
18. Larsen W G. Perfume Dermatitis. A Study of 20 Patients. *Arch Dermatol* 1977; 113: 623-626.
19. Larsen W, Nakayama H, Fischer T, Elsner P, Frosch P, Burrows D, Jordan W, Shaw S, Wilkinson J, Marks J, Jr., Sugawara M, Nethercott M, Nethercott J. Fragrance contact dermatitis: a worldwide multicenter investigation (Part II). *Contact Dermatitis* 2001; 44: 344-346.
20. Belsito D V, Fowler J F, Jr., Sasseville D, Marks J G, Jr., De Leo V A, Storrs F J. Delayed-type hypersensitivity to fragrance materials in a select North American population. *Dermatitis* 2006; 17: 23-28.
21. Zug K A, Warshaw E M, Fowler J F, Jr., Maibach H I, Belsito D L, Pratt M D, Sasseville D, Storrs F J, Taylor J S, Mathias C G, Deleo V A, Rietschel R L, Marks J. Patch-test results of the North American Contact Dermatitis Group 2005-2006. *Dermatitis* 2009; 20: 149-160.
22. Wöhrl S, Hemmer W, Focke M, Götz M, Jarisch R. The significance of fragrance mix, balsam of Peru, colophony and propolis as screening tools in the detection of fragrance allergy. *Br J Dermatol* 2001; 145: 268-273.
23. Goossens A, Merckx L. Allergic Contact Dermatitis from farnesol in a deodorant. *Contact Dermatitis* 1997; 37: 179-180.
24. Malten K E, van Ketel W G, Nater J P, Liem D H. Reactions in selected patients to 22 fragrance materials. *Contact Dermatitis* 1984; 11: 1-10.
25. de Groot A C, Liem D H, Nater J P, van Ketel W G. Patch tests with fragrance materials and preservatives. *Contact Dermatitis* 1985; 12: 87-92.
26. Rudzki E, Grzywa Z, Bruo W S. Sensitivity to 35 essential oils. *Contact Dermatitis* 1976; 2: 196-200.
27. Rudzki E, Grzywa Z. Allergy to perfume mixture. *Contact Dermatitis* 1986; 15: 115-116.
28. Santucci B, Cristaudo A, Cannistraci C, Picardo M. Contact dermatitis to fragrances. *Contact Dermatitis* 1987; 16: 93-95.
29. Mitchell J C. Contact hypersensitivity to some perfume materials. *Contact Dermatitis* 1975; 1: 196-199.
30. Uter W, Schmidt E, Geier J, Lessmann H, Schnuch A, Frosch P J. Contact allergy to essential oils: current patch test results (2000-2008) from the IVDK network. *Contact Dermatitis* 2010; 63: 277-283.
31. Trattner A, David M. Patch testing with fine fragrances: comparison with fragrance mix, balsam of Peru and a fragrance series. *Contact Dermatitis* 2003; 49: 287-289.
32. Handley J, Burrows D. Allergic contact dermatitis from the synthetic fragrances Lylal and acetyl cedrene in separate underarm deodorant preparations. *Contact Dermatitis* 1994; 31: 288-290.

-
33. SCCNFP. The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers: Opinion concerning Fragrance Allergy in Consumers. A Review of the Problem. Analysis of the Need for appropriate Consumer Information and Identification of Consumer Allergens, adopted 8 December 1999. *SCCNFP/0017/98 Final* 1999:
 34. Surburg H, Panten J. *Common fragrance and flavor materials: preparation, properties and uses*. Weinheim: Wiley-VCH, 2006.
 35. Bhatia S P, Wellington G A, Cocchiara J, Lalko J, Letizia C S, Api A M. Fragrance material review on alpha-amylcinnamyl alcohol. *Food Chem Toxicol* 2007: 45 Suppl 1: S32-39.
 36. Lapczynski A, McGinty D, Jones L, Bhatia S P, Letizia C S, Api A M. Fragrance material review on pentyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S460-466.
 37. Franks A. Contact allergy to anethole in toothpaste associated with loss of taste. *Contact Dermatitis* 1998: 38: 354-355.
 38. Garcia-Bravo B, Perez Bernal A, Garcia-Hernandez M J, Camacho F. Occupational contact dermatitis from anethole in food handlers. *Contact Dermatitis* 1997: 37: 38.
 39. Fahlbusch K-G, Hammerschmidt F-J, Panten J, Pickenhagen W, Schatkowski D, Bauer K, Garbe D, Surburg H. Flavors and Fragrances. In: Wiley-VCH, eds. *Ullmann's Encyclopedia of Industrial Chemistry*. Weinheim: Wiley-VCH, 2002:
 40. Hostynek J J, Maibach H I. Is there evidence that anisyl alcohol causes allergic contact dermatitis? *Exog Dermatol* 2003: 2: 230-233.
 - 40a. Bruze M, Svedman C, Andersen KE, Bruynzeel D, Goossens A, Johansen JD, Matura M, Orton D, Vigan M; ESCD. Patch test concentrations (doses in mg/cm²) for the 12 non-mix fragrance substances regulated by European legislation. *Contact Dermatitis* 2012: 66: 131-136
 41. Andersen A. Final report on the safety assessment of benzaldehyde. *Int J Toxicol* 2006: 25 Suppl 1: 11-27.
 42. Seite-Bellezza D, el Sayed F, Bazex J. Contact urticaria from cinnamic aldehyde and benzaldehyde in a confectioner. *Contact Dermatitis* 1994: 31: 272-273.
 43. Opdyke D L, Letizia C. Monographs on fragrance raw materials. *Food Chem Toxicol* 1983: 21: 645-667.
 44. Nair B. Final report on the safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. *Int J Toxicol* 2001: 20 Suppl 3: 23-50.
 45. Sestini S, Mori M, Francalanci S. Allergic contact dermatitis from benzyl alcohol in multiple medicaments. *Contact Dermatitis* 2004: 50: 316-317.
 46. Podda M, Zollner T, Grundmann-Kollmann M, Kaufmann R, Boehncke W H. Allergic contact dermatitis from benzyl alcohol during topical antimycotic treatment. *Contact Dermatitis* 1999: 41: 302-303.
 47. Shoji A. Allergic reaction to benzyl alcohol in an antimycotic preparation. *Contact Dermatitis* 1983: 9: 510.
 48. Cuesta L, Silvestre J F, Toledo F, Lucas A, Perez-Crespo M, Ballester I. Fragrance contact allergy: a 4-year retrospective study. *Contact Dermatitis* 2010: 63: 77-84.
 49. Guin J D, Goodman J. Contact urticaria from benzyl alcohol presenting as intolerance to saline soaks. *Contact Dermatitis* 2001: 45: 182-183.

50. Fisher A A. Allergic paraben and benzyl alcohol hypersensitivity relationship of the "delayed" and "immediate" varieties. *Contact Dermatitis* 1975: 1: 281-284.
51. Shaw D W. Allergic contact dermatitis to benzyl alcohol in a hearing aid impression material. *Am J Contact Dermat* 1999: 10: 228-232.
52. Jacob S E, Barron G S. Benzyl alcohol: a covert fragrance. *Dermatitis* 2007: 18: 232-233.
53. Hausen B M, Brinkmann J, Dohn W. *Lexikon der Kontaktallergene (6. Erg.-Lieferung)*. Landsberg am Lech: Ecomed, 1998.
54. Corazza M, Manovani L, Maranini C, Virgili A. Allergic Contact Dermatitis from benzyl alcohol. *Contact Dermatitis* 1996: 34: 74.
55. Buffet M, Dupin N. Current treatments for scabies. *Fundam Clin Pharmacol* 2003: 17: 217-225.
56. Bhatia S P, Wellington G A, Cocchiara J, Lalko J, Letizia C S, Api A M. Fragrance material review on benzyl cinnamate. *Food Chem Toxicol* 2007: 45 Suppl 1: S40-48.
57. Lapczynski A, McGinty D, Jones L, Bhatia S, Letizia C S, Api A M. Fragrance material review on benzyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S362-380.
58. Bhatia S P, Jones L, Letizia C S, Api A M. Fragrance material review on 2-tert-butylcyclohexyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S44-47.
59. Bhatia S P, Jones L, Letizia C S, Api A M. Fragrance material review on 4-tert-butylcyclohexyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S36-41.
60. Arnau E G, Andersen K E, Bruze M, Frosch P J, Johansen J D, Menne T, Rastogi S C, White I R, Lepoittevin J P. Identification of Lilial as a fragrance sensitizer in a perfume by bioassay-guided chemical fractionation and structure-activity relationships. *Contact Dermatitis* 2000: 43: 351-358.
61. Stevenson O E, Finch T M. Allergic contact dermatitis from rectified camphor oil in Earex ear drops. *Contact Dermatitis* 2003: 49: 51.
62. Vilaplana J, Romaguera C, Campderros L. [Contact dermatitis by camphor present in a flushing solution]. *Actas Dermosifiliogr* 2007: 98: 345-346.
63. Noiles K., M. P. Contact dermatitis to Vicks VapoRub. *Dermatitis* 2010: 21: 167-169.
64. Sköld M, Karlberg A T, Matura M, Börje A. The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. *Food Chem Toxicol* 2006: 44: 538-545.
65. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Selected oxidized fragrance terpenes are common contact allergens. *Contact Dermatitis* 2005: 52: 320-328.
66. Andersen A. Final report on the safety assessment of sodium p-chloro-m-cresol, p-chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol. *Int J Toxicol* 2006: 25 Suppl 1: 29-127.
67. Corazza M, Levratti A, Virgili A. Allergic contact cheilitis due to carvone in toothpastes. *Contact Dermatitis* 2002: 46: 366-367.
68. Worm M, Jeep S, Sterry W, Zuberbier T. Perioral contact dermatitis caused by L-carvone in toothpaste. *Contact Dermatitis* 1998: 38: 338.
69. Hausen B M. Zahnpasta-Allergie. *Dtsch Med Wochenschr* 1984: 109: 300-302.

70. Paulsen E, Andersen K E, Carlsen L, et al. Carvone: an overlooked contact allergen cross-reacting with sesquiterpene lactones? *Contact Dermatitis* 1993: 29: 138-143.
71. Karlberg A T, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Dermatitis* 1992: 26: 332-340.
72. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjö K, Karlberg A T. Patch testing with oxidized R-(+)-limonene and its hydroperoxide fraction. *Contact Dermatitis* 2003: 49: 15-21.
73. Nilsson A M, Gafvert E, Salvador L, Luthman K, Bruze M, Gruvberger B, Nilsson J L, Karlberg A T. Mechanism of the antigen formation of carvone and related alpha, beta-unsaturated ketones. *Contact Dermatitis* 2001: 44: 347-356.
74. Nguyen S H, Dang T P, MacPherson C, Maibach H, Maibach H I. Prevalence of patch test results from 1970 to 2002 in a multi-centre population in North America (NACDG). *Contact Dermatitis* 2008: 58: 101-106.
75. Diba V C, Statham B N. Contact urticaria from cinnamal leading to anaphylaxis. *Contact Dermatitis* 2003: 48: 119.
76. Decapite T J, Anderson B E. Allergic contact dermatitis from cinnamic aldehyde found in an industrial odour-masking agent. *Contact Dermatitis* 2004: 51: 312-313.
77. Cocchiara J, Letizia C S, Lalko J, Lapczynski A, Api A M. Fragrance material review on cinnamaldehyde. *Food Chem Toxicol* 2005: 43: 867-923.
78. Bickers D, Calow P, Greim H, Hanifin J M, Rogers A E, Saurat J H, Sipes I G, Smith R L, Tagami H. A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. *Food Chem Toxicol* 2005: 43: 799-836.
79. Buckley D A, Basketter D A, Smith Pease C K, Rycroft R J, White I R, McFadden J P. Simultaneous sensitivity to fragrances. *Br J Dermatol* 2006: 154: 885-888.
80. Elahi E N, Wright Z, Hinselwood D, Hotchkiss S A, Basketter D A, Pease C K. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. *Chem Res Toxicol* 2004: 17: 301-310.
81. Letizia C S, Cocchiara J, Lalko J, Lapczynski A, Api A M. Fragrance material review on cinnamyl alcohol. *Food Chem Toxicol* 2005: 43: 837-866.
82. Heydorn S, Menne T, Andersen K E, Bruze M, Svedman C, White I R, Basketter D A. Citral a fragrance allergen and irritant. *Contact Dermatitis* 2003: 49: 32-36.
83. Hindle E, Ashworth J, Beck M H. Cheilitis from contact allergy to citral in lip salve. *Contact Dermatitis* 2007: 57: 125-126.
84. Hagvall L, Backtorp C, Svensson S, Nyman G, Borje A, Karlberg A T. Fragrance compound geraniol forms contact allergens on air exposure. Identification and quantification of oxidation products and effect on skin sensitization. *Chem Res Toxicol* 2007: 20: 807-814.
85. Hagvall L, Baron J M, Borje A, Weidolf L, Merk H, Karlberg A T. Cytochrome P450-mediated activation of the fragrance compound geraniol forms potent contact allergens. *Toxicol Appl Pharmacol* 2008: 233: 308-313.
86. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on l-citronellol. *Food Chem Toxicol* 2008: 46 Suppl 11: S110-113.
87. Lapczynski A, Letizia C S, Api A M. Fragrance material review on (+)-(R)-citronellol. *Food Chem Toxicol* 2008: 46 Suppl 11: S114-116.

-
88. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on dl-citronellol. *Food Chem Toxicol* 2008: 46 Suppl 11: S103-109.
 89. Hostynek J J, Maibach H I. Sensitization Potential of Citronellol. *Exog Dermatol* 2004: 3: 307-312.
 90. Mutterer V, Gimenez Arnau E, Lepoittevin J P, Johansen J D, Frosch P J, Menne T, Andersen K E, Bruze M, Rastogi S C, White I R. Identification of coumarin as the sensitizer in a patient sensitive to her own perfume but negative to the fragrance mix. *Contact Dermatitis* 1999: 40: 196-199.
 91. Vocanson M, Goujon C, Chabeau G, Castelain M, Valeyrie M, Floc'h F, Maliverney C, Gard A, Nicolas J F. The skin allergenic properties of chemicals may depend on contaminants--evidence from studies on coumarin. *Int Arch Allergy Immunol* 2006: 140: 231-238.
 92. Bhatia S P, Letizia C S, Api A M. Fragrance material review on cyclohexyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S52-55.
 93. Letizia C S, Cocchiara J, Wellington G A, Funk C, Api A M. Food and chemical toxicology. *Food Chem Toxicol* 2000: 38 Suppl 3: S1-236.
 94. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on damascenone. *Food Chem Toxicol* 2007: 45 Suppl 1: S172-178.
 95. Takanami I, Nakayama H. TMCHB: a possible alternative to DNCB in skin testing for immune competence. *Contact Dermatitis* 1988: 19: 81-83.
 96. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on alpha-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S179-187.
 97. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on cis-alpha-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S188-191.
 98. Lalko J, Lapczynski A, Letizia C S, Api A M. Fragrance material review on cis-beta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S192-198.
 99. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on trans-beta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S199-204.
 100. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on delta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S205-210.
 101. Lapczynski A, Lalko J, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on trans,trans-delta-damascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S211-215.
 102. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on gamma-damascone. *Food Chem Toxicol* 2007: 45 (Suppl. 1): S216-S220.
 103. McGinty D, Letizia C S, Api A M. Fragrance material review on dihydromyrcenol. *Food Chem Toxicol* 2010: 48 Suppl 3: S70-75.
 104. McGinty D, Letizia C S, Api A M. Fragrance material review on 3,7-dimethyl-1,6-nonadien-3-ol. *Food Chem Toxicol* 2010: 48 Suppl 3: S52-55.
 105. Mitchell D M, Beck M H. Contact allergy to benzyl alcohol in a cutting oil reodorant. *Contact Dermatitis* 1988: 18: 301-302.

106. Giusti F, Porcaro V, Seidenari S. Evaluation of eugenol allergy in a patch-test population. *Contact Dermatitis* 2001; 44: 37-38.
107. Quirce S, Fernandez-Nieto M, del Pozo V, Sastre B, Sastre J. Occupational asthma and rhinitis caused by eugenol in a hairdresser. *Allergy* 2008; 63: 137-138.
108. Bhalla M, Thami G P. Acute urticaria due to dental eugenol. *Allergy* 2003; 58: 158.
109. Sarraimi N, Pemberton M N, Thornhill M H, Theaker E D. Adverse reactions associated with the use of eugenol in dentistry. *Br Dent J* 2002; 193: 257-259.
110. Kanerva L, Estlander T, Jolanki R. Dental nurse's occupational allergic contact dermatitis from eugenol used as a restorative dental material with polymethylmethacrylate. *Contact Dermatitis* 1998; 38: 339-340.
111. Hemmer W, Focke M, Leitner B, Gotz M, Jarisch R. Axillary dermatitis from farnesol in a deodorant. *Contact Dermatitis* 2000; 42: 168-169.
112. Schnuch A, Uter W, Geier J, Lessmann H, Frosch P J. Contact allergy to farnesol in 2021 consecutively patch tested patients. Results of the IVDK. *Contact Dermatitis* 2004; 50: 117-121.
113. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on farnesol. *Food Chem Toxicol* 2008; 46 Suppl 11: S149-156.
114. Tamagawa-Mineoka R, Katoh N, Kishimoto S. Allergic contact cheilitis due to geraniol in food. *Contact Dermatitis* 2007; 56: 242-243.
115. Yamamoto A, Morita A, Tsuji T, Suzuki K, Matsunaga K. Contact urticaria from geraniol. *Contact Dermatitis* 2002; 46: 52.
116. Hostynek J J, Maibach H I. Is there evidence that geraniol causes allergic contact dermatitis? *Exog Dermatol* 2004; 3: 318-331.
117. Lapczynski A, Bhatia S P, Foxenberg R J, Letizia C S, Api A M. Fragrance material review on geraniol. *Food Chem Toxicol* 2008; 46 Suppl 11: S160-170.
118. Dearman R J, Wright Z M, Basketter D A, Ryan C A, Gerberick G F, Kimber I. The suitability of hexyl cinnamic aldehyde as a calibrant for the murine local lymph node assay. *Contact Dermatitis* 2001; 44: 357-361.
119. Lapczynski A, Jones L, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on hexyl salicylate. *Food Chem Toxicol* 2007; 45 Suppl 1: S410-417.
120. Karlberg A T. Contact allergy to colophony. Chemical identifications of allergens, sensitization experiments and clinical experiences. *Acta Dermatol Venerol (Stockh) Suppl* 1988; 139: 1-43.
121. Schnuch A, Uter W, Dickel H, Szliska C, Schliemann S, Eben R, Rueff F, Gimenez-Arnau A, Loffler H, Aberer W, Frambach Y, Worm M, Niebuhr M, Hillen U, Martin V, Jappe U, Frosch P J, Mahler V. Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients. *Contact Dermatitis* 2009; 61: 152-162.
122. Hendriks S A, Bousema M T, van Ginkel C J. Allergic contact dermatitis from the fragrance ingredient Lyrall in underarm deodorant. *Contact Dermatitis* 1999; 41: 119.
123. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on hydroxycitronellol. *Food Chem Toxicol* 2008; 46 Suppl 11: S179-181.
124. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on ionone. *Food Chem Toxicol* 2007; 45 Suppl 1: S251-257.

125. Lalko J, Lapczynski A, Politano V T, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on alpha-ionone. *Food Chem Toxicol* 2007: 45 Suppl 1: S235-240.
126. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on beta-ionone. *Food Chem Toxicol* 2007: 45 Suppl 1: S241-247.
127. Lapczynski A, Jones L, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on isoamyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S418-423.
128. Ford R A, Api A M, Letizia C S. Monographs on fragrance raw materials. *Food Chem Toxicol* 1992: 30 Suppl: 1S-138S.
129. White J M, White I R, Glendinning A, Fleming J, Jefferies D, Basketter D A, McFadden J P, Buckley D A. Frequency of allergic contact dermatitis to isoeugenol is increasing: a review of 3636 patients tested from 2001 to 2005. *Br J Dermatol* 2007: 157: 580-582.
130. Tanaka S, Royds C, Buckley D, Basketter D A, Goossens A, Bruze M, Svedman C, Menne T, Johansen J D, White I R, McFadden J P. Contact allergy to isoeugenol and its derivatives: problems with allergen substitution. *Contact Dermatitis* 2004: 51: 288-291.
131. White I R, Johansen J D, Arnau E G, Lepoittevin J P, Rastogi S, Bruze M, Andersen K E, Frosch P J, Goossens A, Menne T. Isoeugenol is an important contact allergen: can it be safely replaced with isoeugenyl acetate? *Contact Dermatitis* 1999: 41: 272-275.
132. Lapczynski A, Lalko J, Politano V T, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on alpha-iso-methylionone. *Food Chem Toxicol* 2007: 45 Suppl 1: S280-289.
133. Hostynek J J, Maibach H I. Is there evidence that alpha-isomethylionone causes allergic contact dermatitis? *Exog Dermatol* 2004: 3: 121-125.
134. Guarneri F, Barbuzza O, Vaccaro M, Galtieri G. Allergic contact dermatitis and asthma caused by limonene in a labourer handling citrus fruits. *Contact Dermatitis* 2008: 58: 315-316.
135. Foti C, Zambonin C G, Conserva A, Casulli C, D'Accolti L, Angelini G. Occupational contact dermatitis to a limonene-based solvent in a histopathology technician. *Contact Dermatitis* 2007: 56: 109-112.
136. Wakelin S H, McFadden J P, Leonard J N, Rycroft R J. Allergic contact dermatitis from d-limonene in a laboratory technician. *Contact Dermatitis* 1998: 38: 164-165.
137. Topham E J, Wakelin S H. D-Limonene contact dermatitis from hand cleansers. *Contact Dermatitis* 2003: 49: 108-109.
138. Martins C, Goncalo M, Goncalo S. Allergic contact dermatitis from dipentene in wax polish. *Contact Dermatitis* 1995: 33: 126-127.
139. Rycroft R J. Allergic contact dermatitis from dipentene in honing oil. *Contact Dermatitis* 1980: 6: 325-329.
140. Meding B, Barregard L, Marcus K. Hand eczema in car mechanics. *Contact Dermatitis* 1994: 30: 129-134.
141. Karlberg A T, Dooms-Gossens A. Contact allergy to oxidized d-limonene among dermatitis patients. *Contact Dermatitis* 1997: 36: 201-206.
142. Matura M, Skold M, Borje A, Andersen K E, Bruze M, Frosch P, Goossens A, Johansen J D, Svedman C, White I R, Karlberg A T. Not only oxidized R-(+)- but

- also S-(-)-limonene is a common cause of contact allergy in dermatitis patients in Europe. *Contact Dermatitis* 2006: 55: 274-279.
143. Matura M, Goossens A, Bordalo O, Garcia-Bravo B, Magnusson K, Wrangsjo K, Karlberg A T. Oxidized citrus oil (R-limonene): a frequent skin sensitizer in Europe. *J Am Acad Dermatol* 2002: 47: 709-714.
 144. Sköld M, Börje A, Harambasic E, Karlberg A T. Contact allergens formed on air exposure of linalool. Identification and quantification of primary and secondary oxidation products and the effect on skin sensitization. *Chem Res Toxicol* 2004: 17: 1697-1705.
 145. Christensson J B, Matura M, Gruvberger B, Bruze M, Karlberg A T. Linalool--a significant contact sensitizer after air exposure. *Contact Dermatitis* 2010: 62: 32-41.
 146. Letizia C S, Cocchiara J, Lalko J, Api A M. Fragrance material review on linalool. *Food Chem Toxicol* 2003: 41: 943-964.
 147. Bickers D, Calow P, Greim H, Hanifin J M, Rogers A E, Saurat J H, Sipes I G, Smith R L, Tagami H. A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. *Food Chem Toxicol* 2003: 41: 919-942.
 148. Hostynek J J, Maibach H I. Is there evidence that linalool causes allergic contact dermatitis? *Exog Dermatol* 2003: 2: 223-229.
 149. de Groot A C, Bruynzeel D P, Bos J D, der Meeren H L v, van Joost T, Jagtman B A, Weyland J W. The allergens in cosmetics. *Arch Dermatol* 1988: 124: 1525-1529.
 150. Sköld M, Hagvall L, Karlberg A T. Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis* 2008: 58: 9-14.
 151. Hagvall L, Skold M, Brared-Christensson J, Borje A, Karlberg A T. Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis* 2008: 59: 143-150.
 152. Letizia C S, Cocchiara J, Lalko J, Api A M. Fragrance material review on linalyl acetate. *Food Chem Toxicol* 2003: 41: 965-976.
 153. Ford R A, Letizia C S, Api A M. Longifolene. *Food Chem Tox* 1992: 30(Suppl.): 67S-68S.
 154. Morton C A, Garioch J, Todd P, et al. Contact sensitivity to menthol and peppermint in patients with intra-oral symptoms. *Contact Dermatitis* 1995: 32: 281-284.
 155. Foti C, Conserva A, Antelmi A, Lospalluti L, Angelini G. Contact dermatitis from peppermint and menthol in a local action transcutaneous patch. *Contact Dermatitis* 2003: 49: 312-313.
 156. Andersson M, Hindsen M. Rhinitis because of toothpaste and other menthol-containing products. *Allergy* 2007: 62: 336-337.
 157. dos Santos M A, Santos Galvao C E, Morato Castro F. Menthol-induced asthma: a case report. *J Investig Allergol Clin Immunol* 2001: 11: 56-58.
 158. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S209-214.
 159. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on d-menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S215-217.

160. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on l-menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S218-223.
161. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on d,l-menthol. *Food Chem Toxicol* 2008: 46 Suppl 11: S224-227.
162. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on menthol racemic. *Food Chem Toxicol* 2008: 46 Suppl 11: S228-233.
163. Nair B. Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. *Int J Toxicol* 2001: 20 Suppl 3: 61-73.
164. Trattner A, David M. Patch testing with fine fragrances: comparison with fragrance mix, balsam of Peru and a fragrance series. *Contact Dermatitis* 2003: 49: 287-289.
165. Mitchell J C, Calnan C D, Clendenning W E, Cronin E, Hjorth N, Magnusson B, Maibach H I, Meneghini C L, Wilkinson D S. Patch testing with some components of balsam of Peru. *Contact Dermatitis* 1976: 2: 57-58.
166. Bhatia S P, Wellington G A, Cocchiara J, Lalko J, Letizia C S, Api A M. Fragrance material review on methyl cinnamate. *Food Chem Toxicol* 2007: 45 Suppl 1: S113-119.
167. Kaidbey K H, Kligman A M. Photocontact allergy to 6-methylcoumarin. *Contact Dermatitis* 1978: 4: 277-282.
168. Cardoso J C, Canelas M M, Goncalo M, Figueiredo A. Photopatch testing with an extended series of photoallergens: a 5-year study. *Contact Dermatitis* 2009: 60: 325-329.
169. Victor F C, Cohen D E, Soter N A. A 20-year analysis of previous and emerging allergens that elicit photoallergic contact dermatitis. *J Am Acad Dermatol* 2010: 62: 605-610.
170. McGinty D, Letizia C S, Api A M. Fragrance material review on 4-methyl-3-decen-5-ol. *Food Chem Toxicol* 2010: 48 Suppl 3: S93-96.
171. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia C S, Api A M. Fragrance material review on methyl ionone (mixture of isomers). *Food Chem Toxicol* 2007: 45 Suppl 1: S300-307.
172. English J S, Rycroft R J. Allergic contact dermatitis from methyl heptene and methyl octene carbonates. *Contact Dermatitis* 1988: 18: 174-175.
173. Hostynek J J, Maibach H I. Is there evidence that methyl heptene carbonate causes allergic contact dermatitis? *Cutan Ocul Toxicol* 2006: 25: 259-271.
174. Heisterberg M V, Vigan M, Johansen J D. Active sensitization and contact allergy to methyl 2-octynoate. *Contact Dermatitis* 2010: 62: 97-101.
175. Bernaola G, Escayol P, Fernandez E, de Corres L F. Contact dermatitis from methylionone fragrance. *Contact Dermatitis* 1989: 20: 71-72.
176. Lalko J, Lapczynski A, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-irone. *Food Chem Toxicol* 2007: 45 Suppl 1: S272-275.
177. Oiso N, Fukai K, Ishii M. Allergic contact dermatitis due to methyl salicylate in a compress. *Contact Dermatitis* 2004: 51: 34-35.
178. Hindson C. Contact eczema from methyl salicylate reproduced by oral aspirin (acetyl salicylic acid). *Contact Dermatitis* 1977: 3: 348-349.

179. Lapczynski A, Jones L, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on methyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S428-452.
180. Sköld M. *Contact allergy to autoxidized fragrance terpenes*. Thesis University of Gothenburg 2005.
181. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on myrtenol. *Food Chem Toxicol* 2008: 46 Suppl 11: S237-240.
182. Lapczynski A, Foxenberg R J, Bhatia S P, Letizia C S, Api A M. Fragrance material review on nerol. *Food Chem Toxicol* 2008: 46 Suppl 11: S241-244.
183. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on nerolidol (isomer unspecified). *Food Chem Toxicol* 2008: 46 Suppl 11: S247-250.
184. Lapczynski A, Letizia C S, Api A M. Fragrance material review on cis-nerolidol. *Food Chem Toxicol* 2008: 46 Suppl 11: S245-246.
185. Christensen L P, Jakobsen H B, Paulsen E, Hodal L, Andersen K E. Airborne Compositae dermatitis: monoterpenes and no parthenolide are released from flowering *Tanacetum parthenium* (feverfew) plants. *Arch Dermatol Res* 1999: 291: 425-431.
186. Lapczynski A, McGinty D, Jones L, Bhatia S, Letizia C S, Api A M. Fragrance material review on phenethyl salicylate. *Food Chem Toxicol* 2007: 45 Suppl 1: S467-471.
187. Sanchez-Politta S, Campanelli A, Pashe-Koo F, Saurat J H, Piletta P. Allergic contact dermatitis to phenylacetaldehyde: a forgotten allergen? *Contact Dermatitis* 2007: 56: 171-172.
188. McGinty D, Letizia C S, Api A M. Fragrance material review on phytol. *Food Chem Toxicol* 2010: 48 Suppl 3: S59-63.
189. Cachao P, Menezes Brandao F, Carmo M, Frazao S, Silva M. Allergy to oil of turpentine in Portugal. *Contact Dermatitis* 1986: 14: 205-208.
190. Lear J T, Heagerty A H M, Tan B B, et al. Transient re-emergence of oil of turpentine allergy in the pottery industry. *Contact Dermatitis* 1996: 34: 169-172.
191. Zacher K D, Ippen H. Kontaktekzem durch Bergamottöl. *Derm Beruf Umwelt* 1984: 32: 95-97.
192. Lapczynski A, Bhatia S P, Letizia C S, Api A M. Fragrance material review on rhodinol. *Food Chem Toxicol* 2008: 46 Suppl 11: S259-262.
193. Lapczynski A, Lalko J, McGinty D, Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-isodamascone. *Food Chem Toxicol* 2007: 45 Suppl 1: S267-271.
194. Bruze M, Zimerson E. Cross-reaction patterns in patients with contact allergy to simple methylol phenols. *Contact Dermatitis* 1997: 37: 82-86.
195. Barbaud A, Reichert-Penetrat S, Trechot P, Granel F, Schmutz J L. [Sensitization to resorcinol in a prescription verrucide preparation: unusual systemic clinical features and prevalence]. *Ann Dermatol Venereol* 2001: 128: 615-618.
196. Aalto-Korte K, Valimaa J, Henriks-Eckerman M L, Jolanki R. Allergic contact dermatitis from salicyl alcohol and salicylaldehyde in aspen bark (*Populus tremula*). *Contact Dermatitis* 2005: 52: 93-95.
197. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on alpha-santalol. *Food Chem Toxicol* 2008: 46 Suppl 11: S267-269.

198. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on santalol. *Food Chem Toxicol* 2008: 46 Suppl 11: S263-266.
199. Bhatia S P, McGinty D, Letizia C S, Api A M. Fragrance material review on sclareol. *Food Chem Toxicol* 2008: 46 Suppl 11: S270-274.
200. Bhatia S P, McGinty D, Foxenberg R J, Letizia C S, Api A M. Fragrance material review on terpineol. *Food Chem Toxicol* 2008: 46 Suppl 11: S275-279.
201. Bhatia S P, Letizia C S, Api A M. Fragrance material review on (-)-alpha-terpineol. *Food Chem Toxicol* 2008: 46 Suppl 11: S204-205.
202. Bhatia S P, Letizia C S, Api A M. Fragrance material review on alpha-terpineol. *Food Chem Toxicol* 2008: 46 Suppl 11: S280-285.
203. Castelain P Y, Camoin J P, Jouglard J. Contact dermatitis to terpene derivatives in a machine cleaner. *Contact Dermatitis* 1980: 6: 358-360.
204. Hausen B M, Reichling J, Harkenthal M. Degradation products of monoterpenes are the sensitizing agents in tea tree oil. *Am J Contact Dermat* 1999: 10: 68-77.
205. Lapczynski A, Foxenberg R J, Bhatia S P, Letizia C S, Api A M. Fragrance material review on tetrahydrolinalool. *Food Chem Toxicol* 2008: 46 Suppl 11: S286-288.
206. Schnuch A, Geier J, Uter W, Frosch P J. Majantol--a new important fragrance allergen. *Contact Dermatitis* 2007: 57: 48-50.
207. Heisterberg M V, Johansen J D. Contact allergy to trimethyl-benzenepropanol (Majantol). *Contact Dermatitis* 2009: 61: 360-361.
208. Hausen B M. Contact allergy to balsam of Peru. II. Patch test results in 102 patients with selected balsam of Peru constituents. *Am J Contact Dermat* 2001: 12: 93-102.
209. Rudzki E, Grzywa Z. Dermatitis from propolis. *Contact Dermatitis* 1983: 9: 40-45.
210. Ferguson J E, Beck M H. Contact sensitivity to vanilla in a lip salve. *Contact Dermatitis* 1995: 33: 352.
211. Bhatia S P, Letizia C S, Api A M. Fragrance material review on tricyclo[5.2.1.0^{2,6}]dec-4-en-8-yl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S100-101.
212. Bhatia S P, Jones L, Letizia C S, Api A M. Fragrance material review on tricyclodecenyl acetate. *Food Chem Toxicol* 2008: 46 Suppl 12: S93-96.
213. Anonymous. *ISO/DIS 9235 Aromatic raw materials - vocabulary*. Geneva, Switzerland: International Standardisation Organisation, 2010.
214. Schmidt E. Production of Essential Oils. In: Husnu Can Baser K, Buchbauer G, eds. *Handbook of Essential Oils - Science, Technology, and Applications*. Boca Raton: CRC Press, 2010: 88-95.
215. Trattner A, David M, Lazarov A. Occupational contact dermatitis due to essential oils. *Contact Dermatitis* 2008: 58: 282-284.
216. Jung P, Sesztak-Greinecker G, Wantke F, Gotz M, Jarisch R, Hemmer W. Mechanical irritation triggering allergic contact dermatitis from essential oils in a masseur. *Contact Dermatitis* 2006: 54: 297-299.
217. Bilsland D, Strong A. Allergic contact dermatitis from the essential oil of French marigold (*Tagetes patula*) in an aromatherapist. *Contact Dermatitis* 1990: 23: 55-56.
218. Cockayne S E, Gawkrödger D J. Occupational contact dermatitis in an aromatherapist. *Contact Dermatitis* 1997: 37: 306-307.

-
219. Boonchai W, Iamtharachai P, Sunthonpalin P. Occupational allergic contact dermatitis from essential oils in aromatherapists. *Contact Dermatitis* 2007: 56: 181-182.
 220. Keane F M, Smith H R, White I R, Rycroft R J. Occupational allergic contact dermatitis in two aromatherapists. *Contact Dermatitis* 2000: 43: 49-51.
 221. Selvaag E, Holm J O, Thune P. Allergic contact dermatitis in an aroma therapist with multiple sensitizations to essential oils. *Contact Dermatitis* 1995: 33: 354-355.
 222. Romaguera C, Vilaplana J. Occupational contact dermatitis from ylang-ylang oil. *Contact Dermatitis* 2000: 43: 251.
 223. Sugawara M, Nakayama H, Watanabe S. Contact hypersensitivity to ylang-ylang oil. *Contact Dermatitis* 1990: 23: 248-249.
 224. Kanerva L, Estlander T, Jolanki R. Occupational allergic contact dermatitis caused by ylang-ylang oil. *Contact Dermatitis* 1995: 33: 198-199.
 225. Sanchez-Perez J, Garcia-Diez A. Occupational allergic contact dermatitis from eugenol, oil of cinnamon and oil of cloves in a physiotherapist. *Contact Dermatitis* 1999: 41: 346-347.
 226. Vilaplana J, Romaguera C. Contact dermatitis from the essential oil of tangerine in fragrance. *Contact Dermatitis* 2002: 46: 108.
 227. Lalko J, Api A M. Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. *Food Chem Toxicol* 2006: 44: 739-746.
 228. Vilaplana J, Romaguera C. Allergic contact dermatitis due to eucalyptol in an anti-inflammatory cream. *Contact Dermatitis* 2000: 43: 118.
 229. Commission M. *List of MAK and BAT Values 2010 (Report No. 46)*. Weinheim: Wiley-VCH, 2011.
 230. Nardelli A, Gimenez-Arnau E, Bernard G, Lepoittevin J P, Goossens A. Is a low content in atranol/chloroatranol safe in oak moss-sensitized individuals? *Contact Dermatitis* 2009: 60: 91-95.
 231. Johansen J D, Heydorn S, Menne T. Oak moss extracts in the diagnosis of fragrance contact allergy. *Contact Dermatitis* 2002: 46: 157-161.
 232. Kanerva L, Jolanki R, Estlander T. Hairdresser's dermatitis caused by oak moss in permanent waving solution. *Contact Dermatitis* 1999: 41: 55-56.
 233. Owen C M, August P J, Beck M H. Contact allergy to oak moss resin in a soluble oil. *Contact Dermatitis* 2000: 43: 112.
 234. Rudzki E, Grzywa Z. Sensitizing and irritating properties of star anise oil. *Contact Dermatitis* 1976: 2: 305-308.
 235. Franz H, Frank R, Rytter M, Haustein U F. Allergic contact dermatitis due to cedarwood oil after dermatoscopy. *Contact Dermatitis* 1998: 38: 182-183.
 236. Adisen E, Önder M. Allergic contact dermatitis from *Laurus nobilis* oil induced by massage. *Contact Dermatitis* 2007: 56: 360-361.
 237. Athanasiadis G I, Pfab F, Klein A, Braun-Falco M, Ring J, Ollert M. Erythema multiforme due to contact with laurel oil. *Contact Dermatitis* 2007: 57: 116-118.
 238. Özden M G, Öztas P, Öztas M O, Önder M. Allergic contact dermatitis from *Laurus nobilis* (laurel) oil. *Contact Dermatitis* 2001: 45: 178.

-
239. Goiriz R, Delgado-Jimenez Y, Sanchez-Perez J, Garcia-Diez A. Photoallergic contact dermatitis from lavender oil in topical ketoprofen. *Contact Dermatitis* 2007; 57: 381-382.
 240. Rademaker M. Allergic contact dermatitis from lavender fragrance in Diffiam gel. *Contact Dermatitis* 1994: 31:
 241. Varma S, Blackford S, Statham B N, Blackwell A. Combined contact allergy to tea tree oil and lavender oil complicating chronic vulvovaginitis. *Contact Dermatitis* 2000; 42: 309-310.
 242. Coulson I H, Khan A S. Facial 'pillow' dermatitis due to lavender oil allergy. *Contact Dermatitis* 1999: 41: 111.
 243. Vermaat H, van Meurs T, Rustemeyer T, Bruynzeel D P, Kirtschig G. Vulval allergic contact dermatitis due to peppermint oil in herbal tea. *Contact Dermatitis* 2008; 58: 364-365.
 244. Kalavala M, Hughes T M, Goodwin R G, Anstey A V, Stone N M. Allergic contact dermatitis to peppermint foot spray. *Contact Dermatitis* 2007; 57: 57-58.
 245. Wilkinson S M, Beck M H. Allergic contact dermatitis from menthol in peppermint. *Contact Dermatitis* 1994: 30: 42.
 246. Andersen K E. Contact allergy to toothpaste flavors. *Contact Dermatitis* 1978: 4: 195-198.
 247. Clayton R, Orton D. Contact allergy to spearmint oil in a patient with oral lichen planus. *Contact Dermatitis* 2004: 51: 314-315.
 248. Skrebova N, Brocks K, Karlsmark T. Allergic contact cheilitis from spearmint oil. *Contact Dermatitis* 1998: 39: 35.
 249. Hänsel R, Keller K, Rimpler H, Schneider G. *Hagers Handbuch der pharmazeutischen Praxis. Drogen E - O*. Berlin, 894-902: Springer, 1993.
 250. Hausen B M, Wollenweber E. Propolis allergy. (III). Sensitization studies with minor constituents. *Contact Dermatitis* 1988: 19: 296-303.
 251. Hausen, M B, Evers, P, Stüwe, T H, et al. Propolis allergy (IV) Studies with further sensitizers from propolis and constituents common to propolis, poplar buds and balsam of Peru. *Contact Dermatitis* 1992: 26: 34-44.
 252. Hausen B M, Simatupang T, Bruhn G, Evers P, König W A. Identification of new allergenic constituents and proof of evidence for coniferyl benzoate in Balsam of Peru. *Am J Contact Dermat* 1995: 6: 199-208.
 253. Hjorth N. Eczematous allergy to balsams, allied perfumes and flavouring agents. *Acta Derm Venereol* 1961: 41 (Suppl. 46): 1-216.
 254. Wurm G. *Hagers Handbuch der pharmazeutischen Praxis. Waren und Dienste*. Berlin, 644-689: Springer, 1990.
 255. Api A M. Only Peru Balsam extracts or distillates are used in perfumery. *Contact Dermatitis* 2006: 54: 179.
 256. Temesvari E, Podanyi B, Ponyai G, Nemeth I. Fragrance sensitization caused by temporary henna tattoo. *Contact Dermatitis* 2002: 47: 240.
 257. Lammintausta K, Maibach H I, Wilson D. Mechanisms of subjective (sensory) irritation. Propensity to non- immunologic contact urticaria and objective irritation in stingers. *Derm Beruf Umwelt* 1988: 36: 45-49.
 258. Forsbeck M, Skog E. Immediate reactions to patch tests with balsam of Peru. *Contact Dermatitis* 1977: 3: 201-205.

-
259. Katsarou A, Armenaka M, Ale I, Koufou V, Kalogeromitros D. Frequency of immediate reactions to the European standard series. *Contact Dermatitis* 1999; 41: 276-279.
 260. Temesvari E, Soos G, Podanyi B, Kovacs I, Nemeth I. Contact urticaria provoked by balsam of Peru. *Contact Dermatitis* 1978; 4: 65-68.
 261. Cancian M, Fortina A B, Peserico A. Contact urticaria syndrome from constituents of balsam of Peru and fragrance mix in a patient with chronic urticaria. *Contact Dermatitis* 1999; 41: 300.
 262. Tanaka S, Matsumoto Y, Dlova N, Ostlere L S, Goldsmith P C, Rycroft R J, Basketter D A, White I R, Banerjee P, McFadden J P. Immediate contact reactions to fragrance mix constituents and Myroxylon pereirae resin. *Contact Dermatitis* 2004; 51: 20-21.
 263. Uter W, Lessmann H. Kontaktallergene. In: Schulze-Werninghaus G, Fuchs T, Bachert C, Wahn U, eds. *Manuale allergologicum*. Deisenhofen: Dustri, 2008: 237-308.
 264. Freireich-Astman M, David M, Trattner A. Standard patch test results in patients with contact dermatitis in Israel: age and sex differences. *Contact Dermatitis* 2007; 56: 103-107.
 265. Lazarov A. European Standard Series patch test results from a contact dermatitis clinic in Israel during the 7-year period from 1998 to 2004. *Contact Dermatitis* 2006; 55: 73-76.
 266. Gupta N, Shenoi S D, Balachandran C. Fragrance sensitivity in allergic contact dermatitis. *Contact Dermatitis* 1999; 40: 53-54.
 267. Kashani M N, Gorouhi F, Behnia F, Nazemi M J, Dowlati Y, Firooz A. Allergic contact dermatitis in Iran. *Contact Dermatitis* 2005; 52: 154-158.
 268. Avalos-Peralta P, Garcia-Bravo B, Camacho F M. Sensitivity to Myroxylon pereirae resin (balsam of Peru). A study of 50 cases. *Contact Dermatitis* 2005; 52: 304-306.
 269. Akyol A, Boyvat A, Peksari Y, Gurgey E. Contact sensitivity to standard series allergens in 1038 patients with contact dermatitis in Turkey. *Contact Dermatitis* 2005; 52: 333-337.
 270. Machovcova A, Dastychova E, Kostalova D, Vojtechovska A, Reslova J, Smejkalova D, Vaneckova J, Vocilkova A. Common contact sensitizers in the Czech Republic. Patch test results in 12,058 patients with suspected contact dermatitis*. *Contact Dermatitis* 2005; 53: 162-166.
 271. Thyssen J P, Carlsen B C, Menne T, Johansen J D. Trends of contact allergy to fragrance mix I and Myroxylon pereirae among Danish eczema patients tested between 1985 and 2007. *Contact Dermatitis* 2008; 59: 238-244.
 272. Lindberg M, Edman B, Fischer T, Stenberg B. Time trends in Swedish patch test data from 1992 to 2000. A multi-centre study based on age- and sex-adjusted results of the Swedish standard series. *Contact Dermatitis* 2007; 56: 205-210.
 273. Uter W, Hegewald J, Aberer W, Ayala F, Bircher A J, Brasch J, Coenraads P J, Schuttelaar M L, Elsner P, Fartasch M, Mahler V, Belloni Fortina A, Frosch P J, Fuchs T, Johansen J D, Menne T, Jolanki R, Krecisz B, Kiec-Swierczynska M, Larese F, Orton D, Peserico A, Rantanen T, Schnuch A. The European standard series in 9 European countries, 2002/2003 - First results of the European Surveillance System on Contact Allergies. *Contact Dermatitis* 2005; 53: 136-145.
 274. Bruynzeel D P, Diepgen T L, Andersen K E, Brandao F M, Bruze M, Frosch P J, Goossens A, Lahti A, Mahler V, Maibach H I, Menne T, Wilkinson J D. Monitoring

-
- the European standard series in 10 centres 1996-2000. *Contact Dermatitis* 2005: 53: 146-149.
275. Vilaplana J, Romaguera C, Grimalt F. Contact dermatitis from geraniol in Bulgarian rose oil. *Contact Dermatitis* 1991: 24: 301.
 276. Nardelli A, Thijs L, Janssen K, Goossens A. Rosa centifolia in a 'non-scented' moisturizing body lotion as a cause of allergic contact dermatitis. *Contact Dermatitis* 2009: 61: 306-309.
 277. Howes M J, Simmonds M S, Kite G C. Evaluation of the quality of sandalwood essential oils by gas chromatography-mass spectrometry. *J Chromatogr A* 2004: 1028: 307-312.
 278. Burdock G A, Carabin I G. Safety assessment of sandalwood oil (*Santalum album* L.). *Food Chem Toxicol* 2008: 46: 421-432.
 279. Treudler R, Richter G, Geier J, Schnuch A, Orfanos C E, Tebbe B. Increase in sensitization to oil of turpentine: recent data from a multicenter study on 45,005 patients from the German-Austrian Information Network of Departments of Dermatology (IVDK). *Contact Dermatitis* 2000: 42: 68-73.
 280. Schnuch A, Lessmann H, Geier J, Frosch P J, Uter W. Contact allergy to fragrances: frequencies of sensitization from 1996 to 2002. Results of the IVDK*. *Contact Dermatitis* 2004: 50: 65-76.
 281. Schäfer T, Böhler E, Ruhdorfer S, Weigl L, Wessner D, Filipiak B, Wichmann H E, Ring J. Epidemiology of contact allergy in adults. *Allergy* 2001: 56: 1192-1196.

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Annex II - Animal Data

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Substance		Vehicle (AOO=acetone:olive oil; DEP=diethyl phthalate; DMF=dimethyl formamide; DMSO=dimethyl sulphoxide; EtOH=ethanol; MEK=methyl ethyl ketone)	Conc. in vehicle (%, generally w/v)	No. animals per dose group	EC3 value *				Comment (deviation from OECD 429 etc)	Reference
INCI name (other name)	CAS no.				%	µg/cm ²	M	lowest for the substance (%)		
<i>Allyl phenoxyacetate</i>	7493-74-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	3.1	775	0.16	3.1		RIFM, 2007a
Amyl cinnamal	122-40-7	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	7.6	1900	0.38	7.6		RIFM, 2006a
Amyl cinnamal	122-40-7	4:1 AOO	-	4	10.6	2650	0.52		Elahi gives ref to Basketter et al 1999, but no data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment	Elahi et al., 2004

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Aptula gives ref to Kimber et al 2003, but no LLNA data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment; original reference is not given.	Aptula et al., 2007
Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Original ref not given.	Roberts et al., 2007
Amylcinnamyl alcohol	101-85-9	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	> 25	>6250	>1.22	> 25	Should have been tested at higher concentrations	RIFM, 2004a
Anise alcohol	105-13-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.9	1475	0.43	5.9		RIFM, 2005a
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref	Roberts et al., 2007
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Benzyl alcohol	100-51-6	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>4.62	> 50	Should have been tested at higher concentrations	RIFM, 2005b

Opinion on fragrance allergens in cosmetic products

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Benzyl benzoate	120-51-4	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>2.36	> 50	Should have been tested at higher concentrations	RIFM, 2005c
Benzyl cinnamate	103-41-3	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	18.4	4600	0.77	18.4		RIFM, 2005d
Benzyl salicylate	118-58-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.9	725	0.13	2.9		RIFM, 2005e
<i>p</i> -tert-Butyl-dihydrocinnamaldehyde	18127-01-0	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	4.3	1075	0.23	4.3		RIFM, 2007b
Butylphenyl methylpropional (BMHCA)	80-54-6	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	2.9	725	0.14	2.9		RIFM, 2001a
Butylphenyl methylpropional (BMHCA)	80-54-6	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	4.1	1025	0.20			RIFM, 2001b
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	13.9	3475	0.68			RIFM, 2001c
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 DEP:EtOH	0.3, 1.0, 3.0, 10.0, 30.0	4	8.8	2200	0.43			RIFM, 2001d
Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	16.8	4200	0.82			RIFM, 2001e

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Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1, 2.5, 10, 25, 50	4	18.7	4675	0.92		Basketter et al., 2001
Camellia sinensis leaf Tea Leaf Absolute	84650-60-2	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	> 5.0	>1250	N/a	> 5.0	Should have been tested at higher concentrations RIFM, 2005m
Cananga odorata leaf / flower oil Ylang Ylang Extra	8006-81-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	6.8	1700	N/a	6.8	RIFM, 2007f
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	10.7	2675	0.71		RIFM, 2007c
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.7	1425	0.38	5.7	RIFM, 2007d
Carvone	6485-40-1	4:1 AOO	6.0, 12, 20	4	13	3250	0.86		Nilsson et al., 2005
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015	0.2	RIFM, 2003a
Cinnamal	104-55-2	0.1% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015		RIFM, 2003b
Cinnamal	104-55-2	2.0% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.6	150	0.045		RIFM, 2003c
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003d

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EtOH:DEP

Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003e
Cinnamal	104-55-2	2.0% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.8	200	0.060		RIFM, 2003f
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.9	225	0.068		RIFM, 2003g
Cinnamal	104-55-2	0.1% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.1	275	0.083		RIFM, 2003h
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.3	325	0.098		RIFM, 2003i
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.4	350	0.11		RIFM, 2003j
Cinnamal	104-55-2	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamal	104-55-2	4:1 AOO	0.5, 1, 2.5, 5, 10	4	3.1	775	0.23		Basketter et al., 2001
Cinnamal	104-55-2	4:1 AOO	-	4	1.3	325	0.10		Elahi et al., 2004
Cinnamal	104-55-2	4:1 AOO	1, 2.5	-	1.4	348	0.11	Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Cinnamal	104-55-2	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	1.7	425	0.13		Wright et al., 1995

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Cinnamal	104-55-2	MEK	1.0, 2.5, 5.0, 10.0, 25.0	4	1.1	275	0.083		Wright et al., 1996
Cinnamal	104-55-2	DMF	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.5	125	0.038		Wright et al., 1997
Cinnamal	104-55-2	propylene glycol	1.0, 2.5, 5.0, 10.0, 25.0	4	1.4	350	0.11		Wright et al., 1998
Cinnamal	104-55-2	DMSO	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.9	225	0.068		Wright et al., 1999
Cinnamal	104-55-2	90:10 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.6	400	0.12		Wright et al., 2000
Cinnamal	104-55-2	50:50 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.2	300	0.091		Wright et al., 2001
Cinnamyl alcohol	104-54-1	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamyl nitrile	1885-38-7	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 10	>2500	>0.77	> 10	Report: systemic toxicity at 25% and 50%. Should have been tested at higher concentrations RIFM, 2005f
Citral	5392-40-5	1:3 EtOH:DEP	0.4, 2.0, 4.0, 8.0, 20.0	4	1.2	300	0.079	1.2	RIFM, 2004b
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	1.5	375	0.099		RIFM, 2003k

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Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	2.1	525	0.14		RIFM, 2003l
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	3.7	925	0.24		RIFM, 2003m
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003n
Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003o
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.3	1325	0.35		RIFM, 2003p
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.8	1400	0.38		RIFM, 2003q
Citral	5392-40-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	6.3	1575	0.41		RIFM, 2003r
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	6.8	1700	0.44		RIFM, 2003s
Citral	5392-40-5	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Citronellol	106-22-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	43.5	10875	2.78	43.5	RIFM, 2004c

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Coumarin	91-64-5	DMF	10, 25, 50	4	>50	>12500	>3.42	>50	Should have been tested at higher concentrations	Vocanson et al., 2006
<i>Dibenzyl ether</i>	103-50-4	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	6.3	1575	0.32	6.3		RIFM, 2007e
Eugenol	97-53-0	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	5.3	1325	0.32	5.3		RIFM, 2001f
Eugenol	97-53-0	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	10.5	2625	0.64			RIFM, 2001g
Eugenol	97-53-0	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	10.7	2675	0.65			RIFM, 2001h
Eugenol	97-53-0	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	15.1	3775	0.92			RIFM, 2001i
Eugenol	97-53-0	4:1 AOO	2.5, 5.0, 10.0, 25.0, 50.0	-	11.9	2975	0.72			Basketter et al., 1999
Eugenol	97-53-0	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	5.0, 10.0, 20	4	> 20	>5000	N/a	> 20	Should have been tested at higher concentrations	RIFM, 2004k
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	10.0, 25.0	4	> 25	>6250	N/a		Too few concentrations tested	RIFM, 2004d

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Evernia prunastri extract <i>Oakmoss</i>	90028-68-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.88	970	N/a	3.88		RIFM, 2004j
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	5.5	1375	0.25		Should also have been tested at lower concentrations	RIFM, 2004d
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	4.1	1025	0.18	4.1	Should also have been tested at lower concentrations	RIFM, 2004d
Geraniol	106-24-1	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	5.6	1400	0.36	5.6		RIFM, 2001j
Geraniol	106-24-1	3:1 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	11.4	2850	0.74			RIFM, 2003t
Geraniol	106-24-1	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	11.8	2950	0.76			RIFM, 2001k
Geraniol	106-24-1	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	20.4	5100	1.32			RIFM, 2001l
Geraniol	106-24-1	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	25.8	6450	1.67			RIFM, 2001m
Geraniol	106-24-1	-	-	-	26	6500	1.69			Roberts et al., 2007
<i>trans-2-Hexenal</i>	6728-26-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5, 10	4	2.6	650	0.26	2.6		RIFM, 2005g
<i>trans-2-Hexenal</i>	6728-26-3	-	-	-	5.5	1375	0.56			Roberts et al., 2007

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Hexyl cinnamal	101-86-0	generally 4:1 AOO		-O162	5.3-14.7	1325-3675	0.25-0.68	5.3		"numerous accounts in the literature"
2-Hexylidene cyclopentanone	17373-89-6	1:3 EtOH:DEP	0.1, 0.5, 1.0, 2.5, 5.0	5	2.4	600	0.14	2.4		RIFM, 2008a
Hexyl salicylate	6259-76-3	1:3 EtOH:DEP	0.05, 0.25, 0.5, 1.0, 2.5	4	0.18	45	0.008	0.18		RIFM, 2006b
Hydroxycitronellal	107-75-5	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.3	4825	1.12	19.3		RIFM, 2001n
Hydroxycitronellal	107-75-5	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.7	4925	1.14			RIFM, 2001o
Hydroxycitronellal	107-75-5	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	22.2	5550	1.29			RIFM, 2001p
Hydroxycitronellal	107-75-5	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	26.4	6600	1.53			RIFM, 2001q
Hydroxycitronellal	107-75-5	AOO	25, 50, 100	-	-	-	-		EC3 value not given	Ashby et al., 1995
Hydroxycitronellal	107-75-5	4:1 AOO	2.5, 5, 10, 25, 50	4	33.0	8250	1.92			Basketter et al., 2001
Hydroxycitronellal	107-75-5	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2002
Hydroxycitronellal	107-75-5	-	-	-	25.25	6313	1.47			Estrada et al., 2003

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Hydroxycitronellal	107-75-5	4:1 AOO	10, 25	-	23	5750	1.34		Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	4:1 AOO	1.0, 2.5, 5, 10, 25, 50	4	17.1	4275	0.81	17.1		RIFM, 2001r
<i>p</i> -Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	70% EtOH	10.0, 25.0, 50.0, 100.0	4	9.5	2375	0.46	9.5	Should also have been tested at lower concentrations	RIFM, 2001w
<i>Isocyclocitral</i>	1335-66-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	7.3	1825	0.48	7.3		RIFM, 2006c
<i>Isocyclogeraniol</i>	68527-77-5	1:3 EtOH:DEP	5.0, 10.0, 25.0, 50.0	4	> 25	>6250	>1.62	> 25	Should have been tested at higher concentrations	RIFM, 2005h
Isoeugenol	97-54-1	4:1 AOO	0.5, 5.0	6	0.54	145	0.033	0.54	Too few concentrations tested	RIFM, 2001s
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.6	150	0.037			RIFM, 2002a
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.76	191	0.046			RIFM, 2002b
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.79	199	0.048			RIFM, 2002c

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Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.19	296	0.072	RIFM, 2001t
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.28	320	0.078	RIFM, 2004e
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	6	1.54	385	0.094	RIFM, 2001u
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.95	488	0.119	RIFM, 2001v
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0		3.3	825	0.20	Basketter et al., 1999
Isoeugenol	97-54-1	-	-	-	-	-	-	No data in the ref (poster abstract) Basketter et al., 2002
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.3	325	0.079	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.3	825	0.20	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.8	450	0.11	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.1	775	0.19	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.6	400	0.097	Loveless et al., 1996
Isoeugenol	97-54-1	AOO	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	MEK	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	1.4	350	0.085	Wright et al., 2001

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Isoeugenol	97-54-1	propylene glycol	0.5, 1.0, 2.5, 5.0, 10.0	4	2.5	625	0.15			Wright et al., 2001
Isoeugenol	97-54-1	DMSO	0.5, 1.0, 2.5, 5.0, 10.0	4	0.9	225	0.055			Wright et al., 2001
Isoeugenol	97-54-1	90:10 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	1.8	450	0.11			Wright et al., 2001
Isoeugenol	97-54-1	50:50 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	4.9	1225	0.30			Wright et al., 2001
<i>Jasmine absolute (Grandiflorum)</i>	8022-96-6	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	5.9	1475	N/a	5.9		RIFM, 2006d
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	35.4	9100	N/a	35.4		RIFM, 2006e
<i>d-Limonene**</i>	5989-27-5	EtOH	10.0, 20.0, 50.0, 75.0, 100.0	4	< 10	< 250	<0.73	< 10	Should also have been tested at lower concentrations	RIFM, 2004l
<i>d-Limonene**</i>	5989-27-5	3:1 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	22.0	5500	1.61			RIFM, 2004m
<i>d-Limonene**</i>	5989-27-5	1:3 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	38.0	9500	2.79			RIFM, 2004n
<i>d-Limonene**</i>	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
<i>d-Limonene**</i>	5989-27-5	4:1 AOO	25, 50, 100	4	68.5	17125	5.03			Warbrick et al., 2001

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Linalool**	78-70-6	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Menthadiene-7-methyl formate	68683- 20-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 10	> 2500	>0.51	> 10	Should have been tested at higher concentrations	RIFM, 2008c
4-Methoxy- α -methyl benzenpropanal	5462- 06-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	23.6	5900	1.32	23.63		RIFM, 2004f
α -Methyl cinnamic aldehyde	101-39- 3	-	-	-	4.5	1125	0.31	4.5		Roberts et al., 2007
Methylenedioxyphenyl methylpropanal	1205- 17-0	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	16.4	4100	0.85	16.4		RIFM, 2005i
6-Methyl-3,5-heptadien-2- one	1604- 28-0	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 5	> 1250	>0.40	> 5	Should have been tested at higher concentrations	RIFM, 2008d
α -iso-Methylionone	127-51- 5	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	21.8	5450	1.06	21.8		RIFM, 2005j
Methyl octine carbonate	111-80- 8	-	-	-	2.5	635	0.15	2.5		Roberts et al., 2007

Opinion on fragrance allergens in cosmetic products

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Methyl 2-octynoate	111-12-6	1:3 EtOH:DEP	0.5, 1.0, 2.0, 5.0, 10.0	4	< 0.5	< 125	<0.032	< 0.5	Should also have been tested at lower concentrations	RIFM, 2005k
2-Methoxy-4-methylphenol	93-51-6	-	-	-	5.8	1450	0.42	5.8		Roberts et al., 2007
1-Octen-3-yl acetate	2442-10-6	1:3 EtOH:DEP	7.5, 15.0, 30.0	5	> 30	> 7500	>1.76	> 30	Should have been tested at higher concentrations	RIFM, 2004g
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	9.3	2325	0.62			RIFM, 2008b
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.95	987	N/a	3.95		RIFM, 2004h
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.5	625	N/a	2.5		RIFM, 2004i
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	0.5, 1.0, 2.5	4	>2.5	>625	N/a			RIFM, 2004i
Phenylacetaldehyde	122-78-1	4:1 AOO	2.5, 5, 10, 25, 50	4	3.0	750	0.25	3.0		Basketter et al., 2001

Opinion on fragrance allergens in cosmetic products

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Phenylacetaldehyde	122-78-1	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2003
3-Propylidenephthalide	17369-59-4	4:1 AOO	5, 10, 20	4 or 5	3.7	925	0.21	3.7	Should also have been tested at lower concentrations	Gerberick et al., 2004
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	25.14	6285	1.07	25.14		RIFM, 2005l
Trimethylbenzenepropanol <i>Majantol</i>	103694-68-4	4:1 AOO	3.0, 10.0, 30.0	4	~30	~7500	~1.68	30	Should have been tested at higher concentrations	RIFM, 2002d
Vanillin	121-33-5	4:1 AOO	2.5, 5, 10, 25, 50	4	>50.0	>1250	>3.3	>50.0		Basketter et al., 2001

* source of EC3 value value: % given in the RIFM report or references; µg/cm2 given in the RIFM report and RIFM poster; M calculated by SCCS working group

**material with low levels of oxidation according to RIFM, 2009

- = no data given; A216

References

- Aptula, N., Roberts, D.W., Schultz, T.W., Pease, C., 2007. Reactivity assays for non-animal based prediction of skin sensitisation potential. *Toxicology*, 231(2-3), 117-118
- Ashby J, Basketter D.A., Patton, D., Kimber I. 1995. Structure activity relationships in skin sensitization using the murine local lymph node assay. *Toxicology* 103:177-194
- Basketter, D.A., Gilmour, N., Dearman, R.J., Kimber, I., Ryan, C.A., Gerberick, F., 2003. Classification of skin sensitisation potency using the Local Lymph Node Assay. *The Toxicologist*, 72(S-1), 101
- Basketter, D. A., Lea, L. J., Dickens, A., Briggs, D., Pate, I., Dearman, R. J., Kimber, I., 1999. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. *Journal of Applied Toxicology*, 19(4), 261-266
- Basketter, D.A., Wright, Z., Gilmour, N.J., Ryan, C.A., Gerberick, G.F., Robinson, M.K., Dearman, R.J., Kimber, I., 2002. Prediction of human sensitization potency using local lymph node assay EC3 values. *The Toxicologist*, 66(1-S), 240
- Basketter, D. A., Wright, Z. M., Warbrick, E. V., Dearman, R. J., Kimber, I., Ryan, C. A., Gerberick, G. F., White, I. R., 2001. Human potency predictions for aldehydes using the local lymph node assay. *Contact Dermatitis*, 45(2), 89-94
- Elahi, E.N., Wright, Z., Hinselwood, D., Hotchkiss, S.A.M., Basketter, D.A., Smith Pease, C.K., 2004. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. *Chemical Research in Toxicology*, 17(3), 301-310
- Estrada, E., Patlewicz, G., Chamberlain, M., Basketter, D., Larbey, S., 2003. Computer aided Knowledge Generation for Understanding Skin Sensitization Mechanisms: The TOPS-MODE Approach. *Chem. Res. Toxicol.*, 16, 1226-1235
- Gerberick, G.F., Ryan, C.A., Kern, P.S., Dearman, R.J., Kimber, I., Patlewicz, G.Y., Basketter, D.A. 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. *Contact Dermatitis* 50, 274-288
- Loveless, S. E., Ladics, G. S., Gerberick, G. F., Ryan, C. A., Basketter, D. A., Scholes, E. W., House, R. V., Hilton, J., Dearman, R. J., Kimber, I., 1996. Further evaluation of the local lymph node assay in the final phase of an international collaborative trial. *Toxicology*, 108(1-2), 141-152
- Nilsson, A.-M., Bergstrom, M.A., Luthman, K., Nilsson, J.L.G., Karlberg, A.-T., 2005. An alpha,beta-unsaturated oxime identified as a strong contact allergen. Indications of antigen formation via several pathways. *Food and Chemical Toxicology*, 43(11), 1627-1636
- RIFM, 2001a. Local Lymph Node Assay on p-t-Butyl- α -methyl-hydrocinnamic aldehyde in EtOH . RIFM report number 37065 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2001b. Local Lymph Node Assay on p-t-Butyl- α -methyl-hydrocinnamic aldehyde in DEP. RIFM report number 37066 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2001c. Local Lymph Node Assay on p-t-Butyl- α -methyl-hydrocinnamic aldehyde in 1:3 EtOH:DEP. RIFM report number 37067 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2001d. Local Lymph Node Assay on p-t-Butyl- α -methyl-hydrocinnamic aldehyde in 1:3 DEP:EtOH. RIFM report number 37068 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2001e. Local Lymph Node Assay on p-t-Butyl- α -methyl-hydrocinnamic aldehyde in 4:1 acetone:olive oil. RIFM report number 41235. (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2001f. Local Lymph Node Assay on eugenol. RIFM report number 37076. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001g. Local Lymph Node Assay on eugenol. RIFM report number 37075. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001h. Local Lymph Node Assay on eugenol. RIFM report number 37073. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001i. Local Lymph Node Assay on eugenol. RIFM report number 37074. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001j. Local Lymph Node Assay on geraniol in ethanol. RIFM report number 37069 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001k. Local Lymph Node Assay on geraniol in DEP. RIFM report number 37070 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001l. Local Lymph Node Assay on geraniol in 1:3 EtOH:DEP. RIFM report number 37071 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001m. Local Lymph Node Assay on geraniol in 3:1 EtOH:DEP. RIFM report number 37072 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001n. Local Lymph Node Assay on hydroxycitronellal in 1:3 EtOH:DEP. RIFM report number 37079 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001o. Local Lymph Node Assay on hydroxycitronellal in DEP. RIFM report number 37078 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001p. Local Lymph Node Assay on hydroxycitronellal in 3:1 EtOH:DEP. RIFM report number 37080 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001q. Local Lymph Node Assay on hydroxycitronellal in EtOH. RIFM report number 37080 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001r. Local Lymph Node Assay on p-t-Butyl- α -methyl-hydrocinnamic aldehyde in 4:1 acetone:olive oil. RIFM report number 41235. Unpublished report from Unilever. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001s. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 59516. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001t. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42122. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001u. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 40676. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001v. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42120. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2001w. Local Lymph Node Assay on p-isobutyl- α -methyl hydrocinnamaldehyde in 70% Ethanol. RIFM report number 41055. Unpublished report from Givaudan. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002a. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42139. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002b. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42145. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002c. Local Lymph Node Assay on isoeugenol in 4:1 acetone:olive oil. RIFM report number 42123. Unpublished report from Firmenich. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2002d. Local Lymph Node Assay on majantol in 4:1 acetone:olive oil. RIFM report number 58693. Unpublished report from Symrise. (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003a. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP. RIFM report number 42032 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003b. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% tocopherol. RIFM report number 42033 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003c. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 2.0% tocopherol. RIFM report number 42040 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003d. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42034 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003e. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% Trolox C. RIFM report number 42036 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003f. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 2.0% tocopherol. RIFM report number 42035 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003g. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP. RIFM report number 42037 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003h. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% tocopherol. RIFM report number 42038 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003i. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42039 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003j. Local Lymph Node Assay on cinnamic aldehyde in 3:1 EtOH:DEP with 0.1% Trolox C. RIFM report number 42041 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003k. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with 0.1% tocopherol. RIFM report number 42028 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003l. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42025 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003m. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42026 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003n. Local Lymph Node Assay on citral in 3:1 EtOH:DEP. RIFM report number 42023 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003o. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42029 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003p. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with antioxidant mix. RIFM report number 42027 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003q. Local Lymph Node Assay on citral in 3:1 EtOH:DEP with 0.1% Trolox C. RIFM report number 42030 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003r. Local Lymph Node Assay on citral. RIFM report number 43822 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003s. Local Lymph Node Assay on citral. RIFM report number 42024 (RIFM, Woodcliff Lake, NJ, USA)

RIFM, 2003t. Local Lymph Node Assay on geraniol in 3:1 EtOH:DEP. RIFM report number 43812 (RIFM, Woodcliff Lake, NJ, USA)

- RIFM, 2004a. Local Lymph Node Assay on α -amylcinnamyl alcohol. RIFM report number 45128 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004b. Local Lymph Node Assay on Citral. RIFM report number 45126 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004c. Local Lymph Node Assay on d,l-Citronellol. RIFM report number 48752 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004d. Local Lymph Node Assay on farnesol RIFM report number 47136. Unpublished report from Symrise (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004e. Local Lymph Node Assay on isoeugenol RIFM report number 47326. Unpublished report from Firmenich (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004f. Local Lymph Node Assay on 4-methoxy- α -methyl benzenpropanal. RIFM report number 47809. Unpublished report from IFF (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004g. Local Lymph Node Assay on 1-Octen-3-yl acetate. RIFM report number 47809. Unpublished report from IFF (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004h. Local Lymph Node Assay on Peru Balsam Oil. RIFM report number 44372. (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004i. Local Lymph Node Assay on Peru Balsam Absolute. RIFM report number 44371. (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004j. Oakmoss absolute: Local lymph node assay. RIFM report number 43861 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004k. Treemoss absolute: Local lymph node assay. RIFM report number 44368 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004l. d-limonene: Local lymph node assay. RIFM report number 45756 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004m. d-limonene: Local lymph node assay. RIFM report number 45753 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004n. d-limonene: Local lymph node assay. RIFM report number 45755 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2004o. d-limonene: Local lymph node assay. RIFM report number 45754 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005a. Local Lymph Node Assay on anisyl alcohol. RIFM report number 45755 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005b. Local Lymph Node Assay on benzyl alcohol. RIFM report number 47376 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005c. Local Lymph Node Assay on benzyl benzoate. RIFM report number 47377 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005d. Local Lymph Node Assay on benzyl cinnamate. RIFM report number 48751 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005e. Local Lymph Node Assay on benzyl salicylate. RIFM report number 47378 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005f. Local Lymph Node Assay on cinnamyl nitrile. RIFM report number 51626 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005g. Local Lymph Node Assay on trans-2-hexenal in 1:3 EtOH:DEP. RIFM report number 48756 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005h. Local Lymph Node Assay on isocyclogeraniol in 1:3 EtOH:DEP. RIFM report number 48755 (RIFM, Woodcliff Lake, NJ, USA)

- RIFM, 2005i. Local Lymph Node Assay on α -Methyl-1,3-benzodioxole- 5-propionaldehyde in 1:3 EtOH:DEP. RIFM report number 50886 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005j. Local Lymph Node Assay on α -iso-Methylionone in 1:3 EtOH:DEP. RIFM report number 48749 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005k. Local Lymph Node Assay on Methyl 2-octynoate in 1:3 EtOH:DEP. RIFM report number 48753 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005l. Local Lymph Node Assay on OTNE in 1:3 EtOH:DEP. RIFM report number 51630 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2005m. Local Lymph Node Assay on tea leaf absolute. RIFM report number 47597. Unpublished report from Robertet (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2006a. Local Lymph Node Assay on α -amylcinnamaldehyde. RIFM report number 52888 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2006b. Local Lymph Node Assay on hexyl salicylate. RIFM report number 51636 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2006c. Local Lymph Node Assay on isocyclocitral. RIFM report number 52892 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2006d. Local Lymph Node Assay on Jasmine Absolute (Grandiflorum). RIFM report number 53024 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2006e. Local Lymph Node Assay on Jasmine Absolute (Sambac). RIFM report number 52885 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2007b. Local Lymph Node Assay on p-t-Butyl-dihydrocinnamaldehyde. RIFM report number 52900 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2007c. Local Lymph Node Assay on carvone. RIFM report number 52902 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2007d. Local Lymph Node Assay on carvone. RIFM report number 52907 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2007e. Local Lymph Node Assay on dibenzyl ether. RIFM report number 52901 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2007f. Local Lymph Node Assay on Ylang Ylang Extra. RIFM report number 52903 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2008a. Local Lymph Node Assay on 2-hexylidene cyclopentanone. RIFM report number 55548 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2008b. Local Lymph Node Assay on p-mentha-1,8-dien-7-al. RIFM report number 54428 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2008c. Local Lymph Node Assay on menthadiene-7-methyl formate. RIFM report number 54429 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM, 2008d. Local Lymph Node Assay on 6-methyl-3,5-heptadien-2-one. RIFM report number 55564 (RIFM, Woodcliff Lake, NJ, USA)
- RIFM. 2009. Research Institute for Fragrance Materials, Inc. Local lymph node assay (LLNA) protocol summaries: Data presented at the 46th Congress of the European Societies of Toxicology
- Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. *Chemical Research in Toxicology*, 20(7), 1019-1030

Smith, C.K., Hotchkiss, S.A.M., 2001. Allergic Contact Dermatitis. Taylor & Francis, New York

Vocanson, M., Goujon, C., Chabeau, G., Castelain, M., Valeyrie, M., Floch, F, Maliverney, C., Gard A., Nicolas, J.F 2006. The skin allergenic properties of chemicals may depend on contaminants - evidence from studies on coumarin. International Archives of Allergy and Immunology, 140, 231-238

Warbrick, E.V.R., Dearman J., Ashby J., Schmezer P. and Kimber I. 2001. Preliminary assessment of the skin sensitizing activity of selected rodent carcinogens using the local lymph node assay. Toxicology, 163(1), 63-69

Wright, Z. M., Basketter, D. A., Blaikie, L., Cooper, K. J., Warbrick, E. V., Dearman, R. J., Kimber, I., 2001. Vehicle effects on skin sensitization potency of four chemicals assessment using the local lymph node assay. International Journal of Cosmetic Science, 23(2), 75-83

Annex III - Tabular summary of dose-elicitation studies in sensitised patients**Contents**

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Chloroatranol

Chloroatranol (allergen in oak moss absolute: <i>Evernia prunastri</i>) (1)	
Design	blinded, randomised with regard to doses and controlled
Test subjects	13 patients previously identified as sensitized to chloroatranol and oak moss absolute
Controls	10 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	200 ppm to 0.0063 ppm (10 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction
ROAT	volar aspect of forearms
area	3 x 3 cm ²
applications/day	two
dose	chloroatranol in ethanol: Step 1: 5 ppm Step 2: 25 ppm
dose/application/cm ²	step 1: 0.025 µg step2: 0.125 µg
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step
Results	
PT ED10% (95% CI)	0.013 (0.002-0.03) ppm =0.0004 µg/cm ²
PT ED50% (95% CI)	0.15 (0.077-0.295) ppm =0.0045 µg/cm ²
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (5 ppm)	12/13 (92%)
Step 2 (25 ppm)	13/13 (100%)
Controls	Negative
Other information	None relevant

In a subsequent study chloroatranol and atranol, both ingredients in *Evernia prunastri*, were tested in equimolar concentrations in serial dilution in 10 eczema patients with known sensitization to chloroatranol and oak moss. A positive response was defined as any degree of reaction. Ethanol was included as the control and gave no response. No use tests were done and no control subjects included.

Results: All patients reacted to the highest concentrations of the two substances. For both substances there was a significant dose-dependence and the estimated difference in elicitation potency of chloroatranol relative to atranol was 217%. The dose-response curve is seen in figure 1 below (2).

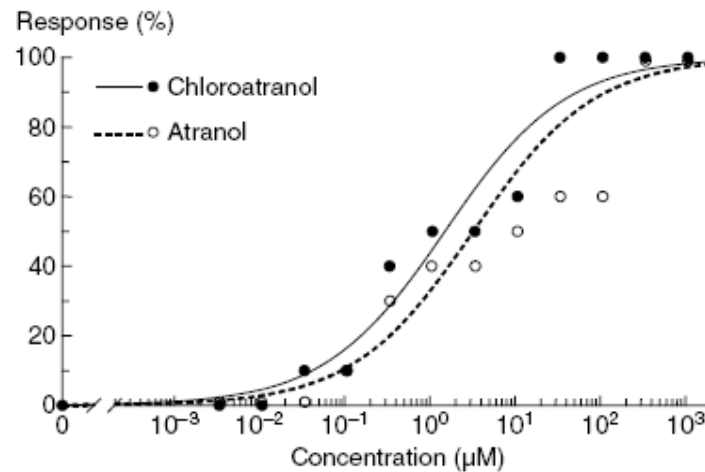


Fig. 1. Observed response rates and fitted parallel logistic dose-response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

Cinnamal

Cinnamal (3)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to cinnamal and additional 4 with a doubtful response
Controls	20 healthy controls
Substance	Purity: >98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (7 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of responses
ROAT	outer aspect of upper arm
area	5 x 5 cm ²
applications/day	two with atomizer pump
dose	Step 1: 0.02% Step 2: 0.1% Step 3: 0.8%
dose/application/cm ²	Not given
control substance	ethanol
definition of positive	The response was classified as positive no matter the degree of reaction.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.24% = 96 µg/cm ² (calculated from the data in the paper)
PT no effect level(observed)	0.01 % in pet. = 0.4 µg/cm ²
ROAT	Cumulative responses
Step 1 (0.02%)	0/18
Step 2 (0.1%)	8/18 (44 %)
Step 3 (0.8%)	13/18 (72 %)
Controls	No eczema reactions were seen
Other information	2 patients and 2 controls developed immediate reactions to the cinnamal solution

Cinnamal (4)	
Design	blinded, randomised doses and controlled
Test subjects	17 patients with a positive patch test to cinnamal (8 patients in part 1 and 9 in part two)
Controls	20 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2 % to 0.00006 % (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting a + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (89-700 mg per application of solution) average cases: 263 mg/application controls: only range given
dose	Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32% Part two: Step 1: 0.01% Step 2: 0.032% Step 3: 0.1%
dose/application/cm ²	Part two estimated: step one: 0.26 µg; step two: 0.84 µg; 2.63 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	0.002%
ROAT	Cumulative responses
Step 1 (0.01)	2/9 (22%)
Step 2 (0.032)	6/9 (67%)
Step 3 (0.1)	8/9 (88%)
Controls	No reactions were seen
Other information	Only reactions seen to the cinnamal-containing deodorants at ROAT, difference to matrix axilla ($p < 0.001$) and all control

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	persons negative ($p < 0.001$)

Hydroxycitronellal

Hydroxycitronellal (5)	
Design	blinded, randomised doses and controlled
Test subjects	7 patients with a positive patch test to hydroxycitronellal
Controls	7 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	4% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (172-591 per application of solution) average cases: 294 mg/application controls: only range given
dose	Step 1: 0.032% Step 2: 0.1% Step: 0.32%
dose/application/cm ²	Estimated: step 1: 0.94 µg; step 2: 2.94 µg; step 3: 9.40 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	<0.00012 %
ROAT	Cumulative responses
Step 1 (0.032)	4/7 (57%)
Step 2 (0.1)	5/7 (71%)
Step 3 (0.32)	7/7 (100%)
Controls	No reactions were seen
Other information	Reactions were only seen to the hydroxycitronellal-containing deodorant at ROAT, difference to matrix treated axilla ($p<0.001$) and all control persons negative ($p<0.001$)

Hydroxycitronellal (6)	
Design	double blinded, randomised
Test subjects	13 patients with a positive patch test to hydroxycitronellal
Controls	/
Substance	purity: unknown
Patch test	confirmatory
-dilution steps	
-control/vehicle	
-definition of threshold	
ROAT	finger immersion in fragrance solution in 10% ethanol
area	/
applications/day	Once per day for 10 min
dose	Step 1: 10 ppm Step 2: 250 ppm
dose/application/cm ²	Not applicable
control substance	10% alcohol
definition of positive	clinical grading scale and laser doppler comparison between active and control
period	two weeks with each concentration: maximum four weeks
Results	
PT ED10% (95% CI)	Not relevant
PT ED50% (95% CI)	Not relevant
PT no effect level(observed)	Not relevant
ROAT	Cumulative responses
Step 1 (10 ppm)	1/13
Step 2 (250 ppm)	5/13
Vehicle control	4/13
Other information	No difference between active substance and control application was found.

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (7)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to HICC
Controls	7 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006%
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	volar aspect of lower arm
area	3 x 3 cm ²
applications/day	two with droplet bottle (theoretical:30 mg per application of solution)
dose	Step 1: 0.5% Step 2: 3%
µg/application/cm ²	Step 1: 15.3 (3.4-22.2) Step 2: 126.2 (40.5-226.2)
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step; total maximum 4 weeks
Results	
PT ED10% (95% CI)	0.9 µg/cm ² 29 (7-69) ppm
PT ED50% (95% CI)	20 µg/cm ² 662 (350-1250)ppm
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (0.5%)	11/18 (61%)
Step 2 (3%)	16/18 (89%)
Controls	No reactions were seen
Other information	Difference between test and control group statistically significant

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (8)	
Design	blinded, randomised and controlled
Test subjects	15 patients with a positive patch test to HICC
Controls	10 healthy controls
Substance	Purity: > 98.8%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006% (5 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	Axilla
area	76 cm ² (template)
applications/day	two with roll on deodorant
dose	Step 1: 200 ppm Step 2: 600 ppm Step 3: 1800 ppm
dose/application/cm ²	median 0.79 µg HICC
control substance	deodorant matrix
definition of positive	spotty erythema involving at least 25% of the exposed area and infiltration represented by at least one papule.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	0.75 µg/cm ² 25 ppm (0.69-120)
PT ED50% (95% CI)	18.3 µg/cm ² 610 ppm (120-2800)
PT no effect level (observed)	< 0.0006%
ROAT	Cumulative responses
Step 1 (200 ppm)	9/14* (64%)
Step 2 (600 ppm)	12/14* (86%)
Step 3 (1800 ppm)	14/14* (100%)
Controls	No reactions were seen
Other information	*14 patients completed the use test study Difference between HICC deodorant and matrix deodorant in cases ($p=0.0001$). Difference between controls and patients ($p=0.004$).

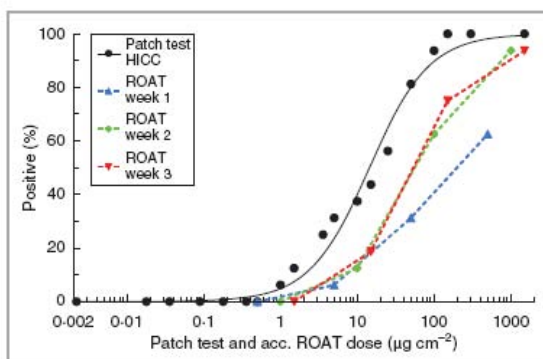
Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (9)	
Design	blinded, randomised and controlled
Test subjects	17 patients with a positive patch test to HICC
Controls	15 healthy controls
Substance	IFF lot SM/8059062
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	1500 to 0.0022 µg/cm ² HICC (19 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of forearms
area	3 x 3 cm (5 areas)
applications/day	two with micropipette (20 µl per application)
dose	Simultaneous application to 5 areas, four doses each and vehicle
µg /application/cm ²	Dose 1:0.0357 Dose 2: 0.357 Dose 3: 3.57 Dose 4: 35.7
control substance	ethanol
definition of positive	at least 5 points on a clinical scale, corresponding to erythema in 25% of test area and at least 1 papule
period	Three weeks. All concentrations applied simultaneously (randomised)
Results	
PT ED10% (95% CI)	0.662 µg/ cm ² (0.052-2.35)
PT ED50% (95% CI)	11.1 µg/ cm ² (3.41- 33.1)
PT no effect level(observed)	<0.0022 µg/ cm ²
ROAT	Cumulative responses
Dose 1 (0.0357)	0/16*
Dose 2 (0.357)	3/16 (19%)
Dose 3 (3.57)	12/16 (75%)
Dose 4 (35.7)	15/16 (94%)
Controls	No reactions were seen
Other information	*16 patients completed the use test study The evaporation rate of HICC was calculated to 72% over a 24-h period. ED10% ROAT: 0.064 µg/cm ² (more info see below)

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Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 1 week	Total accumulated dose after 1 week ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 2 weeks	Total accumulated dose after 2 weeks ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 3 weeks	Total accumulated dose after 3 weeks ($\mu\text{g HICC cm}^{-2}$)
35.7	14	500	28	1000	42	1500
3.57	14	50	28	100	42	150
0.357	14	5	28	10	42	15
0.0357	14	0.5	28	1	42	1.5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

Fig 3. The fitted dose-response curve for the patch test ($n = 16$) and the 1-week, the 2-week 3-week accumulated ROAT doses.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

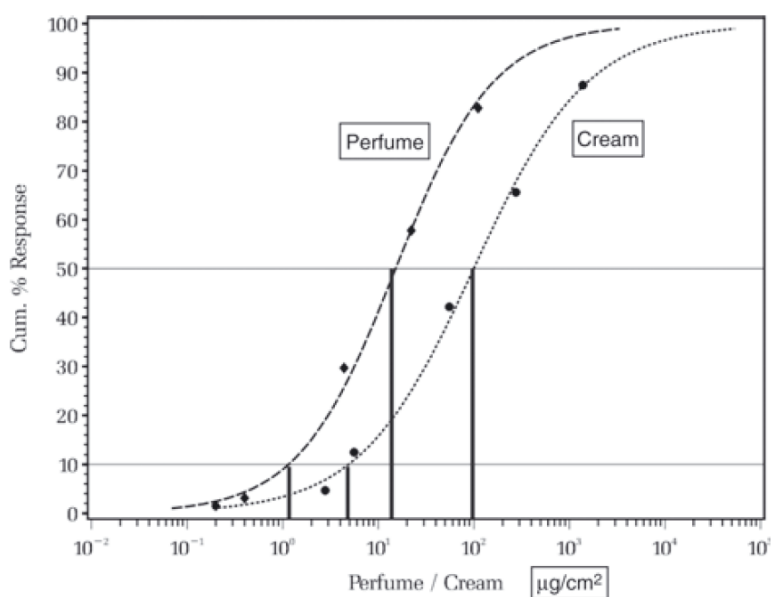
In a study by the German Contact Dermatitis Group, 64 persons previously diagnosed with HICC contact allergy were exposed to increasing doses of HICC in 2 different formulations, a hydrophilic cream and an ethanol solution, to mimic everyday exposures, following a standardised ROAT protocol (10). The concentration of HICC tolerated by 90% of the sensitised was estimated as 1.2 µg/cm² for perfume and 4.9 µg/cm² for cream. The dose-response curve is shown in Fig. 4.3 – 1 below.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (10)			
Design	randomised and vehicle controlled		
Test subjects	67 patients with a previous positive patch test to HICC		
Controls	None		
Substance	Provided by International Flavor & Fragrances Inc, Hilversum, NL		
Patch test			
-dilution steps	2.5% and 5%		
-control/vehicle	petrolatum		
-definition of threshold	lowest concentration giving a positive skin reaction in a continuous line to next higher concentration.		
ROAT	Volar forearms (both sides)		
area	3 x 3 cm (4 areas: one test and one control each for alcoholic solution and cream, respectively)		
applications/day	two		
dose	2.8 µg/cm ² in cream	0.2 µg/cm ² in ethanol	
	5.6 µg/cm ² in cream	0.4 µg/cm ² in ethanol	
	55.6 µg/cm ² in cream	4.4 µg/cm ² in ethanol	
	277.8 µg/cm ² in cream	22.2 µg/cm ² in ethanol	
	1388.9 µg/cm ² in cream	111.1 µg/cm ² in ethanol	
µg /application/cm ²	See above		
control substance	Ethanol 96% and glyceryl stearate 15% in water, resp.		
definition of positive	(spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number		
period	Two weeks for each step until positive reaction or end of study, whichever occurred first		
Results			
PT ED10% (95% CI)	Not calculable; 52 of 60 Patients patch tested positive to 2.5% HICC, 57 / 60 to 5% HICC		
PT ED50% (95% CI)	Not calculable		
PT no effect level (observed)	Not calculable		
ROAT	Cumulative responses:		
	Cream preparation: 2.8 µg/cm ² : 4.7%	Ethanol preparation: 0.2 µg/cm ² : 1.6%	

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	5.6 $\mu\text{g}/\text{cm}^2$: 12.5% 55.6 $\mu\text{g}/\text{cm}^2$: 42.2% 277.8 $\mu\text{g}/\text{cm}^2$: 65.6% 1388.9 $\mu\text{g}/\text{cm}^2$: 87.5%	0.4 $\mu\text{g}/\text{cm}^2$: 3.1% 4.4 $\mu\text{g}/\text{cm}^2$: 29.7% 22.2 $\mu\text{g}/\text{cm}^2$: 57.8% 111.1 $\mu\text{g}/\text{cm}^2$: 82.8%
Controls	No reactions to vehicle in the patients included into analysis	
Other information	See figure below. Three patients were excluded from the study, so results are based on 64 patients.	

Figure 4.3 – 1: Dose-response curve of 64 patients sensitised to HICC, according to a previous PT, regarding two preparations: perfume and cream, the rhomboid and dot symbol, respectively, indicating the observed response. The curve was fitted by a logistic function (10).



Isoeugenol

Isoeugenol (11)	
Design	blinded, randomised doses and controlled
Test subjects	20 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (8 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line
ROAT	outer aspect of upper arms
area	5 x 5 cm (2 areas: one test and one control)
applications/day	two with roll-on
dose	0.2% in ethanol
μg /application/ cm^2	Doses measured to 0.14 -0.13 mg/application the first 14 days = 5.6 $\mu\text{g}/\text{cm}^2$
control substance	ethanol
definition of positive	any degree of reaction
period	Two weeks at upper arm and if negative another two weeks including application to base of neck
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.08% 32 $\mu\text{g}/\text{cm}^2$
PT no effect level (observed)	< 0.01% = 0.4 $\mu\text{g}/\text{cm}^2$
ROAT	
Dose: 0.2%	12/19 (63%)
Controls	No reactions were seen
Other information	

Isoeugenol (12)	
Design	blinded, randomised
Test subjects	27 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of lower arm
area	3 x 3 cm (2 areas)
applications/day	two with droplet bottle (30 mg per application)
dose	0.05% in ethanol and 0.2%
µg /application/cm ²	Doses were calculated as mean 2.2 µg/cm ² (low conc.) and 9 µg/cm ² (high conc.)
control substance	ethanol
definition of positive	clear visible erythema
period	28 days
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	< 0.0005% (5 ppm)
ROAT	Cumulative responses
Dose 1: 0.05%	10/24 (42%)
Dose 2: 0.2%	16/24 (67%)
Controls	No reactions were seen
Other information	Response to the low concentration in the ROAT appeared after median 15 days and to the high concentration after median 7 days.

Isoeugenol (13)	
Design	blinded, randomised and controlled
Test subjects	13 patients with a positive patch test to isoeugenol and 4 in part 1 (pre-test)
Controls	10 healthy controls (dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (w/v) (16 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting at least + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll-on deodorant (117-586 mg per application of solution) average cases: 266 mg/application controls: only range given
dose	Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2% Part 2: Step1:0.0063% Step 2:0.02% Step 3: 0.063%
dose/application/cm ²	Part 2: Step 1: 0.167 Step 2: 0.53 Step 3: 1.67 µg/application/cm ² (calculated based on data)
control substance	deodorant matrix
definition of positive	eczematous response covering 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	<0.0005% (0.15 µg/cm ²)
ROAT	
Step 1 (0.0063%)	3/13 (23%)
Step 2 (0.02%)	9/13 (69%)
Step 3 (0.063%)	10/13 (77%)
Controls	No reactions were seen
Other information	Deodorants containing cinnamal were responsible for all reactions in cinnamal sensitized individuals ($p<0.001$) and all control persons were negative ($p<0.001$)

References

- 1 Johansen J D, Andersen K E, Svedman C, Bruze M, Bernard G, Gimenez-Arnau E, Rastogi S C, Lepoittevin J P, Menne T. Chloroatranol, an extremely potent allergen hidden in perfumes: a dose-response elicitation study. *Contact Dermatitis* 2003; **49**: 180-4.
- 2 Johansen J D, Bernard G, Gimenez-Arnau E, Lepoittevin J P, Bruze M, Andersen K E. Comparison of elicitation potential of chloroatranol and atranol--2 allergens in oak moss absolute. *Contact Dermatitis* 2006; **54**: 192-5.
- 3 Johansen J D, Andersen K E, Rastogi S C, Menne T. Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. *Contact Dermatitis* 1996; **34**: 165-71.
- 4 Bruze M, Johansen J D, Andersen K E, Frosch P, Lepoittevin J P, Rastogi S, Wakelin S, White I, Menne T. Deodorants: an experimental provocation study with cinnamic aldehyde. *J Am Acad Dermatol* 2003; **48**: 194-200.
- 5 Svedman C, Bruze M, Johansen J D, Andersen K E, Goossens A, Frosch P J, Lepoittevin J P, Rastogi S, White I R, Menne T. Deodorants: an experimental provocation study with hydroxycitronellal. *Contact Dermatitis* 2003; **48**: 217-23.
- 6 Heydorn S, Menne T, Andersen K E, Bruze M, Svedman C, Basketter D, Johansen J D. The fragrance hand immersion study - an experimental model simulating real-life exposure for allergic contact dermatitis on the hands. *Contact Dermatitis* 2003; **48**: 324-30.
- 7 Johansen J D, Frosch P J, Svedman C, Andersen K E, Bruze M, Pirker C, Menne T. Hydroxyisohexyl 3-cyclohexene carboxaldehyde- known as Lylal: quantitative aspects and risk assessment of an important fragrance allergen. *Contact Dermatitis* 2003; **48**: 310-6.
- 8 Jorgensen P H, Jensen C D, Rastogi S, Andersen K E, Johansen J D. Experimental elicitation with hydroxyisohexyl-3-cyclohexene carboxaldehyde-containing deodorants. *Contact Dermatitis* 2007; **56**: 146-50.
- 9 Fischer L A, Menné T, Avnstorp C, Kasting G B, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde allergy: relationship between patch test and repeated open application test thresholds. *Br J Dermatol* 2009; **161**: 560-7.
- 10 Schnuch A, Uter W, Dickel H, Szliska C, Schliemann S, Eben R, Rueff F, Gimenez-Arnau A, Loffler H, Aberer W, Frambach Y, Worm M, Niebuhr M, Hillen U, Martin V, Jappe U, Frosch P J, Mahler V. Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients. *Contact Dermatitis* 2009; **61**: 152-62.
- 11 Johansen J D, Andersen K E, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. *Contact Dermatitis* 1996; **34**: 414-8.
- 12 Andersen K E, Johansen J D, Bruze M, Frosch P J, Goossens A, Lepoittevin J P, Rastogi S, White I, Menne T. The time-dose-response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001; **170**: 166-71.
- 13 Bruze M, Johansen J D, Andersen K E, Frosch P, Goossens A, Lepoittevin J P, Rastogi S C, White I, Menne T. Deodorants: an experimental provocation study with isoeugenol. *Contact Dermatitis* 2005; **52**: 260-7.



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

20 January 2012
EMA/67070/2012

Assessment report for suppositories containing terpenic derivatives

INN/active substances: camphor, cineole, niaouli, wild thyme, terpineol, terpine, citral, menthol and essential oils of pine needle, eucalyptus and turpentine

Procedure number: EMEA/H/A-1284

Referral under Article 31(2) of Directive 2001/83/EC, as amended

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.



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1. Background information on the procedure

1.1. Referral of the matter to the CHMP

On 27 October 2010, France triggered a referral under Article 31(2) of Directive 2001/83/EC, as amended. The CHMP was requested to give its opinion on whether the marketing authorisations for suppositories containing terpenic derivatives, should be maintained, varied, suspended or revoked for use in children less than 30 months.

The procedure described in Article 32 of Directive 2001/83/EC, as amended, was applicable.

2. Scientific discussion

2.1. Introduction

Suppositories containing terpenic derivatives have been authorised through national procedures in Europe since the 1950s and are currently authorized and marketed in 7 European countries (France, Belgium, Portugal, Spain, Italy, Luxembourg and Finland). Patient exposure is the largest in France, followed by Spain and Italy but usage patterns do not vary widely between countries. The products are currently marketed with the following indications:

- Adjunctive (or supportive) treatment of benign acute bronchial disorders.
- Airway diseases associated with cough and expectoration
- Fluidizing and expectorant in acute and chronic respiratory tract disorders
- Symptomatic treatment of respiratory disorders accompanied by cough and phlegm
- Symptomatic treatment of non productive cough
- Antipyretic treatment during benign acute bronchial episodes
- Prophylaxis and treatment of inflammatory diseases of upper airways. Cough, flu situations, acute and chronic catarrh of respiratory airways (bronchitis)

French safety review of use of suppositories containing terpenic derivatives in children

In 1996, the French MA Advisory Group established two lists of terpenic derivatives, based on toxicology and pharmacovigilance data. Group A includes terpenic derivatives with well documented potential toxicity (camphor, eucalyptol or cineole, menthol, levomenthol; essential oil of eucalyptus, niaouli, cajepout, and mint) while Group B includes terpenic derivatives with less well documented potential toxicity (terpineol, terpinol, thymol, alpha-pinene, beta-pinene, eugenol, linalol, and carvacrol; essential oil of pine, fir, turpentine, anise, Chinese anise tree, serpolet, clove, and cedar). Terpenic derivatives belonging to group A were contraindicated in children less than 30 months when used by cutaneous or inhaled route. This contraindication was also applied to children with a history of febrile convulsions, for terpenic derivatives belonging to groups A and B.

A first safety review was performed in 2006 and 2007 by the Afssaps and focused on available safety data provided by the MAHs of suppositories containing terpenic derivatives (from 1997 to the end of 2005 or 2006 depending on the MAH).

A second safety review of use of suppositories containing terpenic derivatives in children was completed in May 2010, following the identification of a potential neurological risk, mainly represented by convulsions, based on more recent cases since June 2007 from the national pharmacovigilance database and cases received by MAHs since 1997.

A total of 92 cases of adverse drug reactions (ADRs) were identified in the national pharmacovigilance database and the Periodic Safety Update Reports (PSURs), with about 82% (76/92) of these cases occurring in children less than 30 months. Thirty cases related to neurological disorders, including convulsions without hyperthermia (11 cases), agitation (5 cases), convulsive equivalent, hyperthermic convulsions, status epilepticus with intensive care management, hallucinations, hypothermia and somnolence. Twenty-one serious cases with plausible relationship were reported and for the cases where time to onset was reported, the neurological ADRs occurred on the day of treatment initiation. The outcome was favourable in all cases. There were also 47 cases of drug misuse, of which 42 were not associated with any ADRs. Medication errors included 5 neurological ADRs. In most cases, the

errors concerned the use of a child formulation instead of the infant formulation. Six cases of local irritation and one case of rectorrhagia with favourable outcome were notified, together with 12 cases of cutaneous ADRs and 2 cases of respiratory ADRs.

Based on these results, the National Pharmacovigilance Board and the MA Advisory Group recommended a contraindication of suppositories containing terpenic derivatives in children less than 30 months (in line with other routes of administration of terpenic derivatives in France) and children with history of febrile convulsion or epilepsy.

France subsequently circulated a Non-Urgent Information (NUI) to all member states to gather information on authorised suppository products containing terpenic active ingredients for children less than 30 months, including camphor, cineole, niaouli, wild thyme, terpineol, terpine, citral, menthol and essential oils of pine needle, eucalyptus and turpentine. Based on the NUI responses, France considered that the concerns of a potential neurological risk, mainly represented by convulsions associated with the use in children of terpenic medicines warranted a Community-level assessment. France therefore submitted a notification to the CHMP on 27th October 2010, requesting a referral procedure under Article 31(2) of Directive 2001/83/EC, to assess the benefit-risk of suppositories containing terpenic derivatives in children less than 30 months. The procedure started in November 2010.

2.2. Assessment of clinical efficacy and safety

Camphor, cineole (eucalyptol) and menthol

Regarding suppositories containing camphor, eucalyptol and menthol, the MAH stated that there is a lack of medicinal/scientific data (no available data in the MAH pre-clinical and clinical files and no data published in the scientific literature) but that no post-marketing spontaneous report was received from any source during the period of marketing in France. Consequently, the MAH could not perform any analysis on the possible risk factors/predictability of nervous system adverse reactions for cineole.

The CHMP noted the absence of spontaneous data. The MAH mentioned a recent published study in Pediatrics by Paul et al., 2010, investigating the efficacy and safety of a single topical application of a camphor, menthol and eucalyptus oil ointment on chest and neck as compared to no treatment for nocturnal cough, congestion and sleep difficulty caused by upper respiratory tract infections (URTI) in children aged 2 to 11 years. While the study was not designed to demonstrate the efficacy of terpenic derivatives suppositories in treatment of cough in children, it confirmed the occurrence of AEs with terpenic derivatives. It is noteworthy that some irritant adverse events (AEs) were only reported among children treated with the ointment; especially burning sensation of skin/nose/eyes, but also skin rash, and skin redness. Nervous system disorders (hyperactivity, sleepiness, headache) were also reported.

The CHMP also noted that safety measures have previously been implemented in France with regard to camphor-containing medicinal products, due to neurological adverse events reported in children. In 1980-1982, a national pharmacovigilance inquiry led to the contraindication of camphor-containing products used in topical formulations or on respiratory mucous membrane in children less than 30 months. In 1990-1992, another inquiry was conducted regarding hyperthermic convulsions and drug risk factor, identifying terpenic medicinal products as medications with a risk to induce convulsions. In 1994, the contraindication in children less than 30 months was extended to all medicinal products containing camphor as active substance. Finally, in 1996, following a benefit/risk review of terpenes-containing products, the contraindication was extended to products containing camphor as excipient. A contraindication in children with a history of convulsions was also included in the SmPC.

The CHMP also noted other regulatory actions taken by the Afssaps regarding adverse effects related to cosmetic products containing terpenic derivatives used in the paediatric population. In 2004, the Afssaps requested the withdrawal from the market of a cosmetic product containing about 6% of eucalyptol, because of the occurrence of serious neurological adverse effects (seizures) related to its application in infants. In 2006, the Afssaps requested the withdrawal from the market of a lotion containing high concentrations of terpenic derivatives, as the product did not mention precautions regarding its use in infants and children, and had caused serious neurological adverse events. Finally, in 2007 and 2008, a further product containing eucalyptol was withdrawn following three serious neurological adverse events reported in infants.

Niaouli

Regarding suppositories containing niaouli essential oil or niaouli essence, the MAH stated that only limited toxicity data exists. However, a number of toxicity studies have been performed on the main terpenic component of niaouli essential oil, eucalyptol. While acknowledging that the existing studies do not represent a complete toxicological program as required according to the current regulations, the MAH argued that eucalyptol is a well-known product.

The CHMP noted 6 ADRs (all serious) reported in PSURs during the period from April 2001 to December 2010, including general disorders (anaphylactic shock), skin and subcutaneous disorders (giant urticaria, rash maculopapular and Stevens Johnson-syndrome) and nervous system disorders (disorientation, visual hallucination). In addition, there was one case of dispensing error, without any ADRs, in which a suppository for children instead of a suppository for infants was dispensed to a 18-month-old child weighing 11 kg.

Additional non-serious cases were reported to the French regulatory authority during the review period, including 1 case of local ADR (burning sensation with anal pruritus), 15 cases of administration error of a child formulation instead of the infant formulation. In most of these cases, there was more than one child in the household.

Regarding the assessment of the toxicity of eucalyptol, the main terpene contained in niaouli essence, the CHMP noted a number of preclinical studies on eucalyptol, together with data on acute toxicity of eucalyptol in clinical studies as well as in paediatric population, evidencing neurological ADRs (in particular convulsions) when used by cutaneous and inhaled route, although no studies were identified using the rectal route of administration. Neurological ADRs have also been reported in the context of age-related administration errors. The CHMP listed several literature articles confirming that eucalyptol can induce convulsions in humans (Corrigan D, 1992, Craig J, 1953, Gurr GW & Scroggie JG, 1965, Jonville APE et al., 1991, Millet Y et al., 1981, Patel S & Wiggins J, 1980, Spoerke DG et al., 1989, Steinmetz MD et al. 1987, Stafstrom CE, 2007, Day LM et al. 1997, Tibballs J, 1995, Vincens M, 1982 and Webb NJA & Pitt WR, 1993).

Thymus vulgaris and cineole

Regarding suppositories containing Thymus vulgaris and cineole, the MAH stated that no substantial new safety trends or signals were observed in safety data from the current post-marketing period that would indicate any change to the benefit-risk profile of the product or that would warrant updating reference safety information in the product data sheet. All studies agree that these compounds are effective in remission of symptoms associated with respiratory infections, especially cough, with no report of serious adverse effects. It is not possible to differentiate by age, as no specific studies in children have been published.

The CHMP noted that the products contained liquid extract of Thymus vulgaris, which includes 7 terpenic derivatives (β -myrcene, γ -terpinene, p-cymene, linalol, terpinen-4-ol, thymol, carvacrol). The CHMP assessed the literature review performed by the MAH on the terpenic active ingredient of the product as well as on the terpenic excipient, cineole (eucalyptol). The review did not identify any efficacy and safety study on suppositories using thyme alone, instead the published studies involved oral treatments (syrup or spray form) with thyme combination. In these studies, the products were indicated for treatment of acute bronchitis with productive cough, upper respiratory tract infections (URTIs), productive cough complaints, symptoms of cough. All studies included adults, except one which included children and adolescents between 2 and 17 years of age. The lack of randomised, doubled-blind, controlled studies was also noted, as most studies were open label. In addition, safety data from PSURs covering the period from 1997 to 2010 reported 2 non-serious cases of dermatitis but no serious ADRs, nervous system disorders or other safety concern were identified in spontaneous reports or literature reviews of clinical studies regarding oral products containing thymus.

The CHMP considered that the non-clinical data provided by the MAH indicate that neurological disorders may occur following exposure to several components of suppositories containing terpenic derivatives: overexposure to eucalyptol, sulfogaiacol and sodium camphosulfonate led to effects on vital functions such as neurological effects (mild sedation) or cardiovascular effects (bradypnea). Severe effects were only observed following oral administration of isolated substances e.g. carvacrol or thymol for which CNS depression, ataxia, coma and respiratory failure were observed. In the absence of data investigating the dose-relationship of ADRs (especially nervous system disorders), no formal quantitative risk assessment could be made but the severity of the effects is anticipated to be dose related. Adequate safety pharmacology studies allowing specific assessment of the vital functions are

lacking. Moreover, the provided data did not allow any assessment of the risk in children because of the absence of studies performed in juvenile animal where, as in humans, minimal toxic dose level are expected to be lower due to the immaturity of the hepatic metabolism and the blood brain barrier.

The CHMP concluded that based on the spontaneous reporting (taking into account expected underreporting for non-prescription products) and in the absence of comparative studies, pooled analyses and meta-analyses comparing toxicity of terpenic derivatives used by rectal route the safety concerns raised by terpenic derivatives in children less than 30 months cannot be ruled out for *Thymus vulgaris*.

Citral

Regarding suppositories containing citral, the MAH submitted mainly non clinical studies and focused on experimental properties of citral and other phenolic compounds, to justify efficacy. From January 1998 to August 2009, a total of 18 cases of ADRs involving suppositories containing citral were reported, of which 10 were serious. Nervous system disorders were reported in 11 cases (7 serious): 4 serious cases of convulsions; 3 non-serious cases of agitation (medication errors in 2 cases); 1 case of somnolence (related to medication error); 2 serious cases of hypersomnia; and 1 serious case of hypotony (with malaise). A total of 7 medication errors were reported, all due to errors in the age formulation delivered. No ADRs were reported in the 4 remaining medication error cases.

In 6 cases, the citral-containing suppositories were associated with the reported cases of nervous system disorders, including malaise with loss of consciousness, convulsions and breathing difficulties, somnolence, three cases of agitation including one with mydriasis on one eye and myosis on the other eye and hypotonia. Other etiologies or significant medical history are confounding factors in 5 cases of neurological disorders; however the role of suppositories with citral as triggering factor of convulsion cannot be excluded. The cases included convulsive state, generalized convulsive seizure, convulsions and hypersomnia.

In summary, despite the confounding factors in 5 cases of neurological disorders reported with suppositories for infants containing citral, the CHMP concluded that citral could be a triggering factor of convulsion.

Cineole (eucalyptol), pine essential oil and guaiacol

Regarding suppositories containing cineole (eucalyptol), pine essential oil and guaiacol, the MAH stated that the products have been marketed since the 1960s and used as supportive therapy in benign acute bronchial disorders. From 1998 to 2010, 13 cases were reported in infants, 9 of which were related to administration errors (confusion between infant and child formulations), without any clinical consequence. In the 4 other cases, 2 severe AEs (somnolence and bronchospasm) could be related to the product. The MAH acknowledged that the benefit is modest and based on traditional use but stated that the products could be useful to allow parents to care for infants suffering from acute benign bronchial obstruction. The CHMP noted the case reports and concluded that suppositories containing cineole and pine are very likely to be associated with the 4 cases reported during the review period.

Turpentine and pine essence

Regarding suppositories containing turpentine, the MAH stated that significant therapeutic efficacy in the authorized indication has been reported by doctors prescribing these products and that adverse events have never been reported. However, the CHMP was of the opinion that the available evidence supports the neurological risks of terpenic derivatives as a family of substances.

2.3. Risk management plan

The CHMP did not require the MAH to submit a risk management plan.

2.4. Overall benefit/risk assessment

Benefits

In member states where they are authorised, suppositories containing terpenic derivatives have been used for decades as treatment for benign acute bronchial disorders or oropharynx congestive states,

particularly for non productive cough. Camphor, menthol and eucalyptol are the most studied substances from a preclinical and clinical point of view. Efficacy is based mainly on traditional use of these products and supported by data regarding pharmacodynamic properties and by their effects as cough suppressants and anti-inflammatory drug in pre-clinical models. Most clinical data are derived from open studies as well as data from clinical practice or expert opinions. However, there is no clinical data derived from comparative studies (randomized, double-blind and controlled studies), pooled analyses or meta-analyses comparing efficacy of terpenic derivatives used by rectal route. No studies focused on children less than 30 months are available.

Risks

The main data was obtained from spontaneous reports, the literature and pre-clinical data. The CHMP reviewed a number of publications confirming that terpenic derivatives can induce convulsions in humans. A number of adverse drug reactions, including serious nervous system disorders were reported in paediatric patients, including convulsions, agitation, somnolence, hypersomnia, hypotonia, disorientation, and hallucination. When taking into account all system organ classes, the reported adverse drug reactions (ADRs) were mainly nervous system disorders. Other disorders including skin disorders and respiratory disorders were also identified. Rectal lesions, including rectal burning, are of particular concern because of their severity and because they represent a limiting factor for treatment duration. The CHMP also noted that underreporting due to the non-prescription status can be assumed. Finally, dispensing or administration errors were also identified with cases where the administered/prescribed suppository was not suitable for the age or weight of the child.

The CHMP also reviewed the French safety assessment of the use of suppositories containing terpenic derivatives in children, completed in May 2010. A total of 92 cases of ADRs were identified in the national pharmacovigilance database and the periodic safety update reports, with about 82% (76/92) of these cases occurring in children less than 30 months. Thirty cases related to neurological disorders, and twenty-one serious cases with plausible relationship were reported. For the cases where time to onset was reported, the neurological ADRs occurred on the day of treatment initiation. Medication errors included 5 neurological ADRs. In most cases, medication errors involved the use of a child formulation instead of the infant formulation. Six cases of local irritation and one case of rectorrhagia with favourable outcome were notified, together with 12 cases of cutaneous ADRs and 2 cases of respiratory ADRs.

The CHMP also noted that terpenic derivatives administered by other route of administration (cutaneous and inhaled) are associated with risks of neurological, skin, and local toxicity. While acknowledging that direct comparisons of suppositories containing terpenic derivatives regarding these aspects are lacking and that suppositories could be a therapeutic alternative in children who do not tolerate treatment with ointments, the CHMP was of the opinion that the available data confirms that the safety profile of terpenic derivatives used by rectal route in infants and children is of concern.

From a mechanistic point of view, based on the pharmacological properties of terpenic derivatives, these substances are non-polar (or lipophilic) compounds which show an affinity for non-polar human body structures. This is of particular concern in children and infants, who have little fatty mass, as these substances instead cross into the central nervous system (CNS), practically the only apolar structure at that age. Moreover, suppositories are known to distribute systemically, due to product absorption through the rectal mucous membrane which is particularly vascularised.

The CHMP also noted that the limited available data made it impossible to establish whether a direct relationship exists between the administered dose and the ADRs observed. The CHMP considered this to be of concern, especially when considering cases where children were exposed to an inadequate dosage or suppository formulation, for example due to parents using suppositories dispensed for older children in other younger children or infants in the family.

Risk minimisation measures

In its assessment, the CHMP requested the MAHs to propose risk minimisation measures to address the identified risks. Having assessed the proposals submitted by the MAHs (including the introduction of special warnings, a lower weight limit, limiting treatment duration, a contra-indication in case of history of convulsions or epilepsy and highlighting the risk of interaction with other products containing terpenic which could result in an increased risk of neurological side effects), the CHMP considered that in addition to the measures proposed, a contraindication in children less than 30 months was necessary to adequately manage the risk profile of suppositories containing terpenic derivatives. The CHMP also considered it necessary to restrict the duration of treatment in the remaining approved

paediatric population to 3 days, due to risk of rectal burning and to the risks related to storage of terpenic derivatives in tissues and brain (the rate of metabolism and elimination is unknown, as a consequence of their lipophilic properties) which can lead to neuropsychological disorders.

Benefit/Risk balance

Having considered the totality of the data submitted by the MAHs related to the use of suppositories containing terpenic derivatives in children less than 30 months and taking into account the data identified during the 2010 French safety review, the CHMP was of the opinion that suppositories containing terpenic derivatives can induce neurological disorders, especially convulsions, in children less than 30 months, due to the immaturity of the central nervous system which results in a higher susceptibility to neurological toxicity. In addition, the suppositories can also be associated with the risk of rectal burning. The risk minimisation measures proposed by the MAHs were considered insufficient to reduce the neurological risk to an acceptable level in children less than 30 months.

Limited clinically relevant efficacy has been demonstrated in the paediatric population. In children less than 30 months, no data on efficacy is available.

As a consequence, taking into account the risk of neurological toxicity and other adverse events in the paediatric population, the CHMP considered that the benefit-risk of suppositories containing terpenic derivatives in children less than 30 months is not positive under normal conditions of use.

2.5. Communication plan

As part of this referral procedure, the MAHs and the CHMP agreed the wording of a 'Dear healthcare professional' communication, common to all suppository products containing terpenic derivatives, designed to inform prescribers of the contraindications in children less than 30 months and in children with a history of epilepsy or febrile convulsion, to be sent to relevant health care professionals, including pharmacists.

2.6. Changes to the product information

The CHMP assessed the revised PI proposals submitted by the MAHs and noted extensive divergences. In particular, the minimum age recommendations varied from the neonatal period for some products, to 1 or even 6 months of age for others. Weight was not taken into account and age groups vary according to the composition of the suppository product, as did treatment duration without medical advice. Taking into account the risk of medication error related to age, the CHMP therefore considered that harmonisation was required for certain sections of the product information of suppositories containing terpenic derivatives.

The main changes, agreed by the CHMP and to be included as relevant in the PI of all suppositories containing terpenic derivatives, according to the intended age range of the product, were the introduction in Section 4.3 of contraindications in children less than 30 months because of the risk of neurological disorders, due to immaturity of the central nervous system, as well as in children with a history of febrile convulsion or epilepsy and in children with a recent history of anorectal lesion. These contraindications were also reflected in sections 4.2 and in the labelling and the package leaflet. In addition, the duration of use was limited to 3 days, due to risks related to terpenic derivatives storage in tissues and brain and the risk of rectal burning. A warning stating that the products are flammable and should not be approached to a naked flame was also added, together with a recommendation that the products should not be used in pregnant women and should not be used during breast-feeding.

3. Overall conclusion

In reaching its opinion, the CHMP took into account the totality of the available data on the safety of suppositories containing terpenic derivatives in paediatric populations, noting the limited efficacy data in the paediatric population, the absence of data on efficacy in children less than 30 months, the known neurological toxicity of terpenic derivatives, the risk of potentially severe neurological and local lesions and the risk of medication errors arising from the mistaken use of children formulations in infants.

The CHMP concluded that the data confirms the concerns that suppositories containing terpenic derivatives can induce neurological disorders, especially convulsions, in children less than 30 months.

These concerns are strengthened by the fact that no direct relationships could be established between the quantity of terpenic derivatives administered and the occurrence or severity of the adverse events. The clinical evidence shows that children less than 30 months are more prone to neurological disorders due to immaturity of the central nervous system, which results in a higher susceptibility to neurological toxicity. In addition, limited clinically relevant efficacy has been demonstrated in the paediatric population and no data on efficacy is available in children less than 30 months. The CHMP therefore concluded that the use of suppositories containing terpenic derivatives should be contraindicated in children less than 30 months, as well as in children with a history of epilepsy or febrile convulsion and in children with a recent history of anorectal lesion.

The CHMP was of the opinion that marketing authorisations with a wide approved age range should be varied to contraindicate use in children less than 30 months while marketing authorisations where the only approved age range is children less than 30 months should be revoked.

The MAHs and the CHMP also agreed on the wording of a 'Direct healthcare professional' communication, to inform prescribers on the above agreed contraindications to be sent to relevant health care professionals, including pharmacists.

The relevant sections of the summary of product characteristics, labelling and package leaflet are set out in Annex III to the opinion.

4. Annexes

The list of the names of the medicinal products, marketing authorisation holders, pharmaceutical forms, strengths and route of administration in the Member States are set out Annex I to the opinion.

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25¹

**EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
(CEF)^{2,3}**

European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 16 August 2012, replaces the earlier version published on 11 July 2012⁴

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 21 flavouring substances in the Flavouring Group Evaluation 9, Revision 4, using the Procedure in Commission Regulation (EC) No 1565/2000. The present revision of FGE.09 includes the assessment of four additional flavouring substances, *p*-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], *l*-piperitone [FL-no: 07.255] and menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843]. None of the substances were considered to have genotoxic potential. The

¹ On request from the European Commission, Question No EFSA-Q-2011-01245, EFSA-Q-2011-01244, EFSA-Q-2011-01243, EFSA-Q-2011-01242, adopted on 5 July 2012.

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⁴ Editorial changes have been made on page 1 and 2. The changes do not affect the overall conclusions of the opinion. To avoid confusion, the original version of the Opinion has been removed from the website, but it is available on request.

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substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the 20 substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For the remaining candidate substance [FL-no: 07.207], additional toxicity data are requested (further metabolism and/or toxicity studies). Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have been considered. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all candidate substances.

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KEYWORDS

Flavourings, alcohols, ketones, esters, secondary alicyclic, FGE.09.

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate 21 flavouring substance in the Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These flavouring substances belong to chemical group 8, 25 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.09, FGE.09Rev4, includes the assessment of four additional flavouring substances, *p*-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], *l*-piperitone [FL-no: 07.255] and menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843].

The present flavouring group evaluation deals with 21 secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols. The flavouring substances are structurally related to 27 flavouring substances evaluated at the 51st, 59th and 63rd meetings of the Joint FAO/WHO Expert Committee on Food Additives (the JECFA).

Seventeen of the flavouring substances have one or more chiral centres.

Fourteen of the flavouring substances belong to structural class I, six belong to structural class II and one to structural class III, according to the decision tree approach presented by Cramer et al., 1978.

Thirteen of the flavouring substances have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach the 14 flavouring substances belonging to structural class I have intakes in Europe ranging from 0.0012 to 830 microgram/*capita*/day, for five of the six substances from structural class II the intakes range from 0.0085 to 530 microgram/*capita*/day, and for the substance from structural class III the intake is 1.2 microgram/*capita*/day, which are all below their respective threshold of concern value for structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively. For one substance [FL-no: 09.520] from structural class II the MSDI is 770 microgram/*capita*/day, which is above the threshold of concern of 540 microgram/person/day.

Genotoxicity data are available only for a limited number of the flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

Twenty of the flavouring substances are expected to be metabolised to innocuous products at their estimated level of use as flavouring substances. The remaining substance, cyclotetradecanone [FL-no: 07.207], could not be assumed to be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is considered that, on the basis of the default MSDI approach, 20 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. For one flavouring substance, cyclotetradecanone [FL-no: 07.207], the evaluation has been deferred as additional data on toxicokinetics and/or toxicology are required.

When the estimated intakes were based on the mTAMDI approach they ranged from 420 to 63000 microgram/person/day for 13 flavouring substances in structural class I. For seven of these substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold of concern of 1800 microgram/person/day (class I) and for six substances [FL-no: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] above the threshold. The estimated intakes of the six substances assigned to structural class II, range from 320 to 8700 microgram/person/day, which for five substances [FL-no: 07.059, 07.202, 07.203, 07.207 and 09.520] are above the threshold of concern for structural class II substances of 540 microgram/person/day. The mTAMDI estimate for the one substance from structural class III [FL-no: 06.136] is 0.075 microgram/person/day, which is below the threshold of 90 microgram/person/day. For one flavouring substance [FL-no: 09.929] the mTAMDI could not be calculated due to missing information on use levels. Thus, for eleven flavouring substances [FL-no: 07.059, 07.202, 07.203, 07.207, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], and for [FL-no: 09.929] for which use levels are missing, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

The substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.255, 09.355, 09.621 and 09.870], which have mTAMDI estimates below the threshold of concern, are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 21 flavouring substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 21 flavouring substances evaluated through the Procedure.

For cyclotetradecanone [FL-no: 07.207] additional data are requested (further metabolism and/or toxicity studies). For the remaining 20 substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union List of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

HISTORY OF THE EVALUATION

The Flavouring Group Evaluation 09, FGE.09 dealt with 10 candidate substances, nine secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols, and one ester of a phenol carboxylic acid and a secondary alicyclic alcohol.

The first Revision of FGE.09, FGE.09Rev1, included the assessment of five additional flavouring substances [FL-no: 06.136, 09.154, 09.520, 09.929 and 09.935]. No new toxicity or metabolism data were provided for four of the five substances. For [FL-no: 09.520] acute and short term toxicity data and *in vitro* and *in vivo* genotoxicity data were provided. Additional data on five substances [FL-no: 02.075, 02.167, 09.355, 09.619 and 09.621] was made available since the FGE.09 was published.

The second Revision of FGE.09, FGE.09Rev2, included the assessment of one additional substance, carvyl-3-methylbutyrate [FL-no: 09.870]. No toxicity and/or metabolism data were provided for this substance. Carvyl-3-methylbutyrate has initially been considered in FGE.212 with respect to genotoxicity, together with other alpha,beta-unsaturated substances from subgroup 2.6 of FGE.19, where the Panel concluded that “*d*-Carvone [FL-no: 07.146] was found genotoxic *in vitro*. However, *d*-carvone was not carcinogenic in mice. Therefore, the Panel concluded that this substance together with the structurally related l-carvone as well as carveol and the carvylderivatives [FL-no: 02.062, 07.147, 09.143, 09.215 and 09.870] could be evaluated through the Procedure”.

The third Revision of FGE.09, FGE.09Rev3, included the assessment of one additional substance, *L*-menthyl (S)-3-hydroxybutyrate [FL-no: 09.949]. No toxicity and/or metabolism data were provided for this substance.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.09	9 December 2004	http://www.efsa.eu.int/science/afc/afc_opinions/814_en.html	10
FGE.09Rev1	1 April 2008	http://www.efsa.europa.eu/en/efsajournal/doc/927.pdf	15
FGE.09Rev2	13 May 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1454.htm	16
FGE.09Rev3	28 September 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2396.htm	17
FGE.09Rev4	5 July 2012		21

The present Revision of FGE.09, FGE.09Rev4, includes the assessment of four additional substances [FL-no: 07.059, 07.202, 07.255 and 09.843].

Two of these substances [FL-no: 07.202 and 07.255] are alpha,beta-unsaturated ketones originally allocated to FGE.212Rev1. The two substances have been considered with respect to genotoxicity (EFSA, 2011f) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the two substances can be evaluated through the Procedure.

No toxicity or metabolism data were provided for the new substances. A search in the open literature for these substances did not provide any further data on toxicity or metabolism.

Industry has informed that the substance [FL-no: 07.207] is no longer supported for use as flavouring substance in Europe (EFFA, 2009c).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letter of the 7th May 2010, the Commission requested EFSA, based on additional submitted data on genotoxicity, to carry out re-evaluation of the flavouring substances 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202] and *l*-piperitone [FL-no: 07.255] and depending on the outcome to proceed to the evaluation of this flavouring substance through the Procedure, also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 9, Revision 4

1.1. Description

The present Flavouring Group Evaluation (FGE.09Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Annex I of this FGE), deals with nine secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and 11 esters containing secondary alicyclic alcohols. These 21 flavouring substances (candidate substances) belong to chemical groups 08, 25 and 30 of Annex I of Regulation (EC) No 1565/2000 (EC, 2000a).

The candidate substances under consideration, with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

A summary of the outcome of the safety evaluation of the candidate substances is listed in Table 2a

The hydrolysis products of the candidate substances and their evaluation status as flavouring substances are listed in Table 2b.

The candidate substances are structurally related to 27 flavouring substances (supporting substances) evaluated at the 51st, 59th and 63rd meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the groups “Substances structurally related to menthol”, “Carvone and structurally related substances”, “Alicyclic ketones, secondary alcohols and related esters” and “Monocyclic and bicyclic secondary alcohols, ketones and related esters” (JECFA, 1999a; JECFA, 2003a; JECFA, 2006a). In addition the racemate of menthyl-3-hydroxybutyrate has been evaluated by the JECFA at the 69th meeting (JECFA, 2009a) in the group of “Substances structurally related to menthol”.

The names and structures for the 27 supporting substances are listed in Table 3, together with their evaluation status.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

Two candidate substances possess one chiral centre [FL-no: 07.203 and 07.255] and 15 substances possess two or more chiral centres [FL-no: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949].

1.3. Natural Occurrence in Food

Thirteen candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618 and 09.619] have been reported to occur in fruits, spices, butter, chicken, wine, drinks, tea, juice and essential oils. Quantitative data on the natural occurrence of these substances in food have been reported for three substances (TNO, 2000; TNO, 2011).

These reports include:

- Cyclohexanol [FL-no: 02.070]: up to 0.1 mg/kg in fruits (passionfruit) and 0.006 mg/kg in white wine.
- Cyclopentanol [FL-no: 02.135]: 0.01 - 0.1 mg/kg in passiflora juice, 0.01 - 0.1 mg/kg in passiflora mollissima, 0.01 - 0.02 mg/kg in oysters and 0.01 mg/kg in Chinese quince flesh.
- 2,6,6-Trimethylcyclohex-2-en-1-one [FL-no: 07.202]: 2000mg/kg in maize, 1 mg/kg in tea and up to 0.23 mg/kg in citrus fruits.

According to TNO (TNO, 2000; TNO, 2010; TNO, 2011), the remaining eight candidate substances have not been reported to occur naturally in any food items. These substances are 6-isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [FL-no: 06.136], cyclotetradecanone [FL-no: 07.207], menthyl salicylate [FL-no: 09.621], menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843],

carvyl-3-methylbutyrate [FL-no: 09.870], L-monomenthyl glutarate [FL-no: 09.929], dimenthyl glutarate [FL-no: 09.935] and L-menthyl (S)-3-hydroxybutyrate [FL-no: 09.949].

2. Specifications

Purity criteria for the 21 candidate substances have been provided by the Flavouring Industry (see Table 1 (EFFA, 2003a; EFFA, 2010a; EFFA, 2011e; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f). Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the specifications are adequate for all substances (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995a). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population⁵ (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present FGE.09Rev4, the total annual volume of production of the candidate substances from use as flavouring substances in Europe has been reported to be approximately 19000 kg (EFFA, 2003a; EFFA, 2003b; EFFA, 2011e; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k). For the 26 of 27 supporting substances, for which production figures are available for Europe, the total annual volume of production is approximately 138500 kg (JECFA, 1999a; JECFA, 2003a; JECFA, 2006a).

On the basis of the annual volumes of production reported for the candidate substances, the MSDI values for each of these flavourings have been estimated (Table 2a).

96 % of the total annual volumes of production for the candidate substances is accounted for by four of these flavourings, *p*-menthan-3-one [FL-no: 07.059], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843] and L-monomenthyl glutarate [FL-no: 09.929]. The estimated daily *per capita* intakes from use as flavouring substances are 530, 770, 830 and 110, microgram, respectively. The daily per capita intakes for each of the remaining substances are below 37 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the candidate substances information on food categories and normal and maximum use levels^{6,7,8} were submitted by the Flavour Industry (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k). For 20 candidate substances the use in flavoured food products divided into the food categories as outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), is shown in Table 3.1. For L-monomenthyl glutarate [FL-no: 09.929] the use has not been reported in accordance with the Commission Regulation.

For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

⁵ EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

⁶ "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

⁷ The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

⁸ The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances in Various Food Categories for 20 Candidate Substances for which Data on Use have been provided

Food category	Description	Flavourings used*
01.0	Dairy products, excluding products of category 2	All except [FL-no: 07.059]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 07.059, 07.255, 09.843 and 09.949]
03.0	Edible ices, including sherbet and sorbet	All
04.1	Processed fruits	All except [FL-no: 06.136, 07.059, 07.255 and 09.843]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 09.935]
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 07.059, 07.255 and 09.935]
07.0	Bakery wares	All
08.0	Meat and meat products, including poultry and game	All except [FL-no: 06.136, 07.059, 07.255, 09.843 and 09.949]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 06.136, 07.059, 07.255, 09.843 and 09.949]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no: 06.136 and 07.059]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 07.059, 07.255, 09.843, 09.935 and 09.949]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 09.870]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All
15.0	Ready-to-eat savouries	All except [FL-no: 06.136 and 07.059]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no: 07.059 and 09.843]

* No use levels (in accordance with the Commission Regulation) have been submitted for [FL-no: 09.929]

According to the Flavour Industry the candidate substances, for which Industry has provided data on food categories, normal use levels are in the range of 0.0001 - 500 mg/kg food, and the maximum use levels are in the range of 0.0001 - 2000 mg/kg food (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k).

The mTAMDI values for 13 candidate substances from structural class I (see Section 5), for which exposure data have been submitted, range from 420 to 63000 microgram/person/day. For the six candidate substances from structural class II the mTAMDI values range from 320 to 8700 microgram/person/day, respectively. For the remaining substance from structural class III the mTAMDI is 0.075 microgram/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. Absorption, Distribution, Metabolism and Elimination

The 11 esters [FL-no: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the data available for the supporting substances (Emberger, 1994a; Emberger, 1994b; White et al., 1990). The resulting carboxylic acids are either metabolised through common physiological pathways like *beta*-oxidation and the citric acid cycle or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994) (see Table 2b and Annex III).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, *p*-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-no: 02.070, 02.075, 02.135, 02.167, 07.059, 07.202, 07.203 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, the hydrolysis products of [FL-no: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are also metabolised via this pathway. Neither menthol nor carveol or dihydrocarveol is anticipated to be oxidised to the corresponding ketone (for detailed discussion, see Annex III).

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion (see Annex III). Thus, it may be anticipated that these 20 substances will be metabolised to innocuous products.

No information is available on toxicokinetics (including metabolism) of cyclotetradecanone [FL-no: 07.207] or on structurally related substances. Cyclotetradecanone [FL-no: 07.207] cannot be assumed to be metabolised to innocuous products.

For more detailed information, see Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the candidate substances the Procedure was applied. The stepwise evaluations are summarised in Table 2a.

Step 1

Fourteen of the candidate substances are classified into structural class I [FL-no: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949], six candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207, 07.255 and 09.520] into structural class II and one substance [FL-no: 06.136] is classified into structural class III according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

Step 2 requires consideration of whether detoxification pathways are available to metabolise the substances, at the estimated levels of intake, to innocuous products.

Twenty of the candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and

09.949] are expected to be metabolised to innocuous products and accordingly they proceed via the A-side of the Procedure scheme (Annex I).

It cannot be anticipated that cyclotetradecanone [FL-no: 07.207] is metabolised to innocuous products and accordingly it proceeds via the B-side.

Step A3

The fourteen candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] that have been assigned to structural class I have estimated European daily *per capita* intakes ranging from 0.0012 to 830 microgram (Table 2a). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

The five candidate substance [FL-no: 07.059, 07.202, 07.203, 07.255 and 09.520] assigned to structural class II have European daily *per capita* intakes of 0.0085 and 770 microgram, respectively (Table 2a). For [FL-no: 07.059, 07.202, 07.203 and 07.255] the intakes are below the threshold of concern of 540 microgram/person/day for structural class II. For [FL-no: 09.520] the daily *per capita* intake of 770 microgram is above the threshold of concern for a substance assigned to structural class II. The substance therefore proceeds to step A4.

The candidate substance [FL-no: 06.136] has been assigned to structural class III and has a European daily *per capita* intake of 1.2 microgram (μg). This intake is below the threshold of concern of 90 microgram/person/day for structural class III.

Based on the results of the safety evaluation sequence, 19 candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] proceeding via the A-side of the Procedure do not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substances.

Step A4

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] or its metabolites are not endogenous, the substance therefore proceeds to step A5.

Step A5

A 90 day study in rats has been performed for [FL-no: 09.520] from which a No Observed Adverse Effect Level (NOAEL) of 100 mg/kg body weight (bw)/day could be derived. This NOAEL provides a margin of safety of nearly 10^4 compared to the daily intake of 0.013 mg/kg bw/day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Therefore, [FL-no: 09.520] does not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substance.

Step B3

The estimated intake (MSDI) for cyclotetradecanone [FL-no: 07.207] of 0.061 microgram/capita/day does not exceed the threshold of concern for structural class II (540 microgram/person/day). Accordingly, the evaluation proceeds to step B4.

Step B4

Toxicity data that would permit establishment of a NOAEL are not available for cyclotetradecanone [FL-no: 07.207] or for structurally related substances. Therefore, for the candidate substance cyclotetradecanone [FL-no: 07.207] additional data are required.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes, based on the mTAMDI, range from 420 to 63000 microgram/person/day for 13 candidate substances in structural class I. For seven of these substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870] the mTAMDI is below the threshold of concern of 1800 microgram/person/day and for six substances [FL-no: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] the mTAMDI is above the threshold. For L-monomenthyl glutarate [FL-no: 09.929] no mTAMDI could be calculated due to lack of information on use levels in the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For the six substances [FL-no: 07.059, 07.202, 07.203, 07.207, 07.255 and 09.520] assigned to structural class II, the estimated intakes, based on the mTAMDI, range from 320 to 8700 microgram/person/day, which are all above the threshold of concern for structural class II substances of 540 microgram/person/day, except for [FL-no: 07.255].

For [FL-no: 06.136], assigned to structural class III, the estimated intake based on the mTAMDI is 0.075 microgram/person/day, which is below the threshold of concern for a structural class II substance of 90 microgram/person/day.

Thus, for 11 candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949] further information is therefore required. This would include more reliable intake data and then, if required, additional toxicological data. For L-monomenthyl glutarate [FL-no: 09.929] use levels are needed in accordance with the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/capita/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.070	Cyclohexanol	3.7	1600	Class I	1800
02.075	neo-Dihydrocarveol	2.4	1600	Class I	1800
02.135	Cyclopentanol	0.012	1600	Class I	1800
02.167	Isodihydrocarveol	2.4	1600	Class I	1800
09.154	Menthyl valerate	1.0	3900	Class I	1800
09.355	neo-Dihydrocarvyl acetate	0.012	1600	Class I	1800
09.618	Menthyl formate	0.73	3900	Class I	1800
09.619	Menthyl hexanoate	0.37	3900	Class I	1800
09.621	Menthyl salicylate	0.012	420	Class I	1800
09.843	Menthol 1-and 2-propylene glycol carbonate	830	63000	Class I	1800
09.870	Carvyl-3-methylbutyrate	0.0012	1000	Class I	1800
09.929	L-Monomenthyl glutarate	110		Class I	1800
09.935	Dimenthyl glutarate	30	38000	Class I	1800
09.949	L-Menthyl (S)-3-hydroxybutyrate	37	10600	Class I	1800
07.059	p-Menthan-3-one	530	8700	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	0.12	1600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	0.0085	1600	Class II	540
07.207	Cyclotetradecanone	0.061	3900	Class II	540
07.255	l-Piperitone	12	320	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate	770	3900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one	1.2	0.075	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally

related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003a; EFFA, 2003b; EFFA, 2011e; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k), the combined estimated daily *per capita* intake as flavourings of the 14 candidate substances assigned to class I is 1000 microgram, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day.

The candidate substances from structural class I are structurally related to 15 supporting substances for which European intake data are available (European intake data are only available for 15 of the 16 supporting substances from structural class I). The total combined intake of the 14 candidate and 15 supporting substances is approximately 17000 microgram/capita/day, which is above the threshold for structural class I substances of 1800 microgram/person/day. The major contribution (92 %) is provided by one supporting substance, menthol [FL-no: 02.015] (16 mg/capita/day). An ADI of 0-4 mg/kg bw was allocated to menthol (JECFA-no: 427) at the 51st meeting (JECFA, 2000a). The ADI is 15 times higher than the MSDI of 16 mg/capita/day. The total combined daily *per capita* intake for the remaining substances from structural class I is approximately 1400 microgram, which is below the threshold for structural class I of 1800 microgram/person/day.

On the basis of the reported annual production volumes in Europe, the combined estimated daily *per capita* intake as flavourings of the six candidate substances assigned to structural class II is 1300 microgram, which exceeds the threshold of concern for a compound belonging to structural class II of 540 microgram/person/day. Nearly 60 % of the 1300 microgram derives from one candidate substance, namely methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]. However, for methyl 3-oxo-2-pentyl-1-cyclopentylacetate a NOAEL of 100 mg/kg bw/day has been established, which provides a margin of safety of more than 7000 to the daily *per capita* intake of 770 microgram, corresponding to 13 microgram/kg bw/day. For the remaining five substances the combined daily *per capita* intake is 530 microgram, which is below the threshold of 540 microgram/person/day for a structural class II substance.

The total combined intake from the six candidate and 11 supporting substances from structural class II is approximately 2200 microgram/capita/day, which exceeds the threshold for structural class II substances of 540 microgram/person/day. The major contribution (98 %) is provided by two candidate substances, methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], *p*-menthan-3-one [FL-no: 07.059] and one supporting substance, trans-menthone [FL-no: 07.176]. The estimated intake of [FL-no: 09.520] of 770 microgram/capita/day corresponds to 13 microgram/kg bw/day, which is more than 7000 fold lower than the NOAEL of 100 mg/kg bw/day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate.

In human liver microsomes (-)menthone is reported to be biotransformed to (+)neomenthol as the major metabolite and to a minor extent to 7-hydroxymenthone (Miyazawa & Nakanishi, 2006). For *d,l* menthol, which is isomeric to (+)neomenthol, a NOAEL of 375 mg/kg bw is reported after oral dosing of rats for 2 years (National Cancer Institute, 1979). The combined estimated intake of *p*-menthan-3-one [FL-no: 07.059] and trans-menthone [FL-no: 07.176] of 1420 microgram/capita/day corresponds to 24 microgram/kg bw/day, which is nearly 16,000 fold lower than the NOAEL of 375 mg/kg bw/day for menthol. The total combined daily *per capita* intake for the remaining 14 candidate and supporting

substances from structural class II is approximately 50 microgram, which is below the threshold for structural class II of 540 microgram/person/day.

The only candidate substance from structural class III, 6-isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [FL-no: 06.136] has a daily *per capita* intake of 1.2 microgram, which does not exceed the threshold of 90 microgram/person/day. There are no supporting substances from structural class III.

8. Toxicity

8.1. Acute Toxicity

Data are available for five candidate substances [FL-no: 02.070, 02.135, 06.136, 09.520 and 09.355]. Oral LD₅₀ values from studies in the rat range from 625 mg/kg body weight (bw) to > 5000 mg/kg bw.

Ten supporting substances [FL-no: 02.015, 02.061, 02.062, 02.209, 07.111, 07.148, 07.176, 09.016, 09.215 and 09.216] were tested for acute toxicity in the mouse, rat, rabbit, dog and guinea pig. The LD₅₀ values ranged from 930 mg/kg bw to > 5000 mg/kg bw.

The magnitudes of the LD₅₀ values indicate that the oral acute toxicity is rather low for the candidate substances and supporting substances.

All acute toxicity studies are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Only one conventional subchronic oral study has been conducted on the candidate substances (for [FL-no: 09.520]). For cyclohexanol [FL-no: 02.070] data were available from a study designed to investigate only peripheral neuropathy in which rats were given intraperitoneal doses of 200 mg cyclohexanol once or twice daily for up to six weeks. No effects on the peripheral nervous system were observed but the experiment was terminated early because the animals were in poor condition, there was a decrement of weight gain and two animals died prematurely. No general gross or histopathological examinations were reported and no No Observed Adverse Effect Level (NOAEL) was established. This study was not considered applicable to the evaluation of the oral toxicity of cyclohexanol (Perbellini et al., 1981).

For methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] a study, following the current OECD guidelines, was performed in rats. Male and female Sprague-Dawley CD rats were given in the diet daily doses of 0 (basal diet), 10, 50 or 100 mg/kg bw over a 90-day period (10 male and 10 female rats per dose group). No treatment related changes were observed in mortality, expanded clinical observations, ophthalmic examination, body weight gain, body weight change, food consumption, haematology, clinical chemistry, urinalysis, organ weights and macroscopic examination. There were no treatment related histopathological changes in the tissues from rats of any of the treatment groups. The NOAEL was therefore considered to be 100 mg/kg bw/day (the highest dose tested) (Kelly and Bolte, 2000).

Carcinogenicity studies are available for the two supporting substances, cyclohexanone and menthol [FL-no: 07.148 and 02.015].

Cyclohexanone [FL-no: 07.148] was given to male and female rats in the drinking water (3300 and 6500 ppm) and male and female mice (6500, 13000 and 25000 ppm (only female)) for two years. A reduction in weight gain (15 - 20 %) was observed in all groups at the highest doses. An increase in the incidence of lymphomas was observed at a lower dose level, but it was not dose related (Lijinsky and Kovatch, 1986).

In two other studies, two doses of dl-menthol were given to rats in the diet (3750 and 7500 ppm) and mice (2000 and 4000 ppm) for 103 weeks. A small reduction in survival was seen in the treated female mice. An increase of incidence of mammary gland fibroadenomas or mammary adenocarcinomas was observed in female rats at the lower dose level, but this was not dose related. In male rats, dl-menthol was not considered toxic or carcinogenic. In mice, a small increase in the incidence of hepatocellular carcinomas was observed. However, this increase was within the normal range of tumour incidence in the historical control groups, and the authors concluded that dl-menthol was not carcinogenic in rats and mice in the performed studies (National Cancer Institute, 1979).

For five supporting substances [FL-no: 02.015, 07.095, 07.111, 07.176 and 07.148] there are further oral subchronic toxicity data.

The repeated-dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

There is a study available for one candidate substance [FL-no: 02.070], with a NOAEL of < 1500 mg/kg bw/day. For one supporting substance [FL-no: 02.015] there are several studies (see Annex IV, Table IV.3).

The developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Due to the presence of a structural alert for genotoxicity (“alpha,beta-unsaturated carbonyl moiety”) for three candidate substances [FL-no: 07.202, 07.255 and 09.870] in the current revision of FGE.09, the genotoxicity of these substances was further assessed in FGE.212 and FGE.212Rev1.

In FGE.212 the concern for carvyl-3-methylbutyrate [FL-no: 09.870] was alleviated and the Panel concluded that this substance could be evaluated through the Procedure.

Since it was concluded in FGE.212Rev1, that based on additional information, the concern for genotoxic potential for isophorone [FL-no: 07.126] has been alleviated, a genotoxic potential can also be ruled out for substances structurally related to isophorone, including [FL-no: 07.202 and 07.255]. Therefore, these two substances [FL-no: 07.202 and 07.255] can be evaluated using the Procedure.

Genotoxicity data are available for only three of the remaining candidate substances: cyclohexanol [FL-no: 02.070], cyclopentanol [FL-no: 02.135], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] and for nine supporting substances and one structurally related substance.

Cyclohexanol [FL-no: 02.070] was not genotoxic in two Ames tests (Barsky, 1976; Haworth et al., 1983) and in an *in vivo* micronucleus assay (Gelbke, 1991), which are all considered as valid studies. However, the results of the *in vivo* study are of limited relevance, due to the lack of evidence that the substance did reach the bone marrow. Inconclusive results were reported in an *in vitro* chromosomal aberration assay with human leukocytes (Collin, 1971) and negative results were reported in a dominant lethal mutations assay with *Drosophila melanogaster* (Goncharova, 1970); both studies were considered inadequate.

Cyclopentanol [FL-no: 02.135] was studied in a valid Ames test (McMahon et al., 1979). No mutagenicity was found.

A battery of *in vitro* and *in vivo* genotoxicity studies were conducted on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] including valid negative reverse mutation tests in *Escherichia coli* (Wagner and Klug, 2000) and *Salmonella typhimurium* (Thompson, 2000).

In a mouse lymphoma test on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], pre-dating GLP, a more than 2-fold increase of the mutant frequency over the solvent treated control values was found at the highest tested cytotoxic concentration of 300 µg/ml in the presence of metabolic activation, and at the two highest tested cytotoxic concentrations of 200 and 300 µg/ml in the absence of metabolic activation. Only limited documentation is provided in the study report; together with the fact that several cultures were infected and a lack of a confirmatory test, it is impossible to assess the reliability of these results (Ross and Harris, 1979b).

No induction of forward mutations at the TK locus in L5178Y mouse lymphoma cells were found in a study performed in compliance with the current OECD test guidelines, both in the absence and in the presence of metabolic activation, up to and including cytotoxic concentrations (Cifone, 2001).

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was tested in a bone marrow micronucleus test in mice following a single intraperitoneal administration of 0, 280, 560 or 1120 mg/kg bw in corn oil. The study was performed in compliance with the current OECD test guidelines. The two highest doses chosen induced clear signs of toxicity; slight reductions (up to 12 %) in the ratio of polychromatic erythrocytes to total erythrocytes were found, indicating that the test material had reached the target cells. No increase in micronucleated cells was found in the groups treated with the test material. The positive control induced the expected increases (Gudi and Krsmanovic, 1998).

In an Unscheduled DNA Synthesis (UDS) study, the ability of methyl 3-oxo-2-pentyl-1-cyclopentylacetate to induce DNA repair was studied in isolated rat hepatocytes after administration *in vivo*. The study was performed in compliance with the current OECD Guideline 486 (OECD, 1997a). Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was administered to male Sprague-Dawley CD rats by intraperitoneal injection in doses of 333.3 and 1000 mg/kg bw (the latter dose was the maximum tolerated dose) followed by liver perfusion at 2 or 16 hours after dosing. No marked increase in the incidence of UDS was observed at either dose level or perfusion time. Statistically significant differences were revealed in the positive control groups when compared to the negative control group and the test article (Durward, 2001).

Genotoxicity data are available for nine supporting substances [FL-no: 02.015, 02.062, 07.148, 07.176, 09.027, 09.215, 09.230, 07.149 and 07.045].

Cyclohexanone [FL-no: 07.148], structurally related to the alicyclic ketones and secondary alcohols in this FGE, was not mutagenic in an Ames test, considered to be valid (Haworth et al., 1983). Negative and positive results were reported in several other *in vitro* studies at gene and chromosomal level, as well as a negative result in a sex-linked recessive lethal mutations in *D. melanogaster*. However, these studies were considered inadequate.

Menthol [FL-no: 02.015] gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes (Storer et al., 1996). With the same substance equivocal results in an *in vivo* host mediated mutation assay were observed at high dose levels (Food and Drug Res. Lab., Inc., 1975a) and negative results in several Ames tests, a TK+/- mouse lymphoma assay (Myhr and Caspary, 1991), sister chromatid exchange (SCE) tests in Chinese hamster ovary (CHO) cells (Ivett et al., 1989) and human lymphocytes (Murthy et al., 1991), and chromosomal aberration assays with human embryonic lung cells (Food and Drug Res. Lab., Inc., 1975a), human lymphocytes (Murthy et al., 1991) and CHO cells (Ivett et al., 1989). Negative results were also reported in two *in vivo* micronucleus (Shelby et al., 1993) and chromosomal aberration assays (Food and Drug Res. Lab., Inc., 1975a). However, the results of these studies have a limited relevance, due to the lack of bone marrow toxicity. In addition, an *in vivo* dominant lethal assay was available, from which also negative results were obtained.

trans-Menthone [FL-no: 07.176] was genotoxic in an Ames test (Andersen and Jensen, 1984b) and in a somatic mutation and recombination test (SMART) with *Drosophila* (Franzios et al., 1997). The

observed effects were not very pronounced. Further, *trans*-menthone is easily converted to menthol, which is estimated to be overall negative in genotoxicity tests.

Carveol and carvyl acetate [FL-no: 02.062 and 09.215] were tested in Ames test at various doses from 10 - 560 µg/plate in the *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without S9 mix in dimethyl sulphoxide. Positive and negative controls were used. No mutagenicity was observed (Mortelmans et al., 1986).

Conclusion on genotoxicity

For three of the candidate substances [FL-no: 07.202, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances.

Only for three of the remaining candidate substances some genotoxicity data are available, and for these three mainly negative results were obtained. For the supporting substances mainly negative, but also some positive results were obtained. The positive results were obtained in poorly reported tests, or in tests which are difficult to interpret with respect to their relevance for genotoxicity.

Overall, the genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

Genotoxicity data are summarised in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The present Revision of FGE.09, FGE.09Rev4, includes the assessment of four additional candidate substances compared to FGE.09Rev3, *p*-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], *l*-piperitone [FL-no: 07.255] and menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843].

So, the present FGE.09Rev4 deals with 21 secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols. These flavouring substances belong to chemical groups 8, 25 and 30 of Annex I of Regulation (EC) No 1565/2000.

Two candidate substances [FL-no: 07.203 and 07.255] possess one chiral centre and 15 substances [FL-no: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] possess two or more chiral centres.

Fourteen of the candidate substances belong to structural class I, six substances belong to structural class II and one to structural class III according to the decision tree approach presented by Cramer et al., 1978.

Thirteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach the 14 flavouring substances belonging to structural class I have intakes in Europe ranging from 0.0012 to 830 microgram/*capita*/day, for five of the six substances from structural class II the intakes range from 0.0085 to 530 microgram/*capita*/day, and for the substance from structural class III the intake is 1.2 microgram/*capita*/day, which are all below their respective threshold of concern value for structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively. For one substance [FL-no: 09.520] from structural class II the MSDI is 770 microgram/*capita*/day, which is above the threshold of concern of 540 microgram/person/day.

The total combined intakes of candidate and supporting substances from structural class I and II do not give rise to a safety concern.

For three of the candidate substances [FL-no: 07.202, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances. Genotoxicity data are available only for a limited number of the remaining flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

Twenty of the candidate substances are expected to be metabolised to innocuous products at the estimated levels of use as flavouring substances. The remaining candidate substance, cyclotetradecanone [FL-no: 07.207], could not be assumed to be metabolised to innocuous products due to lack of data on cyclotetradecanone or on closely related substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that 20 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances based on the default MSDI approach. For one candidate substance, cyclotetradecanone [FL-no: 07.207], the evaluation has been deferred as additional data on toxicokinetics and/or toxicology are required.

When the estimated intakes were based on the mTAMDI approach they ranged from 420 to 63000 microgram/person/day for 13 candidate substances in structural class I. For seven substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold of concern of 1800 microgram/person/day and for six [FL-no: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] the mTAMDI is above the threshold. For one flavouring substance [FL-no: 09.929] from structural class I the use levels are missing. The estimated intakes of the six substances assigned to structural class II, range from 320 to 8700 microgram/person/day, which for five substances [FL-no: 07.059, 07.202, 07.203, 07.207 and 09.520] are above the threshold of concern for structural class II substances of 540 microgram/person/day. The mTAMDI estimate for the one substance from structural class III [FL-no: 06.136] is 0.075 microgram/person/day, which is below the threshold of 90 microgram/person/day. Thus, for eleven candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], and for [FL-no: 09.929] for which use levels are missing, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

The nine substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.255, 09.355, 09.621 and 09.870] which have mTAMDI estimates below the threshold of concern are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 21 candidate substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all flavouring substances.

For cyclotetradecanone [FL-no: 07.207] additional toxicity data are requested (further metabolism and/or toxicity studies).

For the remaining 20 substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 9, REVISION 4

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

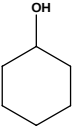
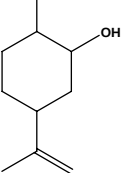
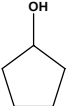
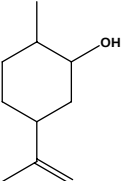
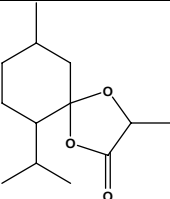
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.070	Cyclohexanol		2138 108-93-0	Solid $C_6H_{12}O$ 100.16	Slightly soluble Freely soluble	158 25 MS 95 %	1.462-1.468 0.942-0.948	
02.075	neo-Dihydrocarveol		2296 18675-34-8	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	107 (33 hPa) MS 95 %	1.476-1.482 0.920-0.926	Register name and CASrn to be changed to (1R,2S,5S)-neo-Dihydrocarveol, 18675-33-7 (ECHA, 2005).
02.135	Cyclopentanol		10193 96-41-3	Liquid $C_5H_{10}O$ 88.15	Slightly soluble Freely soluble	140 MS 95 %	1.449-1.455 0.945-0.951	
02.167	Isodihydrocarveol		18675-35-9	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	90 (6.7 hPa) MS 95 %	1.475-1.481 0.918-0.924	Register name to be changed to (1R,2R,5S)-Isodihydrocarveol.
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		4285 831213-72-0	Liquid $C_{13}H_{22}O_3$ 226.32	Slightly soluble Soluble	259 IR NMR MS 98.9 %	1.4606-1.4609 1.017-1.021	Mixture of isomers: (3S, 5R, 6S,9R)-isomer: 65.6 % and (3S, 5S, 6S,9R)-isomer: 27.4 %, mixture of other diastereomers: 5.86 % (Flavour Industry, 2006).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

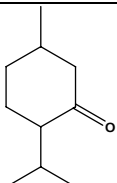
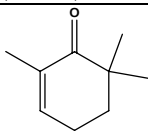
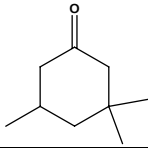
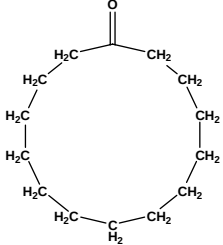
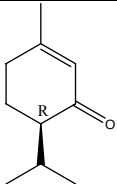
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
07.059	p-Menthan-3-one		2667 2035 10458-14-7	Liquid C ₁₀ H ₁₈ O 154.25	Soluble Soluble	207 MS 96 %	1.448-1.453 0.888-0.895	Mixture of diastereoisomers, approximately 25 % of each (EFFA, 2012b).
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		20013-73-4	Liquid C ₉ H ₁₄ O 138.21	Slightly soluble Freely soluble	63 (16 hPa) MS 95 %	1.470-1.476 0.924-0.930	
07.203	3,3,5-Trimethylcyclohexan-1-one		873-94-9	Liquid C ₉ H ₁₆ O 140.22	Practically insoluble or insoluble Freely soluble	189 MS 95 %	1.442-1.448 0.888-0.894	Racemate (EFFA, 2010a).
07.207	Cyclotetradecanone		3603-99-4	Solid C ₁₄ H ₂₆ O 210.36	Practically insoluble or insoluble Freely soluble	159 (16 hPa) 53 NMR 95 %	n.a. n.a.	Substance no longer supported by Industry (EFFA, 2009c).
07.255 1856	l-Piperitone		4200 4573-50-6	Liquid C ₁₀ H ₁₆ O 152.24	Slightly soluble Freely soluble	246 MS 99 %	1.482-1.488 0.929-0.935	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

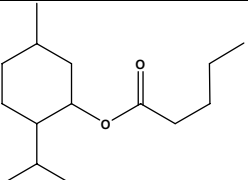
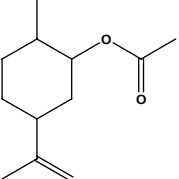
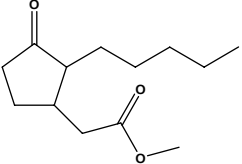
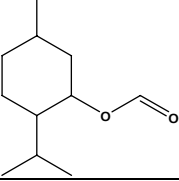
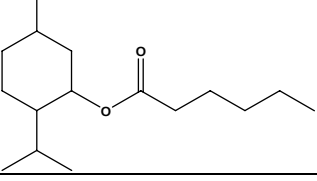
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.154 1852	Menthyl valerate		4156 472 89-47-4	Liquid C ₁₅ H ₂₈ O ₂ 240.39	Practically insoluble or insoluble Freely soluble	261 NMR 95 %	1.445-1.451 0.903-0.909	Register name to be changed to (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl valerate (EFFA, 2010a).
09.355	neo-Dihydrocarvyl acetate		10859 56422-50-5	Liquid C ₁₂ H ₂₀ O ₂ 196.29	Practically insoluble or insoluble Freely soluble	266 MS 95 %	1.453-1.459 0.925-0.931	According to EFFA: Mixture of the two racemic forms (1S,2R,5R) and (1R,2S,5S), which is specified by the name (EFFA, 2005I).
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		3408 10785 24851-98-7	Liquid C ₁₃ H ₂₂ O ₃ 226.32	Slightly soluble Freely soluble	111 (0.1 hPa) NMR 98 %	1.458-1.462 0.997-1.006	According to EFFA: Mixture of the four stereoisomeric forms (RR, RS, SR and SS) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a).
09.618	Menthyl formate		10751 2230-90-2	Liquid C ₁₁ H ₂₀ O ₂ 184.28	Practically insoluble or insoluble Freely soluble	95 (13 hPa) 9 MS 95 %	1.446-1.452 0.933-0.939	According to EFFA: Mixture of the two racemic forms (1S,2R,5S) and (1R,2S,5R), which is specified by the name.
09.619	Menthyl hexanoate		6070-16-2	Liquid C ₁₆ H ₃₀ O ₂ 254.14	Practically insoluble or insoluble Freely soluble	153 (20 hPa) MS 95 %	1.445-1.451 0.898-0.906	Register name to be changed to (1R,2S,5R)-Menthyl hexanoate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

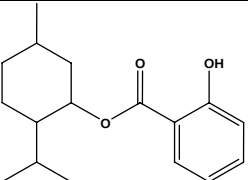
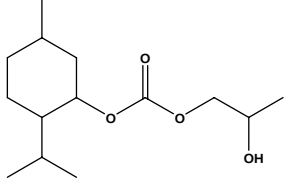
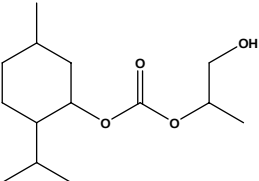
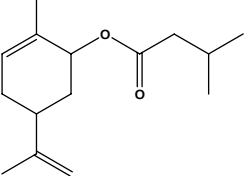
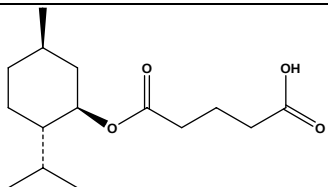
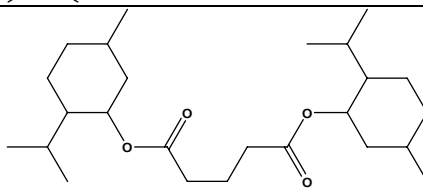
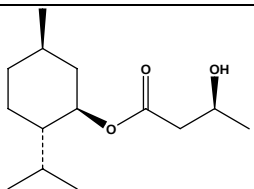
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.621	Menthyl salicylate		89-46-3	Liquid C ₁₇ H ₂₄ O ₃ 276.37	Practically insoluble or insoluble Freely soluble	175 (13 hPa) MS 95 %	1.509-1.515 1.047-1.053	Register name to be changed to (1R,2S,5R)-Menthyl salicylate.
09.843	Menthol 1-and 2-propylene glycol carbonate	 + 	3806 30304-82-6	Liquid C ₁₄ H ₂₆ O ₄ 258.36	Insoluble Soluble	143 IR MS 98 %	1.458-1.458 1.013-1.014	According to EFFA: [FL-no: 09.843] is a mixture of 60 % Menthol 1-propylene glycol carbonate (which is a mixture of four stereoisomers, 15 % of each) and 40 % Menthol 2-propylene glycol carbonate (which is a mixture of four stereoisomers 10 % of each) (EFFA, 2012b)
09.870	Carvyl-3-methylbutyrate		94386-39-7	Liquid C ₁₃ H ₂₄ O ₂ 236.37	Practically insoluble or insoluble Freely soluble	343 MS 95 %	1.462-1.468 0.932-0.938	According to EFFA: Mixture of the four stereoisomeric forms (RR, RS, SR and SS) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.929	L-Monomenthyl glutarate		4006 220621-22-7	Liquid C ₁₅ H ₂₆ O ₄ 270	Sparingly soluble Soluble	390 (decomp) n.a. IR NMR 95 %	1.462-1.470 1.026-1.036	
09.935	Dimenthyl glutarate		406179-71-3	Solid C ₂₅ H ₄₄ O ₄ 408	Insoluble Soluble	48-50 NMR MS 98 %	n.a. n.a.	According to EFFA: Menthyl moiety mixture of the two racemic forms (1S,2R,5S) and (1R,2S,5R), which is specified by the name. Since there are two menthyl moieties, three combinations exist, approximately 25 % +/- 25 % - /- and 50 % +/- (EFFA, 2010a).
09.949	L-Menthyl (S)-3-hydroxybutyrate		4308 115869-76-6	Liquid C ₁₄ H ₂₆ O ₃ 242.35	Slightly soluble Soluble	95-97 (0.7hPa) IR MS 98 %	1.454 - 1.464 0.969 - 0.979	Stereoisomeric composition of (S)-form : > 80 % ee and (R)-form < 20 % ee (ee=enantiomeric excess).

- 1) Solubility in water, if not otherwise stated.
- 2) Solubility in 95 % ethanol, if not otherwise stated.
- 3) At 1013.25 hPa, if not otherwise stated.
- 4) At 20°C, if not otherwise stated.
- 5) At 25°C, if not otherwise stated.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

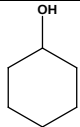
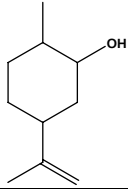
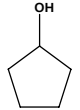
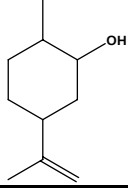
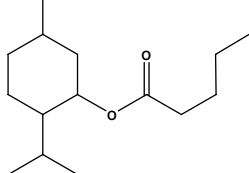
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.070	Cyclohexanol		3.7	Class I A3: Intake below threshold	4)	6)	
02.075	neo-Dihydrocarveol		2.4	Class I A3: Intake below threshold	4)	6)	
02.135	Cyclopentanol		0.012	Class I A3: Intake below threshold	4)	6)	
02.167	Isodihydrocarveol		2.4	Class I A3: Intake below threshold	4)	6)	
09.154 1852	Menthyl valerate		1.0	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

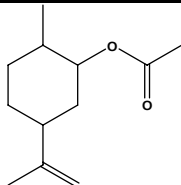
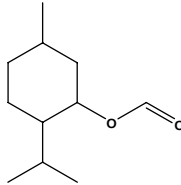
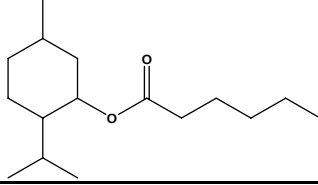
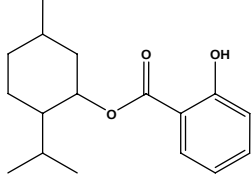
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.355	neo-Dihydrocarvyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.618	Menthyl formate		0.73	Class I A3: Intake below threshold	4)	6)	
09.619	Menthyl hexanoate		0.37	Class I A3: Intake below threshold	4)	6)	
09.621	Menthyl salicylate		0.012	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

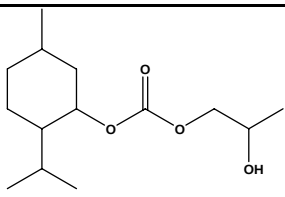
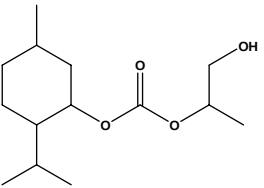
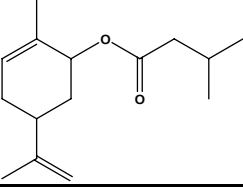
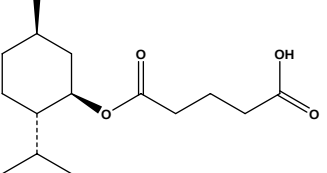
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.843	Menthol 1-and 2-propylene glycol carbonate	 + 	830 380	Class I A3: Intake below threshold	4)	6)	
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.929	L-Monomenthyl glutarate		110	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

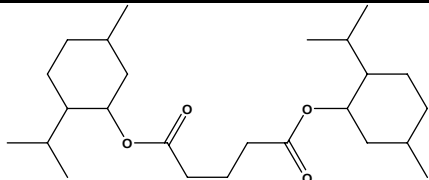
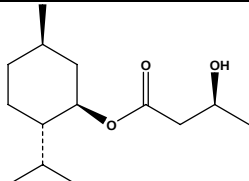
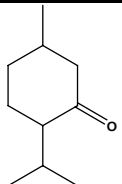
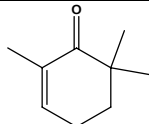
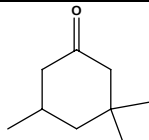
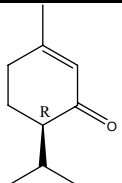
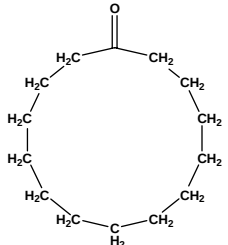
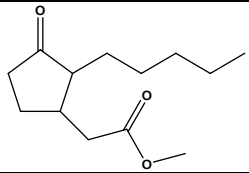
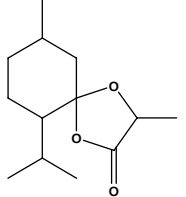
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.935	Dimenthyl glutarate		30	Class I A3: Intake below threshold	4)	6)	
09.949	L-Menthyl (S)-3-hydroxybutyrate		37	Class I A3: Intake below threshold	4)	6)	
07.059	p-Menthan-3-one		530 2500	Class II A3: Intake below threshold	4)	6)	
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		0.12	Class II A3: Intake below threshold	4)	6)	b)
07.203	3,3,5-Trimethylcyclohexan-1-one		0.0085	Class II A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
07.255 1856	l-Piperitone		12	Class II A3: Intake below threshold	4)	6)	b)
07.207	Cyclotetradecanone		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		c)
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		770	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6)	
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		1.2	Class III A3: Intake below threshold	4)	6)	

- 1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
- 2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
- 3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
- 4) No safety concern based on intake calculated by the MSDI approach of the named compound.

- 5) Data must be available on the substance or closely related substances to perform a safety evaluation.
- 6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).
- 7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
- 8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.
 - a) Evaluated in FGE.212, genotoxic concern could be ruled out.
 - b) Evaluated in FGE.212Rev1, genotoxic concern could be ruled out.
 - c) Substance not supported by Industry (EFFA, 2009c).

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

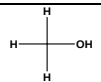
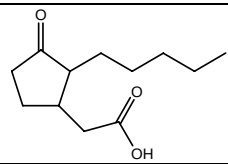
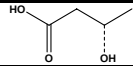
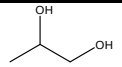
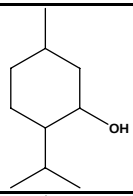
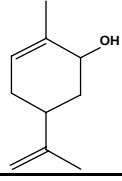
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
Not in Register	Methanol		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	3-Oxo-2-pentyl-1-cyclopentyl acetic acid		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	(S)-3-Hydroxybutyric acid		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	1,2-Propandiol		Not evaluated as flavouring substance		Not a Register substance.
02.015	Menthol 427		No safety concern a) Category A b)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	NOAEL: 380 mg/kg bw/day
02.062	Carveol 381		No safety concern a) Category B b)	Class I A3: Intake below threshold	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

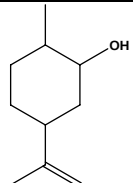
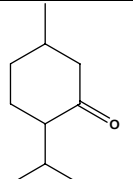
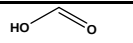
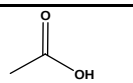
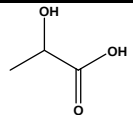
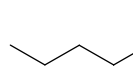
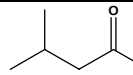
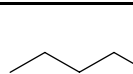
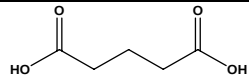
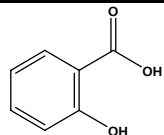
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.075	neo-Dihydrocarveol		Category B b) FGE.09	Class I A3: Intake below threshold	
07.059	p-Menthan-3-one		Category B b) FGE.37	Class II A3: Intake below threshold	
08.001	Formic acid 79		Category 1 c) No safety concern d) Deleted b)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern e) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.082	Glutaric acid		FGE.10	Class I A3: Intake below threshold	
08.112	Salicylic acid 958		No safety concern e)	Class I A3: Intake below threshold	

- 1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.
- 2) No safety concern at estimated levels of intake.
- 3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.
- 4) Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
- 5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
- a) (JECFA, 2000a).
- b) (CoE, 1992).
- c) (SCF, 1995).
- d) (JECFA, 1999b).
- e) (JECFA, 2002b).
- ND: Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

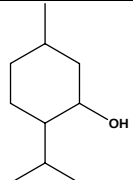
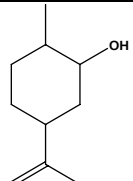
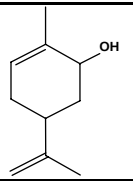
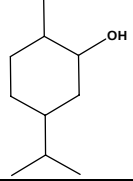
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02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998b)	16000	No safety concern a) Category A b)	ADI: 0-4 (JECFA, 2000a).
02.061	Dihydrocarveol		2379 2025 619-01-2	378 JECFA specification (JECFA, 1998b)	0.37	No safety concern a) Category B b)	
02.062	Carveol		2247 2027 99-48-9	381 JECFA specification (JECFA, 1998b)	9.5	No safety concern a) Category B b)	
02.071	p-Menthan-2-ol		3562 2228 499-69-4	376 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

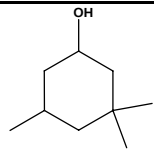
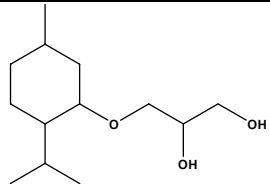
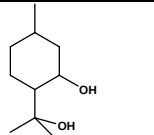
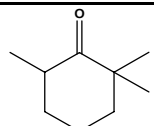
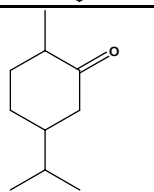
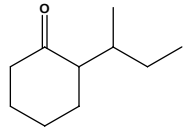
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.209	3,3,5-Trimethylcyclohexan-1-ol		3962 116-02-9	1099 JECFA specification (JECFA, 2002d)	0.12	No safety concern c)	JECFA name: 3,3,5-Trimethyl cyclohexanol.
02.224	3-(1-Menthoxo)propane-1,2-diol		3784 87061-04-9	1408 JECFA specification (JECFA, 2005b).	4.1	No safety concern d)	JECFA name: 3-L-Menthoxopropane-1,2-diol.
02.246	p-Menthane-3,8-diol		4053 42822-86-6	1416 JECFA specification (JECFA, 2005b).	39	No safety concern d)	
07.045	2,2,6-Trimethylcyclohexanone		3473 686 2408-37-9	1108 JECFA specification (JECFA, 2002d)	2.1	No safety concern c) Category B b)	Stereoisomeric composition to be specified.
07.092	p-Menthan-2-one		3176 11128 499-70-7	375 JECFA specification (JECFA, 1998b)	0.012	No safety concern a)	
07.095	2-(sec-Butyl)cyclohexanone		3261 11044 14765-30-1	1109 JECFA specification (JECFA, 2002d)	5.1	No safety concern c)	

Table 3: Supporting Substances Summary

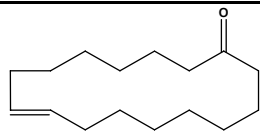
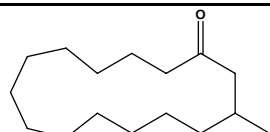
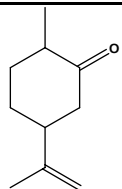
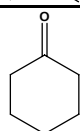
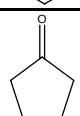
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.110	Cycloheptadec-9-en-1-one		3425 11744 542-46-1	1401 JECFA specification (JECFA, 2005b).	0.24	No safety concern d)	
07.111	3-Methylcyclopentadecan-1-one		3434 11135 541-91-3	1402 JECFA specification (JECFA, 2005b).	0.37	No safety concern d)	JECFA name: 3-Methyl-1-cyclopentadecanone.
07.128	Dihydrocarvone		3565 11703 7764-50-3	377 JECFA specification (JECFA, 2000d)	0.012	No safety concern a)	
07.148	Cyclohexanone		3909 11047 108-94-1	1100 JECFA specification (JECFA, 2002d)	0.12	No safety concern c)	
07.149	Cyclopentanone		3910 11050 120-92-3	1101 JECFA specification (JECFA, 2002d)	0.018	No safety concern c)	

Table 3: Supporting Substances Summary

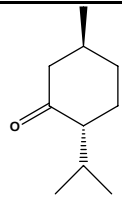
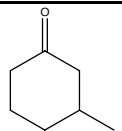
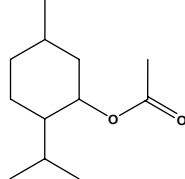
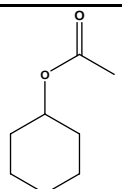
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.176	trans-Menthone	 (-)-menthone shown	2667 2035 89-80-5	429 JECFA specification (JECFA, 1998b)	890	No safety concern a)	JECFA name: Menthone. CASrn in Register refers to cyclohexanone, 5- methyl-2-(1- methylethyl)-, (2R,5S)- rel-.
07.180	3-Methylcyclohexanone		3947 591-24-2	1103 JECFA specification (JECFA, 2002d)	0.12	No safety concern c)	
09.016	Menthyl acetate		2668 206 29066-34-0	431 JECFA specification (JECFA, 1998b)	270	No safety concern a) Category B b)	JECFA evaluated menthyl acetate (CASrn 16409-45-3 which does not specify isomer). CASrn in Register replaced by 89-48-5 which refers to Cyclohexanol, 5-methyl- 2-(1-methylethyl)-, acetate, (1R,2S,5R) (SciFinder).
09.027	Cyclohexyl acetate		2349 217 622-45-7	1093 JECFA specification (JECFA, 2002d)	12	No safety concern c) Category B b)	

Table 3: Supporting Substances Summary

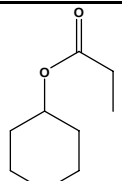
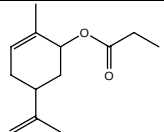
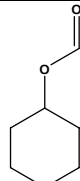
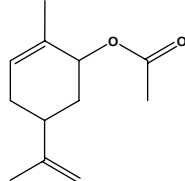
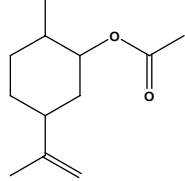
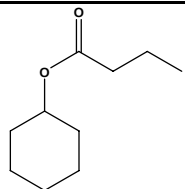
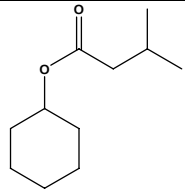
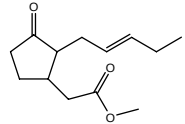
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.140	Cyclohexyl propionate		2354 421 6222-35-1	1097 JECFA specification (JECFA, 2002d)	0.012	No safety concern c) Category B b)	
09.143	Carvyl propionate		2251 424 97-45-0	383 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
09.160	Cyclohexyl formate		2353 498 4351-54-6	1095 JECFA specification (JECFA, 2002d)	0.012	No safety concern c) Category B b)	
09.215	Carvyl acetate		2250 2063 97-42-7	382 JECFA specification (JECFA, 1998b)	4.0	No safety concern a) Category B b)	
09.216	Dihydrocarvyl acetate		2380 2064 20777-49-5	379 JECFA specification (JECFA, 1998b)	9.7	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.230	Cyclohexyl butyrate		2351 2082 1551-44-6	1094 JECFA specification (JECFA, 2002d)	0.89	No safety concern c) Category B b)	
09.464	Cyclohexyl isovalerate		2355 459 7774-44-9	1096 JECFA specification (JECFA, 2002d)	0.28	No safety concern c) Category B b)	
09.521	Methyl 3-oxo-2-pent-2-enyl-1-cyclopentylacetate		3410 10821 39924-52-2	1400 JECFA specification (JECFA, 2005b).	26	No safety concern d)	JECFA evaluated methyl jasmonate (CASrn 1211-29-6). (R)- or (S)- nor (E)- or (Z)- not specified by Register CASrn.

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2000a).

b) (CoE, 1992).

c) (JECFA, 2002c).

d) (JECFA, 2005c).

ND) No intake data reported

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 µg/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products⁹ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous¹⁰ (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

⁹ "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

¹⁰ "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

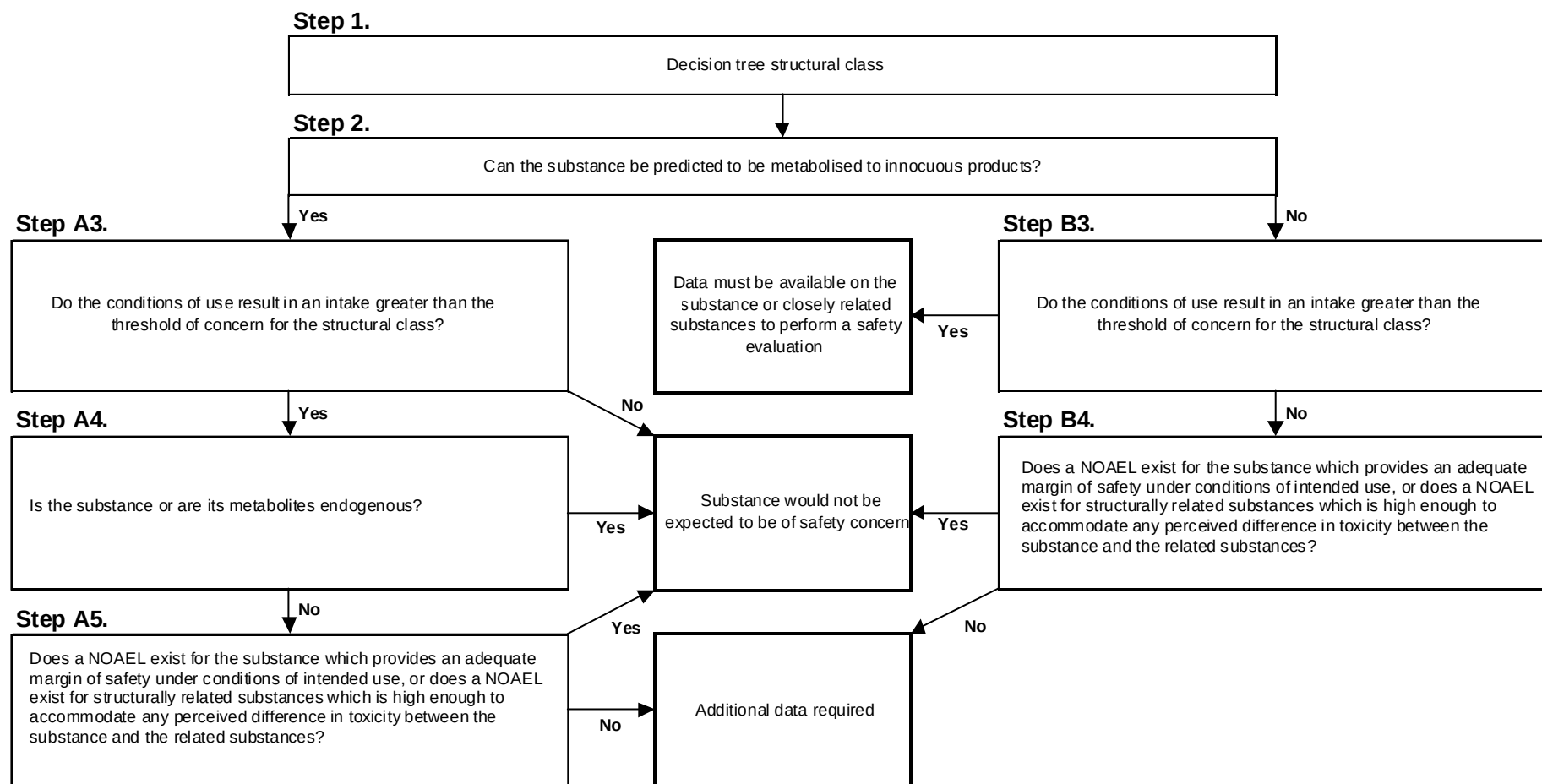


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000a). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic (“soft”) beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The “normal and maximum use levels” are provided by Industry (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k) for 20 of the 21 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.09Rev4 (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k).

FL-no	Food Categories																		
	Normal use levels (mg/kg)																		
	Maximum use levels (mg/kg)																		
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0	
02.07	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2	
0	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10	
02.07	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2	
5	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10	
02.13	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2	
5	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10	
02.16	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2	
7	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10	
06.13	0,000	0,000	0,000	-	-	0,00	0,000	0,000	-	-	-	-	-	0,000	0,000	0,000	-	0,000	
6	1	1	1	-	-	1	1	1	-	-	-	-	-	1	1	1	-	1	
	0,000	0,000	0,000	-	-	0,00	0,000	0,000	-	-	-	-	-	0,000	0,000	0,000	-	0,000	
	5	1	8	-	-	5	5	5	-	-	-	-	-	5	5	8	-	5	
07.05	-	-	15,32	-	-	33,2	-	47,89	-	-	-	-	-	-	4,22	0,87	-	-	
9	-	-	22,99	-	-	7	-	68,1	-	-	-	-	-	-	5,86	2,59	-	-	
						52,9													

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.09Rev4 (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
	7																	
07.20	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
2	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.20	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
3	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.20	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
7	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
07.25	0,5	-	0,2	-	-	0,5	-	1	-	-	-	-	0,2	-	0,2	0,2	5	0,2
5	5	-	2	-	-	5	-	10	-	-	-	-	2	-	1	2	50	2
09.15	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
4	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.35	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
5	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
09.52	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
0	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.61	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
8	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.61	7	5	1	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
9	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.62	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
1	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
09.84	200	-	100	-	-	500	15	60	-	-	-	-	25	-	30	100	25	-
3	800	-	400	-	-	2000	60	250	-	-	-	-	100	-	120	400	100	-
09.87	3	2	3	2	-	4	2	5	1	1	-	-	2	3	-	4	5	2
0	15	10	15	10	-	20	10	25	5	5	-	-	10	15	-	20	25	10
09.93	1	1	10	1	1	100	-	10	1	1	-	-	1	-	100	100	1	1
5	15	15	150	15	15	1500	-	150	15	15	-	-	15	-	1500	1500	15	15
09.94	30	-	10	20	-	50	5	20	-	-	-	-	10	-	10	10	30	10
9	150	-	50	100	-	200	20	100	-	-	-	-	30	-	50	50	150	30

The candidate substances [FL-no. 07.059, 09.843 and 09.949] are also used in chewing gum, which is not covered by any of the above food categories. The normal/maximum use levels for chewing gum are reported to be 14.34/14.34 mg/kg for [FL-no: 07.059], 5000/20000 mg/kg for [FL-no: 09.0843] and 500/1000 mg/kg for [FL-no: 09.949]. Under the assumptions that all of the flavouring substances are released from the chewing gum and that the intake estimate is 2 g chewing gum/day, the calculation of the mTAMDI of the candidate substance based on the 16 food categories and the use of chewing gum sum up to 8700, 63000 and 10600 µg/person/day, respectively. These figures are presented in tables II.2.3 and 6.1.

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for each of the flavouring substances in the present flavouring group, for which Industry has provided use and use levels (Burdock, 1995; ECHA, 2003a; ECHA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.070	Cyclohexanol	1600	Class I	1800
02.075	neo-Dihydrocarveol	1600	Class I	1800
02.135	Cyclopentanol	1600	Class I	1800
02.167	Isodihydrocarveol	1600	Class I	1800
09.154	Menthyl valerate	3900	Class I	1800
09.355	neo-Dihydrocarvyl acetate	1600	Class I	1800
09.618	Menthyl formate	3900	Class I	1800
09.619	Menthyl hexanoate	3900	Class I	1800
09.621	Menthyl salicylate	420	Class I	1800
09.843	Menthol 1-and 2-propylene glycol carbonate	63000	Class I	1800
09.870	Carvyl-3-methylbutyrate	1000	Class I	1800
09.929	L-Monomenthyl glutarate		Class I	1800
09.935	Dimenthyl glutarate	38000	Class I	1800
09.949	L-Menthyl (S)-3-hydroxybutyrate	10600	Class I	1800
07.059	p-Menthan-3-one	8700	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	1600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	1600	Class II	540
07.255	l-Piperitone	320	Class II	540
07.207	Cyclotetradecanone	3900	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate	3900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one	0.075	Class III	90

ANNEX III: METABOLISM

III.1. Absorption, Distribution and Elimination

The candidate substances of secondary alicyclic saturated and unsaturated alcohols, ketones and esters of the present flavouring group evaluation are expected to be rapidly absorbed from the gastrointestinal tract. Supporting substances evaluated by the JECFA sustain this view (JECFA, 1999a; JECFA, 2003a).

III.2. Biotransformation

The candidate substances are expected to be metabolised through several alternative metabolic pathways. Depending on their chemical structure, the possible metabolic reactions are the following:

III.2.1 Ester hydrolysis

III.2.2 Reduction of ketone groups and oxidation of alcohol groups

III.2.3 Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

III.2.4 Metabolism to glucuronides

III.2.5 Metabolism to sulphates

No information is available on the toxicokinetics of the candidate substance cyclotetradecanone [FL-no: 07.207] or any related supporting substances in the present FGE.

III.2.1. Ester hydrolysis

The esters included in this FGE are expected to be hydrolysed enzymatically to carboxylic acids and alcohols via carboxylesterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980). For the one hemiketal ester [FL-no: 06.136] hydrolysis to the corresponding cyclic ketone, p-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004] is expected.

The supporting substances menthyl acetate [FL-no: 09.016] and dihydrocarvyl acetate [FL-no: 09.216] were previously evaluated by the JECFA (JECFA, 1999a), but no metabolism studies were available for these supporting substances, structurally related to the candidate substances menthyl valerate, neo-dihydrocarvyl acetate, menthyl formate and menthyl hexanoate [FL-no: 09.154, 09.355, 09.618 and 09.619]. The JECFA evaluation was based on a study demonstrating about 75 % and 85 % hydrolysis of 1-menthol propylene glycol carbonate and 1-menthol ethylene glycol carbonate, respectively, after four hours in liver homogenate. Less than 20 % of these two substances were hydrolysed in gastric juice and intestinal fluid (Emberger, 1994a; Emberger, 1994b). More than 80 % of a radioactively labelled mandelic acid of 3,3,5-trimethylcyclohexanol, a cyclohexyl ester structurally related to the candidate substance menthyl salicylate [FL-no: 09.621], was hydrolysed after 15 minutes of incubation with rat hepatic microsomes (White et al., 1990).

Based on these data, it is anticipated that candidate esters and the one hemiketal ester [FL-no: 06.136, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949], after intestinal absorption are hydrolysed to the corresponding alcohols/ketone and their corresponding carboxylic acids (see

Table 2b). The simple mono- and di-carboxylic acids [FL-no: 08.001, 08.002, 08.004, 08.007, 08.008, 08.009 and 08.082] and 3-hydroxybutyric acid are expected to be completely metabolised through common routes of biotransformations. The acids salicylic acid [FL-no: 08.112] and 3-oxo-2-pentyl-1-cyclopentyl acetic acid (formed from [FL-no: 09.520]) are anticipated to be conjugated and excreted with the urine.

III.2.2. Reduction of ketone groups and oxidation of alcohol groups

Six of the candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207, 07.255 and 09.520] contain a ketone group, which may be metabolically reduced to a hydroxyl group. This may also be expected for the hemiketal ester [FL-no: 06.136] after hydrolysis to ketone.

Incubation of human liver microsomes with the supporting substance trans-menthone resulted in formation of two metabolites. The major metabolite was a reduction product, (+)neomenthol and a hydroxylation product, 7-hydroxymenthone, was a minor metabolite (Miyazawa & Nakanishi, 2006)

Metabolism of the supporting substance carveol [FL-no: 02.062], the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870], was studied *in vitro*. (+)-Carveol and (+)-carvone were incubated with liver microsomes from dogs, rabbits, guinea pigs, mice, rats, monkeys and humans. (+)-Carveol was oxidized to (+)-carvone by liver microsomes of dogs, rabbits and guinea pigs, but not by those of humans, monkeys, rats and mice. On the other hand the (+)-carvone was reduced to (+)-carveol by liver microsomes of all animals examined. These results suggest a species specific metabolism of (+)-carveol, and shows that carveol is not converted to carvone in the liver of humans (Shimada et al., 2002).

In vivo metabolism of *l*-menthol was studied in adult male rats by giving the rats 800 mg/kg bw *l*-menthol solved in 1 % methyl cellulose solution by gavage every day for 20 days. Control rats were given vehicle only. The following metabolites of *l*-menthol were found in the urine; *p*-menthane-3,8-diol, *p*-menthane-3,9-diol, 3,8-oxy-*p*-menthane-7-carboxylic acid and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. The main urinary metabolites were *p*-menthane-3,9-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. Menthone was not detected (Madyastha and Srivatsan, 1988a).

III.2.3. Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

Oxidation of alkyl groups have been observed for menthol and for the hydrolysis product of the candidate esters neo-dihydrocarvyl acetate [FL-no: 09.355] and menthyl formate [FL-no: 09.618], and for the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] (Truhaut et al., 1970; Yamaguchi et al., 1994).

III.2.4. Metabolism to glucuronides

The hydrolysis product menthol as such or after the oxidation of the alkyl ring substituents is mainly conjugated with glucuronic acid and excreted *via* the bile in rats. Low levels of oxidation products were found in the urine, but no unchanged menthol was detected in the urine, faeces or bile after oral administration of radioactive labelled menthol (Yamaguchi et al., 1994).

The candidate substances isodihydrocarveol and neo-dihydrocarveol [FL-no: 02.167 and 02.075] are also anticipated to be conjugated with glucuronic acid, since dihydrocarveol after application by gavage to rabbits was found in the urine as the glucuronide (Hämäläinen, 1912; JECFA, 1999a). However, dihydrocarveol was also found to be excreted unchanged (Fischer and Bielig, 1940; JECFA, 1999a). In rabbits, carvone is reduced to yield carveol, which then is converted to the glucuronic acid conjugate and excreted in the urine (Fischer and Bielig, 1940). Carveol, the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870] is therefore anticipated to be conjugated with glucuronic acid and excreted in the urine.

Salicylic acid (resulting from hydrolysis of menthyl salicylate [FL-no: 09.621]) is either excreted unchanged, or as salicyluric acid and salicylic glucuronide (Vree et al., 1994).

The candidate alicyclic ketones (p-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203], l-piperitone [FL-no: 07.255] and methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]) are anticipated to be reduced to the corresponding secondary alcohols. These secondary alcohols and the candidate secondary alcohols cyclohexanol [FL-no: 02.070], neo-dihydrocarveol [FL-no: 02.075], isodihydrocarveol [FL-no: 02.167] and cyclopentanol [FL-no: 02.135] are mainly excreted as conjugates with glucuronic acid. Studies in rabbits with the supporting substance cyclohexanone [FL-no: 07.148] and with cyclopentanone and cycloheptanone show that 50 - 70 % of these substances are reduced to the corresponding alcohols (the candidate substances cyclohexanol [FL-no: 02.070] and cyclopentanol [FL-no: 02.135] and cycloheptanol), which are conjugated with glucuronic acid and excreted (Elliott et al., 1959; James and Waring, 1971). Workers employed in a shoe factory were exposed to small amounts of cyclohexane in the air. Cyclohexanol and cyclohexanone were found in the urine of these workers, indicating that the same metabolic pathways are also found in humans (Governa et al., 1987). A recent study in humans shows that the main metabolite in urine after cyclohexanone or cyclohexanol exposure is not cyclohexanol-glucuronide as in rabbit and rats, but 1,2-cyclohexanediol-glucuronide (Mráz et al., 1994; Mráz et al., 1998).

When the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] was given to rats and rabbits glucuronides of 3,5,5-trimethylcyclohexanol were detected in the urine (Truhaut et al., 1979).

III.2.5. Metabolism to sulphates

A small fraction of the two candidate substances, cyclopentanol and cyclohexanol [FL-no: 02.135 and 02.070], is anticipated to be conjugated with sulphate and excreted in the urine. This is based on studies on the structurally related substances cyclopentanone, cyclohexanone and cycloheptanone, which were given by gavage to rabbits (1.7 - 2.3 mmol/kg) and rats (1.8 - 2.5 mmol/kg), and 1 - 3 % of the dose was found in the urine as sulphate conjugates (James and Waring, 1971).

III.3. Summary and Conclusions

The 11 esters [FL-no: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the evaluation of supporting substances (Heymann, 1980; Anders, 1989; Emberger, 1994a; Emberger, 1994b; White et al., 1990). The resulting carboxylic acids are either metabolised through common physiological pathways like *beta*-oxidation and the citric acid cycle or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, *p*-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-no: 02.070, 02.075, 02.135, 02.167, 07.059, 07.202, 07.203 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, hydrolysis products of [FL-no: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are also metabolised via this pathway. Neither menthol nor carveol or dihydrocarveol is anticipated to be oxidised to the corresponding ketone.

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion.

Thus, it may be anticipated that these 20 substances will be metabolised to innocuous products.

No information is available on toxicokinetics (including metabolism) of cyclotetradecanone [FL-no: 07.207] or on structurally related substances. Cyclotetradecanone [FL-no: 07.207] cannot be assumed to be metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for five candidate substances of the present FGE and for ten supporting substances evaluated by the JECFA at the 51st, 59th and 63rd meetings. The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Menthol [02.015])	Mouse	M	Gavage	2652	(Food and Drug Research Laboratories, Inc., 1975a)	
	Mouse	M	Gavage	4384	(Food and Drug Research Laboratories, Inc., 1975a)	
	Mouse	NR	Gavage	3100	(Wokes, 1932)	
	Rat	M, F	Gavage	3180	(Jenner et al., 1964)	
	Rat	M	Gavage	940	(Food and Drug Research Laboratories, Inc., 1975a)	
(trans-Menthone [07.176])	Rat	M, F	Oral	1600 - 1950	(Igimi and Ide, 1974; Levenstein, 1973c)	Testmaterial = racemic menthone
(Menthyl acetate [09.016])	Rat	M, F	Gavage	> 7000	(Levenstein, 1973d)	Testmaterial = racemic menthyl acetate
	Rat	M, F	Oral	> 5000	(Shelanski, 1972a)	Testmaterial = <i>l</i> -menthyl acetate
(Dihydrocarveol [02.061])	Rat	NR	Oral	> 5000	(Moreno, 1977k)	
(Dihydrocarvyl acetate [09.216])	Rat	NR	Oral	> 5000	(Moreno, 1980f)	
neo-Dihydrocarvyl acetate [09.355]	Rat	NR	Oral	> 5000	(Moreno, 1980f)	
Cyclopentanol [02.135]	Rat	NR	Gavage	< 625	(Myers et al., 1980)	
(3,5,5-Trimethylcyclohexanol [02.209]) (Cyclohexanone [07.148])	Rat	M, F	Oral	3250	(Smyth and Carpenter, 1948)	
	Rat	M, F	Oral	1705	(Kohli et al., 1967)	
	Rat	M, F	Oral	1840	(Deichmann and LeBlanc, 1943)	
	Rat	M, F	Oral	1620	(Smyth et al., 1969a)	
	Rat	M, F	Oral	1800	(Gupta et al., 1979)	
	Mouse	M, F	Oral	2070	(Gupta et al., 1979)	
	Rabbit	M	Gavage	1600	(Treon et al., 1943)	
	Rabbit	M	IP	1540	(Gupta et al., 1979)	
	Guinea pig	M	IP	930	(Price, 1951)	
	Rat	M	Gavage	1750	(Miller and Sherman, 1965)	
Cyclohexanol [02.070]	Rat	NR	Oral	1550	(Birch, 1978a)	
	Rat	NR	Oral	2060 ¹	(Smyth et al., 1946c)	
	Rat	NR	Oral	2060	(Bär and Griepentrog, 1967)	
	Rat	M, F	Oral	1120	(Birch et al., 1981)	
	Rabbit	NR	Gavage	2200 - 2600 ²	(Treon et al., 1943)	
	Dog	M, F		> 2000	(You et al., 1997)	
(3-Methylcyclopentadecan-1-one [07.111])	Rat	M, F		> 5000	(Oh et al., 1997)	
	Rat	NR	Oral	>2000	(Flavour Industry, 2006c)	
6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [06.136]	Rat	NR	Oral	> 2000	(Flavour Industry, 2006c)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Rat	NR	Oral	> 2000	(Flavour Industry, 2006c)	
(Carveol [02.062])	Rat	NR	Oral	3000	(Keating, 1972a)	

Table IV.1: ACUTE TOXICITY

Chemical Name	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Carvyl acetate [09.215])	Rat	NR	Oral	> 5000	(Levenstein, 1976c)	

NR: Not reported, M: Male, F: Female.

¹ Administered as 10 % solution in Tergitol 7.

² Minimum lethal dose.

Subacute / subchronic / chronic toxicity data are available two candidate substance of the present flavouring group and for five supporting substances evaluated at the 51st, 59th and 63rd JECFA meetings. The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
(Menthol [02.015])	Mouse; M, F 2/50	Diet	2000, 4000 ppm	103 weeks	600 ¹	(National Cancer Institute, 1979)	Good quality
	Mouse; F 2/30	Intraperitoneal injection (IP)	500 and 2000 mg/kg 3 times week	24 weeks	A NOAEL was not determined	(Stoner et al., 1973)	Good quality
	Rat; M, F 3/20	Gavage	0, 200, 400 and 800 mg/kg bw day	28 days	< 200 ²	(Thorup et al., 1983a)	Relative good quality
	Rat; M, F 2/80	Diet	100 and 200 mg/kg bw	5.5 weeks	200 ¹	(Herken, 1961)	Limited information
	Rat; M, F 2/50	Diet	3750 and 7500 ppm	103 weeks	375 ¹	(National Cancer Institute, 1979)	Good quality
(trans-Menthone [07.176])	Rat; M, F 3/20	Gavage	200, 400 and 800 mg/kg bw day	28 days	400	(Madsen et al., 1986)	Good quality
	Mouse; F 2/30	IP	1900 and 4750 mg/kg 3 times week	24 weeks	A NOAEL was not determined ²	(Stoner et al., 1973)	Good quality
(Cyclohexanone [07.148])	Mouse; M, F 7/20	Drinking Water	400 - 47000 ppm	13 weeks	M: approx. 3300, F: approx. 6500	(Lijinsky and Kovatch, 1986)	Good quality
	Rat; M, F 7/10	Drinking water	190 – 6500 ppm	25 weeks	Approx. 330	(Lijinsky and Kovatch, 1986)	Good quality
	Mouse; M, F 3/84-104	Drinking water	6500, 13000 and 25000 (F) ppm	2 years	Approx. 1600	(Lijinsky and Kovatch, 1986)	Good quality
	Rat; M, F 2/104	Drinking water	3300 and 6500 ppm	2 years	Approx. 330	(Lijinsky and Kovatch, 1986)	Good quality
	Rat 1/7	IP	200 mg/kg bw (twice a day) 5 days/week	13 weeks	A NOAEL was not determined ¹	(Perbellini et al., 1981)	Only neurotoxicity was checked. Limited experimental design
Cyclohexanol [02.070]	Rats 1/7	IP	200 mg/kg bw (twice a day) 5 days/week	3 weeks (twice a day) plus 3 weeks (once a day)	A NOAEL was not determined ¹	(Perbellini et al., 1981)	Limited experimental design
	Rat; M 1/6	Gavage	455 mg/kg day	7 days	455 ¹	(Lake et al., 1982)	Limited quality
	Rat; M 1/NR	Drinking water	10 ppm	30 days	1 ¹	(Messiha and Lox, 1985)	Limited quality
(2-sec-Butylcyclohexanone [07.095])	Rat 3/NR	Diet		91	370	(Hummler, 1969)	Study not available
(3-Methylcyclopentadecan-1-one [07.111])	Rat, M, F 3/20	Gavage		30	1000 ³	(Oh et al., 1997)	
	Dog, M, F 3/6	Gavaga		28	20 ³	(You et al., 1997)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Rat M, F 10/10	Diet	0, 10, 50 or 100 mg/kg bw day	90	100	(Kelly and Bolte, 2000)	

NR: Not reported.

M: Male, F: Female.

¹ The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

² The test substance was administered 3 times per week for 8 weeks; animals were observed for an additional 16 weeks.

³ Study was performed with either a single dose or multiple doses that produced no adverse effect. The value is therefore not a true NOEL, but is the highest dose tested that produced no adverse effects. The actual NOEL may be higher.

Developmental and reproductive toxicity data are only available for one candidate substance of the present FGE and for one supporting substance evaluated at the 51st JECFA meeting. Supporting substance is listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name	Study type Duration	Species/Sex No/group	Route	Dose levels (mg/kg/day)	NOAEL (mg/kg/day) Including information on possible maternal toxicity	Reference
(Menthol [02.015])	Teratology Gestation days 6-15	Mouse; F 22	Gavage	0, 1.85, 8.59, 39.9, 185	185 ¹	(Food and Drug Research Laboratories, Inc., 1973)
	Teratology Gestation days 6-15	Rat; F 22-23	Gavage	0, 2.18, 10.15, 47.05, 218	218 ¹	(Food and Drug Research Laboratories, Inc., 1973)
	Teratology Gestation days 6-15	Hamster; F 20-22	Gavage	0, 4.05, 21.15, 98.2, 405	405 ¹	(Food and Drug Research Laboratories, Inc., 1973)
	Teratology Gestation days 6-18	Rabbit; F 9-11	Gavage	0, 4.25, 19.75, 91.7, 425	425 ¹	(Food and Drug Research Laboratories, Inc., 1973)
Cyclohexanol [02.070]	Reproductive NR ²	Mouse; M, F NR	Diet	ca. 1500 (1 %)	< 1500 (< 1 %)	(Gondry, 1972)

NR: Not reported.

M: Male, F: Female.

¹ The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

² Animals were exposed during gestation, lactation and weaning over multiple generations. Total length of exposure not reported.

In vitro mutagenicity/genotoxicity data are available for three candidate substances of the present flavouring group evaluation and for nine supporting substances evaluated at the 51st, 59th and 63rd JECFA meetings and one structurally related substance evaluated at the 69th meeting. Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
(Menthol [02.015])	Ames test	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537	0, and 6 concentrations up to 5000 µg/plate	Negative ¹	(Ishidate et al., 1984)	d,l-Menthol was used. The study is considered valid.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535	3 - 666 µg/plate	Negative ¹	(Zeiger et al., 1988)	d,l-Menthol was used. The study is considered valid.
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA2637	0, 5 - 500 µg/plate	Negative ¹	(Nohmi et al., 1985)	d,l-menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA2637	0, 20 - 500 µg/plate	Negative ¹	(Nohmi et al., 1985)	l-menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 6.4, 32, 160, and 800 µg/plate	Negative ¹	(Andersen and Jensen, 1984b)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.
	Ames test	<i>E. coli</i> WP2 uvrA (Trp ⁻)	100 - 800 µg/plate	Negative	(Yoo, 1986)	l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.
	Ames test	<i>S. typhimurium</i> TA97A, TA98, TA100, TA102	0, 5 - 800 µg/plate	Negative ¹	(Gomes-Carneiro et al., 1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.
	Rec assay	<i>B. subtilis</i> H17, M45	Up to 10000 µg/disk	Positive	(Yoo, 1986)	l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method (rec-assay) has poor predictive value.
	Rec assay	<i>B. subtilis</i> H17, M45	20 µg/disk	Negative	(Oda et al., 1979)	l-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (rec-assay) has poor predictive value.
	Alkaline elution assay	Rat hepatocytes	0, 0.1 - 1.3 mM (203.2 µg/ml ⁴)	Negative	(Storer et al., 1996)	The experiment employed d-Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.
	Sister chromatid exchange	Chinese hamster ovary cells	5 - 50 and 0, 2 - 25 µg/ml ³ 0, 16 - 167 µg/ml ²	Negative ¹	(Ivett et al., 1989)	d,l-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/ml ⁴)	Negative ¹	(Murthy et al., 1991)	The study is considered valid.
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1, 10 µg/ml	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 and three concentrations up to 200 µg/ml	Negative ³	(Ishidate et al., 1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.
	Chromosome aberration	Chinese hamster ovary cells	0, 50 - 250 µg/ml	Negative ¹	(Ivett et al., 1989)	d,l-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Chromosome aberration	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/ml ⁴)	Negative ¹	(Murthy et al., 1991)	The study is considered valid.
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0, 12.5 - 200 µg/ml	Negative ¹	(Myhr and Caspary, 1991)	d,l-Menthol was used. The maximum concentration was selected by a preliminary test. The study is considered valid.
(trans-Menthone [07.176])	Ames test	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537	0, 6.4 - 800 µg/plate	Positive ¹	(Andersen and Jensen, 1984b)	Concentrations were selected based on preliminary experiments. In absence of metabolic activation, menthone was mutagenic only to strain TA1537 at 6.4 and 32 µg/ml (slightly less than 2-fold increase in mutation frequency), but not at higher (toxic) concentrations. Also in absence of metabolic activation, there was a concentration dependent increase in number of TA97 strain revertants (up to 4-fold increase at 600 µg/l). It was stated that metabolic activation did not enhance the mutagenicity of menthone. The study is considered valid.
Cyclopentanol [02.135]	Modified Ames test	<i>S. typhimurium</i> G46, TA98, TA100, TA1535, C3076, TA1537, D3052, TA1538 <i>E. coli</i> WP2, WP2 <i>uvrA</i>	0, 0.1 - 1000 µg/ml	Negative ¹	(McMahon et al., 1979)	The study was performed with agar plates containing the following concentration gradients: 0.1 - 1, 1 - 10, 10 - 100, and 100 - 1000 µg/ml. The study is considered valid, although tabulated data on cyclopentanol were not presented.
(Cyclohexanone [07.148])	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	33 - 3333 µg/plate	Negative ¹	(NTP, 2007)	
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 33 - 10000 µg/plate	Negative ¹	(Haworth et al., 1983)	The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid.
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 3 µmol/plate	Negative ¹	(Florin et al., 1980)	A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03 - 30 µmol/plate. The validity of the study cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	NR	Positive	(Massoud et al., 1980)	Only an abstract is available. No reporting with respect to metabolic activation. The substance was also tested with <i>Bacillus subtilis</i> . With this specie,

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
Cyclohexanol [02.070]	Cytogenetic assay	Human leukocytes	0.1 - 10 mM	Inconclusive ₃	(Collin, 1971)	toxicity was found as well as a positive response. The validity of the study cannot be evaluated because of lack of experimental information.
	Chromosomal aberration	Human lymphocytes	0, 0.005 - 0.1 µg/ml	Positive	(Dyshlovoi et al., 1981)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.
	Gene mutation (HPRT)	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative ¹	(Aaron et al., 1985)	Article is not in English. Only an abstract available in English. The validity of the study cannot be evaluated.
	Chromosomal aberration	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative ¹	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Sister chromatic exchange	Chinese hamster ovary cells	0, 7.5 µg/ml	Positive ³ Negative ²	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Mutation	Mouse lymphoma L5178Y Tk ^{+/+} cells	312.5–5000 µg/ml	Negative	(NTP, 2007)	
	Ames test	<i>S. typhimurium</i> TA98, TA1535, TA1537, TA1538	500 - 10000 µg/plate ¹ 500 - 15000 µg/plate ²	Negative ¹	(Barsky, 1976)	The highest concentrations showed cytotoxicity. The study is considered valid.
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 10 - 3333 µg/plate	Negative ¹	(Haworth et al., 1983)	The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. Both rat and hamster liver S9 were used. The test was run twice. The study is considered valid.
	Chromosomal aberration	Human leukocytes	0.1 - 10 mM	Inconclusive ₃	(Collin, 1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.
(Cyclohexyl acetate [09.027])	DNA damage	<i>B. subtilis</i> H17(<i>rec</i> ⁺), M45 (<i>rec</i> ⁻)	19 mg/disc	Negative ¹	(Yoo, 1986)	
(Cyclohexyl butyrate [09.230])	DNA damage	<i>B. subtilis</i> H17(<i>rec</i> ⁻), M45 (<i>rec</i> ⁻)	19 mg/plate	Negative ¹	(Oda et al., 1979)	
(Cyclopentanone [07.149])	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	2.5 - 2500 mg/plate	Negative ¹	(Florin et al., 1980)	
(2,2,6-Trimethyl cyclo-hexanone	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	4.2 - 3600 mg/plate	Negative ¹	(Florin et al., 1980)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
[07.045])						
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	5 mg/plate	Negative ¹	(Thompson, 2000)	Valid study in compliance with the OECD Guideline -471.
	Reverse mutation	<i>E. coli</i> WP2 <i>uvrA</i>	5 mg/plate	Negative ¹	(Wagner and Klug, 2000)	Valid study in compliance with the OECD Guideline -471.
	Forward mutation Test	Mouse lymphoma cells <i>L5178y</i>	200 & 300 µg/L 300 µg/L	Positive ³ Positive ²	(Ross and Harris, 1979b)	Pre-GLP study - not possible to assess the reliability of these studies.
	Forward mutation Test	Mouse lymphoma cells <i>L5178y</i>	100 - 325 µg/L	Negative ¹	(Cifone, 2001)	Valid study and in compliance with OECD Guideline 476.
(Carveol [02.062])	Ames test (pre-incubation)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	560 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
(Carvyl acetate [09.215])	Ames test (pre-incubation)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	333 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
(L-menthyl (R,S)-3-hydroxybutyrate)	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and TA1538	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative ^{1,6}	(Morimoto, 2005)	The JECFA evaluated the racemate of L-menthyl (R,S)-3-hydroxybutyrate.
	Reverse mutation	<i>E. coli</i> WP2uvrA	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative ^{1,6}	(Morimoto, 2005)	

NA: Not applicable.

NR: Not reported.

¹ With and without S9 metabolic activation.

² With S9 activation.

³ Without S9 activation.

⁴ Calculated based on molecular weight of menthol = 156.3 g/mol.

⁵ Marked differential toxicity was seen at dose levels above 25 µmol/plate. No observations were noted at lower dose levels.

⁶ Modified preincubation method.

In vivo mutagenicity/genotoxicity data are available for two candidate substance of the present FGE and for three supporting substances evaluated by JECFA at the 51st and 59th meetings. Supporting substances are listed in brackets.

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Menthol [02.015])	Host mediated mutation assay	<i>S. typhimurium</i> TA1530 and G46; <i>S. cerevisiae</i> D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Equivocal	(Food and Drug Research Laboratories, Inc., 1975a)	Negative results, with exception of the combination <i>S. typhimurium</i> TA1530 – 5000 mg/kg bw and <i>S. cerevisiae</i> D3 – 1150 mg/kg bw/day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels.
	<i>In vivo</i> cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	Oral DL ₅₀ was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.
	<i>In vivo</i> micronucleus assay	B6C3F1 male mouse bone marrow cells	Intra peritoneal	0, 250 - 1000 mg/kg bw/day, during 3 days	Negative	(Shelby et al., 1993)	d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	<i>In vivo</i> dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	This study is considered valid.
(trans-Menthone [07.176])	<i>In vivo</i> SMART assay	<i>D. melanogaster</i> – flr3 x mwh cross	Whole body	0, 1.3 µl/disk	Positive	(Franzios et al., 1997)	Somatic Mutation and Recombination Test. Only one dose level (1.29 µl/disk; slightly higher than the LD ₅₀) was tested. A two-fold increase in mutation frequency as compared to control was observed. Menthone was not recombinogenic. The validity of this study is unclear.
(Cyclohexanone [07.148])	<i>In vivo</i> sex-linked recessive lethal mutation	<i>D. melanogaster</i>	NR 3 days exposure	0, 1 µl/ml	Negative	(Goncharova, 1970)	Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.
Cyclohexanol [02.070]	<i>In vivo</i> sex-linked recessive lethal mutation	<i>D. melanogaster</i>	NR 3 days exposure	0, 1 µl/ml	Negative	(Goncharova, 1970)	The validity of the study cannot be evaluated.
	<i>In vivo</i> micronucleus test	NMRI mouse bone	Oral	500 - 1500 mg/kg bw	Negative	(Gelbke, 1991)	The study is considered valid. The

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
		marrow					negative result of this study is of limited relevance, since no bone marrow toxicity could be detected. Testing at higher dose levels might not have been possible due to observed general toxicity at the highest dose.
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Micronucleus test	ICR mice	Intraperitoneal	280, 560 & 1120 mg/kg bw	Negative	(Gudi and Krsmanovic, 1998)	Valid study in compliance with the OECD Guideline 474.
	Unscheduled DNA Synthesis	Rat hepatocytes	Intraperitoneal	333.3 & 1000 mg/kg bw	Negative	(Durward, 2001)	Valid study in compliance with the OECD Guideline 486.

NR: Not reported.

REFERENCES

- Aaron CS, Brewen JG, Stetka DG, Bleicher WT and Spahn MC, 1985. Comparative mutagenesis in mammalian cells (CHO) in culture: multiple genetic endpoint analysis of cyclohexanone in vitro. *Environmental Mutagenesis* 7 (Suppl. 3), 60-61.
- Anders MW, 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson DH, Caldwell J and Paulson GD (Eds.). *Intermediary xenobiotic metabolism in animals*. Taylor and Francis, New York, pp. 81-97.
- Andersen PH and Jensen NJ, 1984b. Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. *Mutation Research* 138, 17-20.
- Bär F and Griepentrog F, 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. [Where we stand concerning the evaluation of flavoring substances from the viewpoint of health]. *Ernährung und Medizin* 8, 244-251.
- Barsky FC, 1976. *In vitro* microbial mutagenicity studies of cyclohexanol, with cover letter dated 10/3/1995. Cyclohexanol. E. I. Dupont De Nemour & Co. Lab. no. 9822, study no. 755-75. EPA Doc 86960000143S, microfiche no. OTS0558283. January 7, 1977. Unpublished report submitted by EFA to FLAVIS Secretariat.
- Birch MD, Evans MJ, Birch RE and Woods WR, 1981. Initial submission: Toxicological investigation of: RAKA with cover letter dated 08/13/92. Cyclohexanol-cyclohexanone mixture. Monsanto Co. Project no. Y0-81-049. EPA Doc 88-920007866, microfiche no. OTS0546011. July 7, 1981. Unpublished data submitted by EFA to SCF.
- Birch MD, 1978a. Toxicological investigation of: crude cyclohexanol with cover letter dated 09/29/95. Cyclohexanol. Monsanto Co. Project no. Y-78-73. EPA Doc 86960000003, microfiche no. OTS0572833. June 28, 1978. Unpublished data submitted by EFA to FLAVIS Secretariat.
- Burdock GA (Ed.), 1995. *Fenaroli's Handbook of Flavor Ingredients*. 3rd Ed. Vol I + II. CRC Press, Inc., Florida.
- Cifone MA, 2001. ST 08 C 99: L5178Y TK +/- mouse lymphoma forward mutation assay with a confirmatory assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Covance Laboratories Inc, Vienna, Virginia. Study no. 21997-0-431 ICH. February 27, 2001. Unpublished report submitted by EFA to FLAVIS Secretariat.
- CoE, 1992. *Flavouring substances and natural sources of flavourings*. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.
- Collin J-P, 1971. Effet cytogénétique du cyclamate de soude, de la cyclohexanone et du cyclohexanol. [Cytogenetic effect of cyclamate, cyclohexanone and cyclohexanol]. *Le Diabète* 19, 215-221. (In French)
- Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. *Food and Cosmetics Toxicology* 16(3), 255-276.

- Deichmann WB and LeBlanc TJ, 1943. Determination of the approximate lethal dose with about six animals. *Journal of Industrial Hygiene and Toxicology* 25(9), 415-417.
- Durward R, 2001. ST 41 C 00: *In vivo* Liver Unscheduled DNA Synthesis (UDS) assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Safepharma Laboratories Limited, Derby, U.K. Project no. 161/266.08 August 2001. Unpublished report submitted by EFTA to the FLAVIS Secretariat.
- Dyshlovoi VD, Boiko NL, Shemetun AM and Kharchenko TI, 1981. [Cytogenetic action of cyclohexanone]. *Gigiena i sanitariia* 5, 76-77. (In Russian)
- EC, 1996a. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. *Official Journal of the European Communities* 23.11.1996, L 299, 1-4.
- EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. *Official Journal of the European Communities* 27.3.1999, L 84, 1-137.
- EC, 2000a. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. *Official Journal of the European Communities* 19.7.2000, L 180, 8-16.
- EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. *Official Journal of the European Communities* 12.4.2002, L 95, 10-11.
- EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. *Official Journal of the European Union* 27.2.2009, L 55, 41.
- EFTA, 2002i. Letter from EFTA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
- EFTA, 2003a. Submission 2002-2. Flavouring group evaluation of 14 flavouring substances (candidate chemicals) of the chemical group 8 (Annex I of 1565/2000/EC), structurally related to menthol, carvone and ionones [FAO/WHO JECFA 42/51], and alicyclic ketones, secondary alcohols and related esters [considered during the 59th meeting of JECFA] used as flavouring substances 31. December 2002. SCOOP/FLAV/8.18.
- EFTA, 2003b. Submission 2002-2. Flavouring group evaluation of 14 flavouring substances (candidate chemicals) of the chemical group 8 (Annex I of 1565/2000/EC), structurally related to menthol, carvone and ionones [FAO/WHO JECFA 42/51], and alicyclic ketones, secondary alcohols and related esters [considered during the 59th meeting of JECFA] used as flavouring substances 31. December 2002. SCOOP/FLAV/8.18. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Unpublished report submitted by EFTA to SCF.
- EFTA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFTA to Torben Hallas-Møller, EFSA. May 31, 2004.
- EFTA, 2005l. EFTA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
- EFTA, 2007a. E-mail from Jan Demyttenaere, EFTA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages. FLAVIS/8.70.

- EFFA, 2009c. Supplement list of EU-only Footnote-10 materials for Commission. Unpublished communication submitted by EFFA to the FLAVIS secretariat. 14 December 2009.
- EFFA, 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
- EFFA, 2011e. Specifications and poundage data for 42 Register substances submitted by EFFA/Industry to FLAVIS Secretariat. August 2011. FLAVIS/8.124
- EFFA, 2012b. Privat Communication forwarded to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 17 January 2012, 14, 23 and 24 February 2012 and 19 March 2012. Specification data related to substances in FGE.29.Rev1: [FL-no: 01.015]; FGE.09Rev4 [FL-no: 07.059, 09.843 and 09.920] and FGE.51Rev1 [FL-no: 07.034, 07.035, 07.095, 07.129, 07.172, 07.257 and 09.930]. FLAVIS/8.143.
- EFSA, 2004a. Minutes of the 7th Plenary Meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true
- EFSA, 2011f. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 212, Revision 1 (FGE.212Rev1): alpha,beta-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 25 November 2010. EFSA-Q-2010-01251.
- Elliott TH, Parke DV and Williams RT, 1959. Studies in detoxication. The metabolism of cyclo[14C]hexane and its derivatives. *Biochemical Journal* 72, 193-200.
- Emberger D, 1994a. *In vitro* hydrolysis test. Menthyl glycarbonate (MGC). Flavor and Extract Manufacturers' Association of the United States. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Emberger D, 1994b. *In vitro* hydrolysis test. Menthyl propyleneglycol carbonate (MPC). Flavor and Extract Manufacturers' Association of the United States. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.
- Fischer FG and Bielig HJ, 1940. Über die hydrierung ungesättigter stoffe im tierkörper. [On the hydrogenation of unsaturated materials in the animal body]. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* 266, 73-98. (In German)
- Flavour Industry, 2004h. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09rev1.
- Flavour Industry, 2006c. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09.

- Flavour Industry, 2006o. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09rev1.
- Flavour Industry, 2010f. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-09rev3 [Fl-no. 09.949].
- Flavour Industry, 2010k. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09rev3 [FL-no: 06.136].
- Flavouring Industry, 2007n. Addendum of one Flavouring Substances (candidate chemicals) to the Flavouring Group Evaluation of Chemical Group 8 (Annex I of 1565/2000/EC), structurally related to menthol, carvone and ionones [FAO/WHO JECFA 42/51], and alicyclic ketones, secondary alcohols and related esters [considered during the 59th meeting of JECFA] used as flavouring substances. Addendum to FGE.09 (EFFA submission 2002-2). 10 January 2007. Unpublished data submitted by Flavour Industry to FLAVIS Secretariat. A-09Rev4
- Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology* 18, 219-232.
- Food and Drug Research Laboratories, Inc., 1973. Teratologic evaluation of FDA 71-57 (menthol natural, Brazilian). Food and Drug Research Laboratories, Inc. Morgareidge K Lab. no. 1573k, study no. FDABF-GRAS-134, June 1, 1973. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Food and Drug Research Laboratories, Inc., 1975a. Mutagenic evaluation of compound FDA 71-57, menthol. Litton Bionetics, Inc. Weir, R.J. January 14, 1975. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Franzios G, Mirotsoou M, Hatzia Apostolou E, Kral J, Scouras ZG and Mavragani-Tsipidou P, 1997. Insecticidal and genotoxic activities of mint essential oils. *Journal of Agricultural and Food Chemistry* 45(7), 2690-2694.
- Gelbke H-P, 1991. Cytogenetic study *in vivo* of cyclohexanol in mice: Micronucleus test: Single oral administration of cyclohexanol, with cover letter dated 9/11/95. BASF Abteilung Toxikologie. Engelhardt, G. Project no. 26H0843/894490. EPA Doc 86950000355, microfiche no. OTS0557795. September 11, 1995. Unpublished report submitted by EFFA to SCF.
- Gomes-Carneiro MR, Felzenszwalb I and Paumgarten FJ, 1998. Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay. *Mutation Research* 416, 129-136.
- Goncharova RI, 1970. [Genetic activity of some cyclohexane derivatives]. *Tsitologiya i genetika* 137-142. (In Russian)
- Gondry E, 1972. [Research on the toxicity of cyclohexamin, cyclohexanone and cyclohexanol, metabolites of cyclamate]. *Toxicologie expérimentale* 4, 227-238. (In French)
- Governa M, Calisti R, Coppa G, Tagliavento G, Colombi A and Troni W, 1987. Urinary excretion of 2,5-hexanedione and peripheral polyneuropathies in workers exposed to hexane. *Journal of Toxicology and Environmental Health* 20, 219-228.
- Gudi R and Krsmanovic L, 1998. Mammalian erythrocyte micronucleus test. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. MA BioServices, Inc, Rockville, MD. Lab no. G98AN94.123. August 4, 1998. Unpublished report submitted by EFFA to FLAVIS Secretariat.

- Gupta PK, Lawrence WH, Turner JE and Autian J, 1979. Toxicological aspects of cyclohexanone. *Toxicology and Applied Pharmacology* 49, 525-533.
- Hämäläinen J, 1912. [The conduct of the alicyclical compounds in the glycuronic acid matching in the organism]. *Skandinavisches Archiv für Physiologie* 27, 141-226. (In German)
- Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. *Environmental Mutagenesis* 5(Suppl. 1), 3-142.
- Herken H, 1961. Pharmakologisches gutachten über die vertraglichkeit von natürlichem (l--) und synthetischem (d,l-) menthol. Unpublished report from the director, Pharmakologischen Institute der Freien Universität, Berlin-Duhlem, submitted to the World Health Organization by Schering AG. Cited in JECFA, 1976. Toxicological evaluation of certain food additives. Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 21-29 April, 1976. Food Additive Series 10, pp. 64-69.
- Heymann E, 1980. Carboxylesterases and amidases. In: Jakoby WB (Ed.). *Enzymatic basis of detoxication*. 2nd Ed. Academic Press, New York, pp. 291-323.
- Hummler, 1969. Private communication. Submitted to WHO by Flavor and Extract Manufacturers' Association of the United States.
- Igimi H and Ide H, 1974. Improvements in or relating to substances for use in the treatment of gallstones. Patent 1343561, Application no. 13606/72. Filed 23 March, 1972. Complete specification published 9 January, 1974. International Classification A61K 27/00.
- IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.
- Ishidate Jr M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food and Chemical Toxicology* 22(8), 623-636.
- Ivett JL, Brown BM, Rodgers C, Anderson BE, Resnick MA and Zeiger E, 1989. Chromosomal aberrations and sister chromatid exchange tests in chinese hamster ovary cells *in vitro*. IV. Results with 15 chemicals. *Environmental and Molecular Mutagenesis* 14, 165-187.
- James SP and Waring RH, 1971. The metabolism of alicyclic ketones in the rabbit and rat. *Xenobiotica* 1, 573-580.
- JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.
- JECFA, 1996a. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.
- JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

- JECFA, 1998b. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9-18 June 1998. FAO Food and Nutrition paper 52 Add. 6.
- JECFA, 1999a. Safety evaluation of certain food additives. The fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 42. IPCS, WHO, Geneva.
- JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.
- JECFA, 2000a. Evaluation of certain food additives. Fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.
- JECFA, 2000d. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Committee of Food Additives. Fifty-fifth Meeting. Geneva, 6-15 June 2000. FAO Food and Nutrition paper 52 Add. 8.
- JECFA, 2002b. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 909. Geneva, 5-14 June 2001.
- JECFA, 2002c. Evaluation of certain food additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 913. Geneva, 4-13 June 2002.
- JECFA, 2002d. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Add. 10.
- JECFA, 2003a. Safety evaluation of certain food additives. Fifty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.
- JECFA, 2005b. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.
- JECFA, 2005c. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.
- JECFA, 2006a. Safety evaluation of certain food additives and contaminants. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.
- JECFA, 2009a. Safety evaluation of certain food additives and contaminants. Sixty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 60. IPCS, WHO, Geneva 2009 http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf (May 2009).
- Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh OG, 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food and Cosmetics Toxicology 2, 327-343.

- Keating JW, 1972a. Acute oral toxicity (rat-5 gms/kg body weight dose). Dermal toxicity (rabbit-5 gms/kg body weight dose). Amyris acetylated, Bois de rose acetylated, Cadinene, Castoreum, Lavandin acetylated, Dihydrojasnone, Trans-2-hexenol, Methyl isoeugenol, Methyl eugenol, Santalyl acetate, Phenyl propyl cinnamate, Phenylacetic acid, 1-Carveol, Santatol, Methyl heptenone. Biological Science Laboratories. Unpublished report submitted by ECHA to SCF.
- Keefer LK, Streeter AJ, Leung LY, Perry WC, Hu HS-W and Baillie TA, 1987. Pharmacokinetic and deuterium isotope effect studies on the metabolism of formaldehyde and formate to carbon dioxide in rats *in vivo*. *Drug Metabolism and Disposition* 15(3), 300-304.
- Kelly CM and Bolte HF, 2000. A 3-month dietary toxicity study in rats. Final report. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Huntingdon Life Sciences, East Milestone, New Jersey. Project no. 99-2643. 15 December 2000. Unpublished report submitted by ECHA to FLAVIS Secretariat.
- Kohli RP, Kishor K, Dua PR and Saxena RC, 1967. Anticonvulsant activity of some carbonyl containing compounds. *Indian Journal of Medical Research* 55(11), 1221-1225.
- Lake BG, Foster JR, Collins MA, Stubberfield CR, Gangolli SD and Srivastava SP, 1982. Studies on the effects of orally administered dicyclohexyl phthalate in the rat. *Acta Pharmacology & Toxicology* 51, 217-226.
- Levenstein I, 1973c. To determine the oral LD50, in fasted rats, of the test material as submitted. Methone racemic pure. Leberco Laboratories, Inc. Assay no. 30969. January 10, 1973. Unpublished report submitted by ECHA to FLAVIS Secretariat.
- Levenstein I, 1973d. To determine the oral LD50, in fasted rats, of the test material as submitted. Menthyl acetate racemic. Leberco Laboratories, Inc. Assay no. 30970. January 10, 1973. Unpublished report submitted by ECHA to SCF.
- Levenstein I, 1976c. Acute oral toxicity (rats 5 gms./kg body weight dose). Dermal toxicity (rabbits 5 gms./kg. body weight dose). 1-Carvyl acetate. Leberco Laboratories, Inc. Assay no. 62970. August 18, 1976. Unpublished report submitted by ECHA to FLAVIS Secretariat.
- Lijinsky W and Kovatch RM, 1986. Chronic toxicity study of cyclohexanone in rats and mice. *Journal of the National Cancer Institute* 77(4), 941-949.
- Madsen C, Wurtzen G and Carstensen J, 1986. Short-term toxicity study in rats dosed with menthone. *Toxicology Letters* 32(1-2), 147-152.
- Madyastha KM and Srivatsan V, 1988a. Studies on the metabolism of l-menthol in rats. *Drug Metabolism and Disposition* 16(5), 765-772.
- Massoud A, Aly A and Shafik H, 1980. Mutagenicity and carcinogenicity of cyclohexanone. *Mutation Research* 74(3), 174.
- McMahon RE, Cline JC and Thompson CZ, 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the ames test for bacterial mutagens. *Cancer Research* 39, 682-693.
- Messiha FS and Lox CD, 1985. Effect of selected organic solvents on hepatic alcohol and aldehyde-dehydrogenase. *Neurobehavioral Toxicology and Teratology* 7(2), 207-208.
- Miller L and Sherman H, 1965. Oral LD50 test of cyclohexanol with cover letter dated 10/3/95. p-tert-butylpyrocatechol. Haskell Laboratory. Report no. 103-65. EPA Doc 86960000139S, microfiche no. OTS0558279. July 28, 1965. Unpublished report submitted by ECHA to FLAVIS Secretariat.

- Miyazawa M and Nakanishi K, 2006. Biotransformation of (-)-Menthone by Human Liver Microsomes. *Bioscience, Biotechnology and Biochemistry* 70(5), 1259-1261.
- Moreno OM, 1977k. Acute toxicity study in rats. Dermal toxicity in rabbits. Dihydro carveol. MB Research Laboratories, Inc. Project no. MB 77-1748. August 22, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1980f. Oral toxicity in rats. Dermal toxicity in rabbits. Dihydro carvyl acetate. Project no. MB 80-4888, date 12/02/80. Test for oral toxicity in rats. Dihydro carvyl acetate, project no. MB 80-4888A, date 9/05/80. Test for acute dermal toxicity in rabbits. Dihydro carvyl acetate, project no. MB 80-4888B, date 10/08/80. MB Research Laboratories, Inc. Study director: Cerven, D.R. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Morimoto T, 2005. Bacterial reverse mutation study of menthyl 3-hydroxybutyrate. Study No. 235. February 21, 2005. Private communication to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavour Industry, Brussels, Belgium.
- Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E, 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. *Environmental and Molecular Mutagenesis* 8(Suppl. 7), 1-119.
- Mráz J, Gálová E, Nohová H and Vitková D, 1994. Uptake, metabolism and elimination of cyclohexanone in humans. *International Archives of Occupational and Environmental Health* 66(3), 203-208.
- Mráz J, Gálová E, Nohová H and Vitková D, 1998. 1,2- and 1,4-cyclohexanediol: major urinary metabolites and biomarkers of exposure to cyclohexane, cyclohexanone, and cyclohexanol in humans. *International Archives of Occupational and Environmental Health* 71(8), 560-565.
- Murthy PBK, Ahmed MM and Regu K, 1991. Lack of genotoxicity of menthol in chromosome aberration and sister chromatid exchange assays using human lymphocytes *in vitro*. *Toxicology In Vitro* 5(4), 337-340.
- Myers RC, Homan ER, Weil CS and Frank FR, 1980. Initial submission: Cyclopentanol: Range finding toxicity studies (final report) with attachment and cover letter dated 121091. Bushy Run Research CTR. Kuryla, W.C. EPA Doc 88-920000476, microfiche no. OTS0534929. February 2, 1980. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Myhr BC and Caspary WJ, 1991. Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program. *Environmental and Molecular Mutagenesis* 18, 51-83.
- National Cancer Institute, 1979. Bioassay of dl-menthol for possible carcinogenicity. U.S. Department of Health, Education, and Welfare. April 1978. NCI Technical Report Series, no. 98.
- Nohmi T, Miyata R, Yoshikawa K and Ishidate M, 1985. [Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests]. *Eisei Shikenjo hokoku. Bulletin of National Institute of Hygienic Sciences* 103(60), 60-64. (In Japanese)
- NTP, 2007. Search Result on cyclohexanone. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchresults&searchterm=cyclohexanone. [14th September, 2007].

- Oda Y, Hamono Y, Inoue K, Yamamoto H, Niihara T and Kunita N, 1979. [Mutagenicity of food flavors in bacteria]. Osaka Furitsu Kosho Eisei Kenkyusho kenkyu hokoku. Shokuhin eisei hen 9, 177-181. (In Japanese)
- OECD, 1997a. OECD Guidelines for the Testing of Chemicals. Section 4: Health Effects. Test No. 486: Unscheduled DNA synthesis (UDS) Test with Mammalian Liver Cells in vivo. Publication date: 21 July 1997. ISBN: 9789264071520. OECD Code: 979948601E1. Can be downloaded from: <http://browse.oecdbookshop.org/oecd/pdfs/browseit/9748601E.PDF>
- Oh S-M, Yeon J-D, Nam H-Y, Park D-K, Cho M-H and Chang K-H, 1997. [Acute and subacute toxicity studies of l-muscone in rats]. Korean Journal of Toxicology 13(4), 435-447. (In Korean)
- Perbellini L, De Grandis D, Semenzato F and Bongiovanni LG, 1981. Studio sperimentale sulla neurotossicità del cicloesano e del cicloesanone. La Medicina del Lavoro 2, 102-106. (In Italian)
- Price TD, 1951. Studies on the metabolism of acetone. A Dissertation. Univ. Microfilms Pub. No. 2549. 82.
- Ross C and Harris WJ, 1979b. Testing of compound 0478/5 in the mouse lymphoma specific locus mutation assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Inveresk Research International, Edinburgh, Scotland. Project no. 410917. October 1979. Unpublished report submitted by EFA to FLAVIS Secretariat.
- SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.
- SCF, 1999a. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.
- Shelanski MV, 1972a. Report to RIFM, 14 July. l-Menthyl acetate. Cited in Opdyke DLJ (Ed.). Fragrance raw materials monographs. Pergamon Press, p. 477.
- Shelby MD, Erexson GL, Hook GJ and Tice RR, 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Environmental and Molecular Mutagenesis 21(2), 160-179.
- Shimada T, Shindo M and Miyazawa M, 2002. Species differences in the metabolism of (+) and (-)-limonenes and their metabolites, carveols and carvones, by cytochrome P450 enzymes in liver microsomes of mice, rats, guinea pigs, rabbits, dogs, monkeys, and humans. Drug Metabolism and Pharmacokinetics 17(6), 507-515.
- Smyth Jr HF and Carpenter CP, 1948. Further experience with the range-finding test in the industrial toxicology laboratory. Journal of Industrial Hygiene and Toxicology 30, 63-68.
- Smyth Jr HF, Carpenter CP, Shaffer CB and Weil CS, 1946c. Letter from Union Carbide Corp to USEPA regarding toxicology studies of cyclohexanol, with attachments dated 08/25/95. Cyclohexanol. EPA Doc 86950000304, microfiche no. OTS0557744. August 25, 1995. Unpublished report submitted by EFA to FLAVIS Secretariat.
- Smyth Jr HF, Carpenter CP, Weil CS, Pozzani UC, Striegel JA and Nycum JS, 1969a. Range-finding toxicity data: List VII. American Industrial Hygiene Association Journal 30(5), 470-476.

- Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK and Gori GB, 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain a mice. *Cancer Research* 33(12), 3069-3085.
- Storer RD, McKelvey TW, Kraynak AR, Elia MC, Barnum JE, Harmon LS, Nichols WW and DeLuca JG, 1996. Revalidation of the *in vitro* alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. *Mutation Research* 368(2), 59-101.
- Thompson PW, 2000. ST 41 C 00: Reverse mutation assay "Ames test" using *Salmonella typhimurium*. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Safepharma Laboratories Limited, Derby, U.K. Project no. 161/265. 11 October 2000. Unpublished report submitted by ECHA to FLAVIS Secretariat.
- Thorup I, Wurtzen G, Carstensen J and Olsen P, 1983a. Short-term toxicity study in rats dosed with pulegone and menthol. *Toxicology Letters* 19(3), 207-210.
- TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- TNO, 2010. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- TNO, 2011. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- Treon JF, Crutchfield WE and Kitzmiller KV, 1943. The physiological response of rabbits to cyclohexane, methylcyclohexane and certain derivatives of these compounds. *Journal of Industrial Hygiene and Toxicology* 25, 199-214.
- Truhaut R, Dutertre-Catella H and Phu-Lich N, 1970. Biochemical toxicology. First results of a study of metabolism in rabbits, which were administered isophorone, an industrial solvent. *Comptes Rendus de l'Académie des Sciences* 271, 1333-1336.
- Truhaut R, Lich NP, Cluet JL and Dutertre-Catella H, 1979. [Dismutation as a metabolic pathway: Transformation of trimethyl-3,5,5-cyclohexanone]. *Toxicological European research* 2(2), 71-76. (In French)
- Vree TB, Kolmer EWJV, Verweyvanwissen CPWG and Hekster YA, 1994. Effect of urinary pH on the pharmacokinetics of salicylic-acid, with its glycine and glucuronide conjugates in human. *International Journal of Clinical Pharmacology and Therapeutics* 32, 550-558.
- Wagner VO and Klug ML, 2000. ST 08 C 99: Bacterial reverse mutation assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. BioReliance, Rockville, MD. Study no. AA31NK.502.BTL. August 28, 2000. Unpublished report submitted by ECHA to FLAVIS Secretariat.
- White DA, Heffron F, Miciak A, Middleton B, Knights S and Knight D, 1990. Chemical synthesis of dual radiolabelled cyclandelate and its metabolism in rat hepatocytes and mouse J774 cells. *Xenobiotica* 20(1), 71-79.
- Wokes F, 1932. The antiseptic value and toxicity of menthol isomers. *Quarterly Journal of Pharmacy and Pharmacology* 5, 233-244.
- Yamaguchi T, Caldwell J and Farmer PB, 1994. Metabolic fate of [3H]-l-menthol in the rat. *Drug Metabolism and Disposition* 22(4), 616-624.

- Yoo YS, 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka City Medical Journal 34(3-4), 267-288.
- You A-S, Kweon O-K, Sung H-J, Kwak H-I, Fang M-Z, Park D-K, Chung K-H, Yoon H-I and Cho M-H, 1997. Acute and subacute toxicity of l-muscone in beagle dogs. Korean Journal of Toxicology 13(4), 449-460.
- Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1988. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environmental and Molecular Mutagenesis 11(Suppl. 12), 1-158.

ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis

WHO World Health Organisation

Amended Safety Assessment of *Mentha piperita* (Peppermint)-derived Ingredients as Used in Cosmetics

Status: Draft Final Amended Report for Panel Review
Release Date: February 9, 2018
Panel Date: March 5-6, 2018

The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst.

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Memorandum

To: CIR Expert Panel Members and Liaisons

From: Wilbur Johnson, Jr.
Senior Scientific Analyst

Date: February 9, 2018

Subject: Draft Final Amended Report on *Mentha piperita* (Peppermint)-Derived Ingredients

At the December 4-5, 2017 Panel meeting, the Panel issued a revised tentative amended report for public comment with a safe with qualifications conclusion on 7 of the 10 *Mentha piperita* (peppermint)-derived ingredients (mentioned below) that are being reviewed, and a conclusion stating that the available data are insufficient to make a determination of safety for the following 3 ingredients: *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Water, and *Mentha Piperita* (Peppermint) Meristem Cell Culture. The rationale for issuing a revised tentative amended report relates to the new skin irritation and chemical characterization data that were received (see CIR report history in this package: *pepper032018hist.docx*). The data that are needed in order for a conclusion on the safety of the 3 ingredients with insufficient data to be issued are:

- Composition data
 - Depending on the composition data that are received, other toxicological endpoints may be needed.
- Skin irritation and sensitization data

The Panel also determined that the following 7 ingredients are safe in the present practices of use and concentration in cosmetics, as described in the safety assessment, when formulated to be non-sensitizing:

Mentha Piperita (Peppermint) Oil
Mentha Piperita (Peppermint) Extract
Mentha Piperita (Peppermint) Leaf
Mentha Piperita (Peppermint) Leaf Cell Extract

Mentha Piperita (Peppermint) Leaf Extract
Mentha Piperita (Peppermint) Leaf Juice
Mentha Piperita (Peppermint) Leaf Water

Comments on this safety assessment that were received from the Council prior to the December 2017 Panel and in response to the tentative amended report that was issued after the Panel meeting (*pepper032018pcpc1* and *pepper032018pcpc2* – both included in this package) have been addressed. No responses to data requests pertaining to the insufficient data conclusion on *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Water, and *Mentha Piperita* (Peppermint) Meristem Cell Culture have been received.

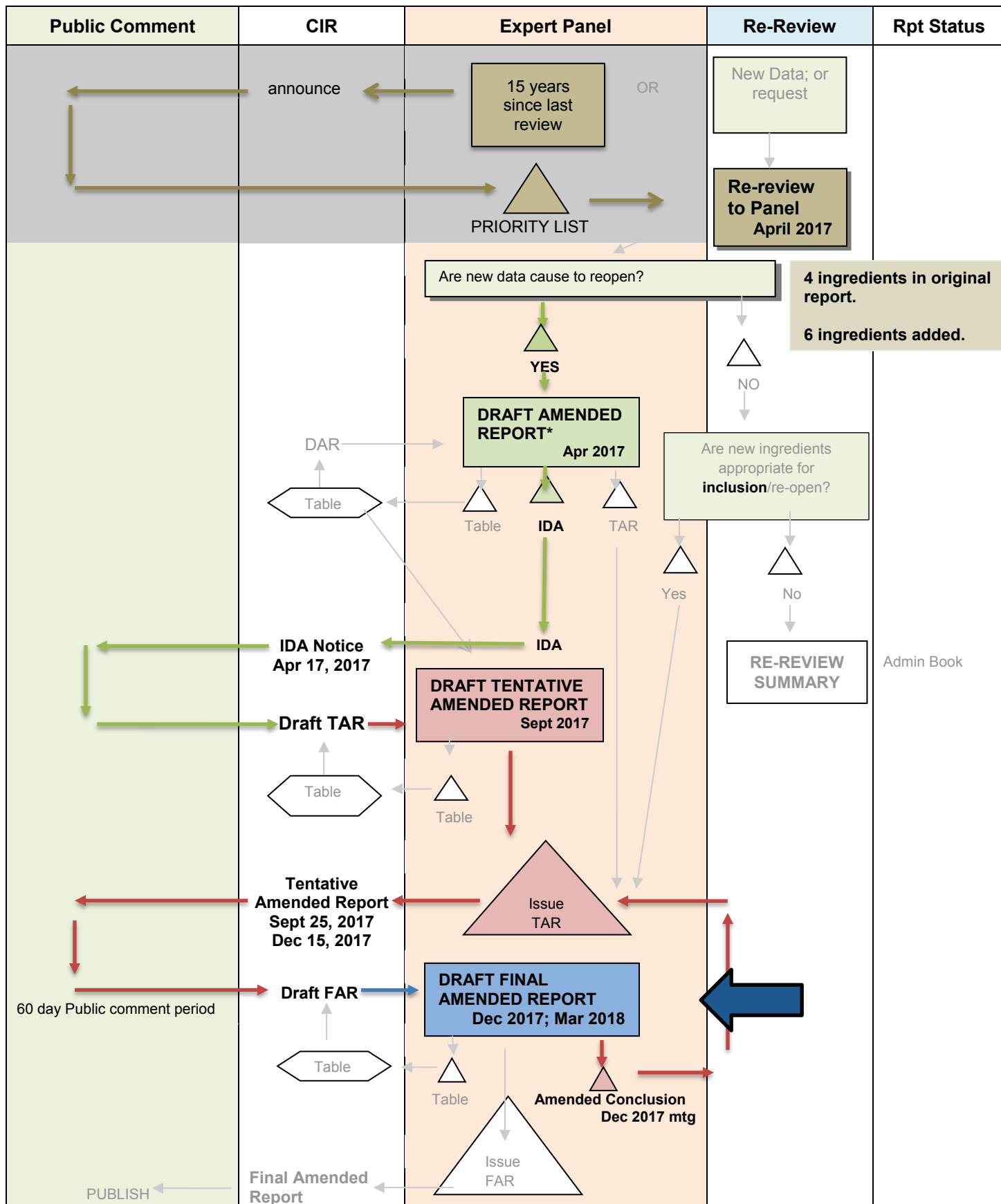
Also included in this package for your review are the Draft Final Amended Report (*pepper032018rep*), Flow chart (*pepper032018flow.docx*), Literature search strategy (*pepper032018strat.docx*), Ingredient data profile (*pepper032018prof.docx*), 2017 FDA VCRP data (*pepper032018FDA.docx*), and minutes from the December 4-5, 2017 Panel meeting and prior Panel meetings (*pepper122017min.docx*). The original final report that was published in 2001 may be accessed at the CIR website (<https://www.cir-safety.org/ingredients>).

After reviewing these documents, the Panel will determine whether a final amended report with the split conclusion stated above should be issued.

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Mentha Piperita (Peppermint)-Derived Ingredients

MEETING March 2018



CIR History of:

Mentha Piperita-derived Ingredients

A Final Report with a conclusion stating that the following ingredients are safe as used in cosmetic formulations was issued at the September 10-11, 1998 CIR Expert Panel meeting and published in 2001: Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. The conclusion also contains a statement indicating that the concentration of pulegone in these ingredients should not exceed 1%.

Re-review document, Teams/Panel: April 10-11, 2017

Use concentration data on Mentha Piperita-derived ingredients and human skin irritation, sensitization, and ocular irritation data on Mentha Piperita (Peppermint) Leaf Water that were received from the Council have been added to the report text.

The Panel agreed that the 2001 final report (published in 2001) on Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water should be reopened to add 6 Mentha piperita (peppermint)-derived ingredients:

Mentha Piperita (Peppermint) Extract
Mentha Piperita (Peppermint) Flower/Leaf/Stem
Extract
Mentha Piperita (Peppermint) Flower/Leaf/Stem
Water

Mentha Piperita (Peppermint) Leaf Cell Extract
Mentha Piperita (Peppermint) Leaf Juice
Mentha Piperita (Peppermint) Meristem Cell Culture

The Panel issued an Insufficient Data Announcement (IDA) with the following data requests for all ten ingredients:

- Skin irritation and sensitization data
- Composition, method of manufacture, and impurities data

Draft Tentative Amended Report, Teams/Panel: September 11-12, 2017

The following data were received in response to the Panel's IDA: (1) use concentration data on the 6 ingredients that are being added; (2) method of manufacture, composition, and impurities data on Peppermint Leaf Extract (butylene glycol/water), Peppermint Leaf Extract (water/ethanol), Peppermint Leaf Extract Powder, and Peppermint Leaf Water [It should be noted that, according to the International Cosmetic Ingredient Dictionary and Handbook, Peppermint Leaf Extract Powder is not an ingredient name that is associated with any of the Mentha Piperita (Peppermint)-derived ingredients that are included in this safety assessment.]; and *in vitro* skin irritation data on Peppermint Leaf Extract (water/ethanol) (at concentrations of 10% and 100%) and an HRIPT on Peppermint Leaf Extract (water/ethanol) (at a concentration of 100%).

The Panel issued a tentative report for public comment with a conclusion stating that the available data are insufficient to make a determination of safety for 9 out of the 10 Mentha piperita (peppermint)-derived ingredients. These 9 ingredients, and the data that are needed to complete this safety assessment, are stated below.

Mentha Piperita (Peppermint) Leaf Extract
Mentha Piperita (Peppermint) Leaf
Mentha Piperita (Peppermint) Leaf Water
Mentha Piperita (Peppermint) Extract
Mentha Piperita (Peppermint) Flower/Leaf/Stem
Extract

Mentha Piperita (Peppermint) Flower/Leaf/Stem
Water*
Mentha Piperita (Peppermint) Leaf Cell Extract*
Mentha Piperita (Peppermint) Leaf Juice*
Mentha Piperita (Peppermint) Meristem Cell
Culture*

*Not reported to be in use.

The data needed to formulate a conclusion of safety include:

- ☐ Composition data on each of the above ingredients.
 - o Depending on the composition data that are received, other toxicological endpoints may be needed.
- ☐ Skin irritation and sensitization data on all of the above ingredients, except *Mentha Piperita* (Peppermint) Leaf Extract and *Mentha Piperita* (Peppermint) Leaf Water.

However, it was determined that *Mentha Piperita* (Peppermint) Oil is safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing.

The Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients in one formulation may each contribute to the final concentration of a single shared constituent. Therefore, when formulating products, manufacturers should avoid reaching levels, in final formulations, of botanical constituents that may cause sensitization or other adverse effects.

This group of ingredients was established at the April 2017 Expert Panel meeting, whereby the Panel agreed that the original final report (published in 2001) on *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water should be reopened to add 6 *Mentha piperita* (peppermint)-derived ingredients. Therein, the Panel also issued an IDA relating to all 10 ingredients, and composition, irritation, and sensitization data were requested.

Data were received in response to the IDA. The Panel agreed that the available composition data on *Mentha Piperita* (Peppermint) Oil are sufficient, but the data relating to composition of the other ingredients, are inadequate. After considering the available skin irritation and sensitization data, the Panel determined that skin sensitization data on all ingredients, except for the *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, and *Mentha Piperita* (Peppermint) Leaf Water, are still insufficient.

The Panel considered the positive effects that were observed in female rats, and in male and female mice, dosed with pulegone (component of *Mentha Piperita* (Peppermint) Oil) in the 2011 National Toxicology Program (NTP) oral carcinogenicity study. However, the Panel did not express concern over these findings relative to pulegone as a component of *Mentha Piperita* (Peppermint) Oil in cosmetic products, based on the understanding that the cytotoxic dose-response relationship (renal and liver toxicity) that was associated with cancer development would not be relevant to pulegone exposure from a cosmetic product. The Panel also reconsidered the 1% concentration limit on pulegone in the published final report on *Mentha piperita* (peppermint)- derived ingredients that appears to have been based on observations of brain lesions in rats. As the brain lesions were an artifact of the fixation method, the Panel determined that this study was not relevant to cosmetic safety. It was therefore agreed, that the 1% concentration limit and the carcinogenicity of pulegone should be addressed in the report discussion and not in the conclusion.

Draft Amended Final Report, Teams/Panel: December 4-5, 2017

The following data were received in response to the insufficient data conclusion on 9 *Mentha piperita* (peppermint)-derived ingredients (listed above) that was announced at the September 2017 Panel Meeting: (1) Human 48-h occlusive patch test, evaluating skin irritation potential, on a lipstick product containing 0.2961% *Mentha Piperita* (Peppermint) Leaf Extract; (2) Composition, method of manufacturing, and physical properties data on *Mentha Piperita* (Peppermint) Leaf Extract, and (3) Updated use concentration data on *Mentha piperita* (peppermint)-derived ingredients. According to the updated use concentration data, use concentrations of *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract are no longer being reported and *Mentha Piperita* (Peppermint) Leaf Extract is being used at concentrations ranging from 0.3% to 0.5% in lipstick products. The Draft Amended Final Report has been revised to include these data.

The Panel issued a revised tentative amended report for public comment with a conclusion that 7 of the 10 *Mentha piperita* (peppermint)-derived ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing:

Mentha Piperita (Peppermint) Oil
Mentha Piperita (Peppermint) Extract
Mentha Piperita (Peppermint) Leaf
Mentha Piperita (Peppermint) Leaf Cell Extract*

Mentha Piperita (Peppermint) Leaf Extract
Mentha Piperita (Peppermint) Leaf Juice*
Mentha Piperita (Peppermint) Leaf Water

*Not reported to be in use. Were the ingredients in this group not currently in use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

The Panel also concluded that the available data are insufficient to make a determination of safety for Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract, Mentha Piperita (Peppermint) Flower/Leaf/Stem Water*, and Mentha Piperita (Peppermint) Meristem Cell Culture*. (*Not reported to be in use.)

The following data needed to formulate a conclusion of safety:

- Composition data on each of the above ingredients
 - Depending on the composition data that are received, other toxicological endpoints may be needed
- Skin irritation and sensitization data

A revised tentative amended report was issued because the Panel determined that the data on Mentha Piperita (Peppermint) Leaf Extract and Mentha Piperita (Peppermint) Extract that were received in response to the data needs associated with the insufficient data conclusion support the safe use of Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaf, and the 4 ingredients that are derived from the leaf, when these ingredients are formulated to be non-sensitizing. However, the available data remain insufficient for making a determination of safety in cosmetic products for Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract, Mentha Piperita (Peppermint) Flower/Leaf/Stem Water, and Mentha Piperita (Peppermint) Meristem Cell Culture.

The Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients in one formulation may each contribute to the final concentration of a single shared constituent. Therefore, when formulating products, manufacturers should avoid reaching levels, in final formulations, of botanical constituents that may cause sensitization or other adverse effects.

Draft Amended Final Report, Teams/Panel: March 5-6, 2018

Comments that were received in response to the revised tentative amended report issued at the December 2017 Panel meeting have been addressed. Data were not received in response to the insufficient data conclusion that was issued on the following ingredients: Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract, Mentha Piperita (Peppermint) Flower/Leaf/Stem Water, and Mentha Piperita (Peppermint) Meristem Cell Culture.

Mentha Piperita (Peppermint) Meristem Cell Culture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

X = data; 0 = no data*

[Mentha Piperita-derived Ingredients (5/16/2016; 2/5/2017; 7/31/2017; 10/23/2017; 1/16/18)]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	Web
Mentha Piperita (Peppermint) Oil	8006-90-4 84082-70-2	1/1	164/14	164/11	165/11	1	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Leaf Extract	84082-70-2	1/1	4/1	1/1	5/2	1	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Leaf		1/1	197/19	10/3	34/4	1	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Leaf Water	84082-70-2	1/1	1/1	2/1	4/1	1	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Extract		1/1	0/1	0/69	0/207	1	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract		1/1	0/1	0/0	0/0	1	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Flower/Leaf/Stem Water		1/1	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Leaf Cell Extract		1/1	0/12	0/15	0/0	0	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Leaf Juice		1/1	0/93	0/0	0/0	1	0	0	0	0	0	0	0	0	0	0	0	
Mentha Piperita (Peppermint) Meristem Cell Culture		1/1	0/1	0/0	0/0	0	0	0	0	0	0	0	0	0	0	0	0	

Search Strategy

[document search strategy used for SciFinder, PubMed, and Toxnet]

[identify total # of hits /# hits that were useful or examined for usefulness]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - <http://www.personalcarecouncil.org/science-safety/line-infobase>
SciFinder (usually a combined search for all ingredients in report; list # of this/# useful) - <https://scifinder.cas.org/scifinder>
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - <http://www.ncbi.nlm.nih.gov/pubmed>
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) - <https://toxnet.nlm.nih.gov/> (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm> (CFR); then, list of all databases: <http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm>; then, <http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true> (EAFUS); <http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm> (GRAS); <http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm> (SCOGS database); <http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives> (indirect food additives list); <http://www.fda.gov/Drugs/InformationOnDrugs/default.htm> (drug approvals and database); <http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf> (OTC ingredient list); <http://www.accessdata.fda.gov/scripts/cder/iig/> (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - <http://ec.europa.eu/growth/tools-databases/cosing/>
ECHA (European Chemicals Agency – REACH dossiers) – <http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1>
IUCLID (International Uniform Chemical Information Database) - <https://iuclid6.echa.europa.eu/search>
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- <http://webnet.oecd.org/hpv/ui/Search.aspx>
HPVIS (EPA High-Production Volume Info Systems) - <https://ofmext.epa.gov/hpvis/HPVISlogin>
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- <https://www.nicnas.gov.au/>
NTIS (National Technical Information Service) - <http://www.ntis.gov/>
NTP (National Toxicology Program) - <http://ntp.niehs.nih.gov/>
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/> (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - <http://www.ecetoc.org/>

Botanical Websites, if applicable

Dr. Duke's <https://phytochem.nal.usda.gov/phytochem/search>
Taxonomy database - <http://www.ncbi.nlm.nih.gov/taxonomy>
GRIN (U.S. National Plant Germplasm System) - <https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx>
Sigma Aldrich plant profiler <http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html>

Fragrance Websites, if applicable

IFRA (International Fragrance Association) – <http://www.ifraorg.org/>

RIFM (the Research Institute for Fragrance Materials) should be contacted

Day 2 of the June 3-4, 1996 CIR Expert Panel Meeting

Peppermint Oil

Dr. Schroeter noted that an informal data request on Peppermint Oil was issued at the March 4-5, 1996 Panel meeting. With the exception of the RIFM (Research Institute For Fragrance Materials) monograph on Peppermint Oil that was received, there was no response to the informal data request that was issued. Thus, the Panel voted unanimously in favor of issuing an Insufficient Data Announcement on Peppermint Oil with the following data requests:

- (1) Concentration of use
- (2) UV absorption; if significantly absorbed in the UVA or UVB range, then a photosensitization study may be needed
- (3) Rate of dermal absorption (of either peppermint oil or its principal component, menthol); if significantly absorbed, then a 28-day dermal toxicity study and reproductive and developmental toxicology data are needed
- (4) Human dermal irritation and sensitization

The Panel also requested that the present review be expanded to include literature citations on menthol.

Day 2 of the December 16-17, 1996 CIR Expert Panel Meeting

Peppermint (*Mentha piperita*) Oil, Peppermint (*Mentha piperita*) Extract, and Peppermint (*Mentha piperita*) Leaves

Dr. Belsito noted that an Insufficient Data Announcement on this group of ingredients was issued at the June 3-4, 1996 Panel meeting. Also, in considering the data on menthol that were incorporated into the present report after this announcement was issued, the Belsito Team determined that the available data are still insufficient for evaluating the safety of these three ingredients. Dr. Belsito stated his Team's data needs as follows: (1) Concentration of use; (2) Impurities of cosmetic grade Peppermint Oil, specifically pulegone; or, in the absence of impurities data, (3) a 28-day dermal toxicity study on cosmetic grade Peppermint Oil.

Dr. Schroeter recalled that the potential need for 28-day dermal toxicity data is expressed in item #2 of the Insufficient Data Announcement as follows: Rate and extent of dermal absorption of Peppermint Oil or menthol; if there is significant skin penetration, then both a 28-day dermal toxicity study to assess general skin and systemic toxicity and a reproductive and developmental toxicity study are needed.

Drs. Shank and Schroeter noted that if the systemic toxicity resulting from the dermal route is the same (or less) compared to that resulting from oral exposure, then a dermal reproductive and developmental toxicity test will not be needed.

Dr. Klaassen wanted to know which toxic effects observed in oral studies were of concern.

Dr. Carlton noted that spongiform encephalopathy was observed in oral toxicity studies, and that the impurity pulegone was probably responsible for this finding.

Dr. Andersen brought to the Panel's attention that an oral reproductive and developmental toxicity study on menthol is included in the present report on Peppermint Oil. He noted that menthol is a significant component of Peppermint Oil.

Regarding the issue of exposure, especially regarding pulegone as a contaminant of Peppermint Oil, Dr. Bailey said that the Panel may want to consider more accurate skin penetration data relative to Peppermint Oil and/or the contaminant.

Dr. Andersen said that the Panel's concern about the safety of cosmetic grade Peppermint Oil seems to be based on the assumption that it may contain the impurity, pulegone. He also said that if pulegone is not detected in the impurities analysis, then there should be no concern about its absorption and, also, no need for a 28-day dermal toxicity study.

The Panel voted unanimously in favor of issuing a Tentative Report with an insufficient data conclusion. The data needed in order for the Panel to complete its safety assessment of Peppermint Oil, Peppermint Extract, and Peppermint Leaves are listed in the discussion section of the report as follows:

- (1) Concentration of use
- (2) Impurities (specifically pulegone) in cosmetic grade Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, and Peppermint (Mentha Piperita) Leaves or in the absence of impurities data, a 28-day dermal toxicity study using cosmetic grade ingredients. (Note: If changes found following dermal exposure are the same as those reported in existing oral-dosing studies, then there will be no need for reproductive and developmental toxicity studies; if the results are not similar, then there may be a need for dermal reproductive and developmental toxicity studies.)

Day 2 of the June 5-6, 1997 CIR Expert Panel Meeting – Full Panel

Peppermint (Mentha Piperita) Oil **Peppermint (Mentha Piperita) Extract** **Peppermint (Mentha Piperita) Leaves**

Dr. Schroeter noted that the Panel issued a Tentative Report with an insufficient data conclusion at the December 16-17, 1996 Panel meeting. The data needed in order for the Panel to complete its safety assessment were stated as follows:

- (1) Concentration of use
- (2) Impurities (especially pulegone) in cosmetic grade Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, and Peppermint (Mentha Piperita) Leaves or in the absence of impurities data, a 28-day dermal toxicity study using cosmetic grade ingredients. (Note: If changes found following dermal exposure are the same as those reported in existing oral-dosing studies, then there will be no need for reproductive and developmental toxicity studies; if the results are not similar, then there may be a need for dermal reproductive and developmental toxicity studies.)

Dr. Schroeter said that, in response to the Tentative Report, the Panel received impurities data on Peppermint Oil (pulegone detected at concentrations of 1 to 4%) and data indicating use concentrations of Peppermint Oil in the range of 0.02 to 3.0%. Dr. Schroeter noted that, based on the impurities analysis, his Team determined that pulegone is at a level that is not significantly toxic. Thus, the Schroeter Team concluded that Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, and Peppermint (Mentha Piperita) Leaves are safe as used in cosmetics.

Dr. Bronaugh noted that the levels of the impurity, pulegone reported by industry fall within the range of the short-term oral toxicity on pages 15-16 of the Tentative Report. The Peppermint Oil sample tested contained 1.7% pulegone, and the no-effect-level for Peppermint Oil in this study was 10 mg/kg body weight.

Dr. Bronaugh also noted that FDA has done an exposure assessment based on some of the new data submitted on a body splash lotion containing 0.2% Peppermint Oil. He said that if one assumes whole-body application of the lotion and 100% absorption (since there are no absorption data on Peppermint Oil), approximately 1 mg of Peppermint Oil per day is absorbed. He also said that the use of a 100-fold safety factor reduces the 10 mg/kg no-effect-level to 0.1 mg/kg. Therefore, without knowing absorption, it is possible that as much as 1 mg of Peppermint Oil/kg will be absorbed if the body splash lotion is applied over the entire body. Dr. Bronaugh noted that there is some concern over the use of Peppermint Oil in cosmetics, not knowing the extent of its dermal absorption.

Dr. Bailey said that Dr. Bronaugh's comments become particularly relevant if one considers that the body splash lotion may be used on infants and children. Dr. Bailey reiterated that a no-effect-level has been determined,

and acknowledged that an extremely conservative exposure estimate was used in the calculation. However, he noted that even if one assumes 10% area and 50% absorption, the safety factor is 180. According to Dr. Bailey, this means that an area in which safety becomes more of a question is being approached. Furthermore, he said that this becomes even more relevant when infants and children are taken into consideration.

Dr. McEwen asked Dr. Bronaugh if, based on his calculation, 1 mg of Peppermint Oil would be applied to the body.

Dr. Bronaugh said that 1 mg of Peppermint Oil/kg would be absorbed per day (by a human) by applying the body splash lotion that reportedly contains 0.2% Peppermint Oil. He also said that 68 mg/day would be absorbed, and, if one divides this by a 60 kg person, this translates into 1.12 mg/kg/day absorption. This estimate assumes 2 mg/cm² application over the whole body.

Dr. Andersen wanted to know if the concerns expressed by Drs. Bailey and Bronaugh relate to Peppermint Oil or the pulegone component.

Dr. Bronaugh said that the theory is based on Peppermint Oil. However, he noted that Peppermint Oil tested in the study by Thorup et al., 1983a (pp.15-16 of Tentative Report) contains 1.7% pulegone.

Dr. McEwen said that it would probably be difficult, if not impossible, to apply a body splash at a concentration of 2 mg/cm².

Dr. Bronaugh said that 2 mg is approximately 2 μ l, which is a small amount.

Dr. Schroeter said that it takes an ounce of content, 28 ml, to cover a total body (for an average adult, this is actually 1.7 m²). With this in mind, he said that he is also concerned about the data.

Dr. Bergfeld said that the Panel has the option of tabling the Tentative Report on Peppermint Oil, placing it in Teams for further discussion.

The Panel voted unanimously in favor of tabling the Tentative Report until the September 22-23, 1997 Panel meeting.

Dr. Bergfeld asked the Panel to elaborate on additional information that would be needed to address Dr. Bronaugh's concerns regarding Peppermint Oil/pulegone exposure.

Dr. Schroeter said that the Panel needs to review the data on which FDA's calculations are based, as well as the actual calculations.

Dr. Bailey noted that the no-effect-level of 10 mg/kg/day for Peppermint Oil was taken from the Tentative Report. He added that an ADI of 0.1 mg/kg/day has been reported for Peppermint Oil.

Dr. Bailey stated that the Panel will be provided with a copy of FDA's calculations.

Dr. Bergfeld said that because the extent of percutaneous absorption of Peppermint Oil is not known, it would be appropriate for the Panel to informally request any available absorption studies on Peppermint Oil that have not been submitted to CIR.

Day 2 of the September 22-23, 1997 CIR Expert Panel Meeting

Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, and Peppermint (Mentha Piperita) Leaves

At the June 5-6, 1997 Panel meeting, The Panel voted unanimously in favor of tabling the Tentative Report (insufficient data conclusion issued at December 1996 Panel meeting) on this group of ingredients until the September 22-23, 1997 Panel meeting. [Note: Prior to the June 5-6, 1997 review, the concentration of use and impurities data requested by the Panel were received.] This action resulted from statements made by Drs. Bailey and Bronaugh relating to the fact that FDA had done an exposure assessment of Peppermint Oil based on the

concentration of use data supplied by industry. In this assessment (made available for present meeting), a concentration of 0.2% (reported use concentration in a body splash) was selected, and 100% absorption was assumed per whole body application (2 mg/cm²). This translated into the absorption of 34 mg Peppermint Oil/day (0.6 mg/kg/day exposure for a 60 kg person).

It was also noted in the exposure assessment that an acceptable daily intake (ADI) of 0.2 mg/kg body weight day for menthol (major constituent of Peppermint Oil) was derived by the Joint Expert Committee on Food Additives, and that the ADI calculated using the data in the CIR report on Peppermint Oil was 0.1 mg/kg body weight/day for this ingredient.

During deliberations at the present meeting, the Panel expressed concern over oral-dosing studies on Peppermint Oil in which cyst-like spaces in the cerebellum of rats were reported, and the difficulty in interpreting these study results (due to inconsistent findings when results were compared). Though the lesions appeared to depend on the pulegone content (a greater NOAEL [no observable adverse effects level] was reported in a 90-day oral toxicity study in which Peppermint Oil containing 1.1% pulegone was tested, compared to a 28-day oral toxicity study in which Peppermint Oil containing 1.7% pulegone was tested), no definitive conclusion could be made.

Given the results for Peppermint Oil containing pulegone in short-term and subchronic oral toxicity studies, Dr. Belsito recommended that pulegone, as an impurity, should be limited to a concentration of 1% in Peppermint Oil, Peppermint Extract, and Peppermint Leaves.

Dr. Bronaugh noted that the toxicity of menthol is similar to that of pulegone.

The Panel also expressed concern over menthol, major component of Peppermint Oil. In the absence of dermal absorption studies on Peppermint Oil, it was noted that its absorption is expected to be rapid, like menthol. Furthermore, there was concern that this rapid rate of absorption may induce systemic toxicity that was not observed following oral exposure. Thus, the Panel decided to limit the cosmetic use of Peppermint Oil and other ingredients in this review such that the menthol content would not exceed the ADI (0.2 mg/kg/day for menthol) that was established by the World Health Organization.

The Panel agreed that the inconsistencies between results from short-term and subchronic oral toxicity studies on Peppermint Oil should be addressed in the report discussion, along with the Panel's basis for using the ADI for menthol in its safety assessment.

The Panel voted unanimously in favor of issuing a Revised Tentative Report with the following conclusion: On the basis of the available data, the CIR Expert Panel concludes that Peppermint (*Mentha Piperita*) Oil, Peppermint (*Mentha Piperita*) Extract, and Peppermint (*Mentha Piperita*) Leaves are safe for use in cosmetic formulations at levels such that the menthol content does not exceed the ADI of 0.2 mg/kg/day. The pulegone level should not exceed 1% in these ingredients.

Day 2 of the March 19-20, 1998 CIR Expert Panel Meeting

Peppermint (*Mentha Piperita*) Oil, **Peppermint (*Mentha Piperita*) Extract,** **and Peppermint (*Mentha Piperita*) Leaves**

Dr. Schroeter said that questions relating to the menthol component of Peppermint Oil remain. One of the questions raised by the Panel related to whether it would be logical to make an exception in the conclusion regarding the concentration of menthol, that it not exceed the ADI (average daily intake) of 0.2 mg/kg/day. Dr. Schroeter noted that his Team determined that there is no need for any specific limitation on menthol in either the report discussion or conclusion. He said that the issue of the dermal absorption of menthol may be managed by explaining in the discussion that the amount of menthol that may be absorbed through the skin is safe, if compared to the same amount in Peppermint Oil that is absorbed from the gastrointestinal tract, which is already considered a safe amount.

Based on the preceding comments, Dr. Schroeter said that the statement in the report discussion that mentions the ADI of 0.2 mg/kg/day for menthol will be replaced with statements based on the following: The dermal absorption of menthol will be no greater than that of oral Peppermint Oil. The clinical data will provide adequate evidence of safety at current concentrations of use [that is, since oral data are adequate for demonstrating that menthol is equal to or not greater than that absorbed through the skin].

Dr. Bailey wanted to know if clinical data (ingestion data) obviate the need for dermal absorption data.

Dr. Shank said that for systemic toxicity, the oral data are sufficient, because it is believed that the extent of absorption through the skin would not be any greater than the absorption through the GI tract and that the "first pass" consideration is not important. He noted that "first pass" effects were considered, and it was determined that the available data are sufficient. Dr. Shank also said that as far as skin effects are concerned, clinical data support the safety of current concentrations of use.

Dr. Bergfeld confirmed with Dr. Schroeter that the substance of Dr. Shank's explanation will be included in the report discussion.

Dr. Schroeter said that Dr. Shank's explanation is logical, and, therefore, does not limit the concentration.

Dr. McEwen said that it is not that "first pass" kinetics are not the primary argument, but, taking this into consideration, there was a sufficient justification for considering the oral doses administered and assuming that the clinical epidermal doses would not sufficiently increase systemic toxicity.

Dr. Andersen recalled that at the last Panel meeting, FDA expressed concern over a calculation that compared the reported concentrations of menthol that could be present in levels of Peppermint Oil that were actually reported to be used. He said that based on that combination of the concentration of Peppermint Oil and the concentration of menthol in it, it was concluded that those levels approached the reported toxicity that was actually included in the CIR report. Dr. Andersen asked FDA for further comments.

Dr. Bronaugh said that because a limitation on the concentration of pulegone is included in the report discussion, one would think that this is adequate.

Dr. Bergfeld said that the revisions in both the report discussion and conclusion need to be discussed and that the Panel needs to vote on the new conclusion. She also said that the Panel needs to discuss the merit of the conclusion and determine whether the present report needs to be reissued as a Tentative Report.

Dr. Bergfeld asked if there was any further discussion regarding the current revised conclusion and the editorial changes in the report discussion that are being considered. Seeing that there was no further discussion, she called for the vote.

The old conclusion that is being revised reads as follows: On the basis of the available data, the CIR Expert Panel concludes that Peppermint (*Mentha Piperita*) Oil, Peppermint (*Mentha Piperita*) Extract, Peppermint (*Mentha Piperita*) Leaves, and Peppermint (*Mentha Piperita*) Water are safe for use in cosmetic formulations at levels such that the menthol content does not exceed the ADI of 0.2 mg/kg/day. The Pulegone level should not exceed 1% in these ingredients.

The Panel voted unanimously in favor of issuing a Revised Tentative Report with the following revised conclusion: On the basis of the available data, the CIR Expert Panel concludes that Peppermint (*Mentha Piperita*) Oil, Peppermint (*Mentha Piperita*) Extract, Peppermint (*Mentha Piperita*) Leaves, and Peppermint (*Mentha Piperita*) Water are safe as used in cosmetic formulations. The concentration of pulegone in these ingredients should not exceed 1%.

Day 2 of the September 10-11, 1998 CIR Expert Panel Meeting

Peppermint (*Mentha Piperita*) Oil, Peppermint (*Mentha Piperita*) Extract, and Peppermint (*Mentha Piperita*) Leaves

Dr. Schroeter stated that his Team concluded that these ingredients are safe as used in cosmetic formulations, and that the concentration of pulegone in each should not exceed 1.0%. The restriction on pulegone is based on the sensitization potential of this impurity.

Additionally, Dr. Schroeter noted that the percutaneous absorption of other chemicals is enhanced in the presence of Peppermint Oil, and that this issue should be addressed in the report discussion. He suggested that the

statement addressing this issue in the CIR report on Peanut Oil should be incorporated into the report on Peppermint Oil.

Dr. Belsito read the following statement from the report on Peanut Oil, relating it to Peppermint Oil: The Panel noted the evidence that Peppermint Oil can enhance penetration. Formulators are cautioned that this enhanced penetration can affect the use of other ingredients whose safety assessment was based on their lack of absorption.

Dr. Bergfeld recommended inclusion of the preceding statement, with any necessary modifications, in the Peppermint Oil report discussion.

Dr. Belsito recommended that it be clarified in the last sentence of the report discussion that use of Peppermint Oil at a concentration of $\leq 3\%$ in rinse-off formulations and $\leq 0.2\%$ in leave-on formulations, coupled with the restriction of pulegone to $\leq 1\%$, alleviates the Panel's concern about pulegone toxicity.

The Panel voted unanimously in favor of issuing a Final Report with the following conclusion: On the basis of the available data, the CIR Expert Panel concludes that Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, Peppermint (Mentha Piperita) Leaves, and Peppermint (Mentha Piperita) Water are safe as used in cosmetic formulations. The concentration of pulegone in these ingredients should not exceed 1%.

Day 1 of the April 10-11, 2017 CIR Expert Panel Meeting – Dr. Belsito's Team

DR. BELSITO: Okay, peppermint. Were previously reviewed in 2001 for ingredients safe as used in the concentration of pulegone in these ingredients shouldn't exceed 1 percent. So, we, basically, agreed that the assessment was primarily based off of peppermint oil; that the data was considered relevant to the other reports with some new available data on leaf extract, leaf water were added, as well as NNTP data on culergon and then it was really reopened because six ingredients were proposed for addition to the family, and that's what we're looking at today; and then there was a wave 2 submission on 20 percent peppermint oil in a HRIPT that was pretty good.

DR. SNYDER: 50 percent leaf water.

MR. JOHNSON: By the way, we received those from RIFM.

DR. BELSITO: Yeah. So, the oil I think is okay, but we never really got constituents for the other parts of the plant, so I had a question about whether we need repro and gentox for the leaf; or do we at least need --

DR. LIEBLER: You feel we should reopen?

DR. BELSITO: What?

DR. LIEBLER: You feel we should reopen, right?

DR. BELSITO: If we want to add those ingredients. I don't think we can add the other ingredients without reopening and getting some data.

DR. LIEBLER: I just thought we hadn't taken a

(inaudible). I figure reopening as well to add the new ingredients.

DR. BELSITO: Was that the question at this point?

DR. KLAASSEN: Yeah.

DR. BELSITO: It was, okay. So, we can add the new -- it's not a no-brainer.

DR. ANSELL: Well, this is a re-review; so, there are some new ingredients that are proposed, but there're issues with -- for whatever reason you want to use (inaudible).

DR. LIEBLER: There almost always some data needs when we reopen for new ingredients anyway; so, I mean, I didn't think this was unreasonable. Maybe no-brainer is not the right term to use.

DR. SNYDER: Well, I thought there was some language related to our -- maybe our initial reasoning for using the oil for the safety of all the leaf stuff was flawed. Wasn't that in here? Didn't we have some language in here about that because there's (inaudible).

MR. JOHNSON: I know with respect to the initial review, the oil was classified as the worst case scenario.

DR. SNYDER: Right; was the basis for the safety of everything.

MR. JOHNSON: Right.

DR. ANSELL: And has now been -- the definition has been revised to be explicit that the oil is the whole plant.

DR. BELSITO: But then when you look on page 18 of the PDF -- it's the major constituents of peppermint oil, terpenes and menthols, yada-yada-yada; and then you get to the leaf extract and it's flavons and flavanones and rosmarinic acid -- these seem to be very different chemicals coming out of different parts of the plant; and then this toxic compound, pennyroyal, in the leaf.

DR. SNYDER: The young leaf.

DR. BELSITO: What?

DR. SNYDER: The young leaf; yeah, you've got to watch out for those young ones.

DR. BELSITO: But, you know, would lead me to believe that, you know, what we're getting out of the -- I didn't pick up the peppermint as the whole plant -- but, if peppermint oil is very different from leaf water and other isolated parts of the plant from which we never had data.

DR. LIEBLER: I think the pennyroyal is the pulegone.

MR. JOHNSON: I checked that publication. As a matter of fact, we received a comment from (inaudible) today, and pennyroyal is basically another species of peppermint plant;

so, really, there's no component of pennyroyal that's a little toxic.

DR. SNYDER: The way you word it and you say the leaf contains a toxic compound, pennyroyal.

MR. JOHNSON: Right; and that's what was stated in the publication, the primary source, but we found that was not correct.

DR. SNYDER: All right; we need to find out what that is.

MR. JOHNSON: That's another peppermint plant.

DR. LIEBLER: Mentha Pulegium, (European) pennyroyal.

DR. SNYDER: The monoturpi?

DR. LIEBLER: No; the plant, Mentha Pulegium.

DR. KLAASSEN: Can't get out of these plants.

DR. LIEBLER: But I know -- I remember because one of our late colleagues getting, Sid Nelson, did all of the really definitive magnistic tox work on pulegone; and I remember how many of the publications talked about, you know, a constituent in pennyroyal oil as being the source of the pulegone that they studied. Anyway, the pennyroyal that they're referring to in this context here, it sounds like they're probably talking about pulegone.

DR. SNYDER: (Inaudible) we already put a limit on it, so.

DR. LIEBLER: And it damages the liver.

DR. SNYDER: Well, I think we need to go with composition for all these conditional ingredients to see if there's any significant differences.

DR. LIEBLER: That was my need too, composition, method of manufacturing impurities.

DR. SNYDER: Yep.

MR. JOHNSON: And that's on all of the ingredients that we're going to be adding --

DR. SNYDER: All the new ingredients.

MR. JOHNSON: New ingredients, right?

DR. BELSITO: So, the oil is okay.

DR. SNYDER: Yeah; I think so.

DR. BELSITO: We're fine with that. So, we want --

DR. ANSELL: I think if you reopen and fill their data needs for the existing materials that's okay; but if you have data needs on the new materials, then they should not be added to this report, and it shouldn't be done through reopening.

DR. BELSITO: Well, that was my question; but I thought some of these materials were in the original report. I'm wondering how we signed off on it.

DR. SNYDER: Well, that's why I was making a comment because we -- the 2001 report is for the oil, the leaf, the leaf extract, and the leaf water.

DR. BELSITO: Right.

DR. SNYDER: So, we used the oil as the margin for everything which was flawed.

DR. BELSITO: Which was so different than what we did; because we hung up rice for three years over the constituents of brand or something. I mean, I think it must have been an impending snowstorm or -- what year was this, 2001? It was 9/11.

DR. SNYDER: 9/11.

DR. LIEBLER: You know, if you've got the oil and the leaf, and the leaf extract already in previous reports, and then you're adding a peppermint extract, which is probably whole plant -- Jay just confirmed for us, I think, if I'm not mistaken --

DR. ANSELL: Yeah.

DR. LIEBLER: -- and then a flower leaf stem extract and a flower leaf stem water, and a leaf cell extract, and a leaf juice, those are all typical of things that are similar enough to what's already been covered that I think we could probably -- if we get (inaudible) --

DR. BELSITO: Well, quite honestly, when I read it, I didn't know why we went safe with this because I didn't think there was data that convinced me that the leaf was the same as the oil. I mean, so that's why I thought we needed to reopen because I'm not even certain that the conclusion we made is the correct one.

DR. LIEBLER: Wasn't this the one where Ron Shank, basically, it was the

Shank Approximation, or whatever we called it -- the Shank Doctrine -- that the oil represented a worst case scenario because it was most enriched with respect to the constituents concerns?

DR. BELSITO: But, when you look at what we're seeing in this report which is some material that we didn't have before, the constituents in the leaf, particularly the leaf water; I mean, now, we're actually talking about waters and oils which are, obviously, going to contain different things are quite different. I mean, I think we need to reopen to clearly look at --

DR. SNYDER: It's a sensitizer.

DR. BELSITO: -- yeah, I think we need to reopen it simply to look at was our original conclusion correct, and during that process I think we can add everything else in.

DR. LIEBLER: So, basically, we're on the same page. I agree we should reopen it. We should add the new ingredients. We do have some data needs for a composition, method of manufacturing impurities. I think, I don't really have any doubt that we can get there for most or all of these ingredients to get sufficient data to draw our conclusions. And the main question I had has to do with having, in the previous conclusion, a 1 percent limit, I think, on the pulegone; and I'm wondering if -- you know, given the way we do things now -- if that's the way we would handle the pulegone issue now or whether we should consider like a QRA approach rather than speaking in numbers?

DR. BELSITO: Well, particularly, in light of the fact that we know that other botanicals contain pulegone and we're restricting those into restricteds, it's the same thing as sensitization. To say that we're going to limit eugenol in this plant based upon the highest level eugenol can be used in, and then, you know, it's supplied and mixed with another botanical that contains eugenol, and suddenly you're passed the standards.

DR. LIEBLER: Yeah. I just think that 1 percent
(inaudible) reflect in a way that the panel can reason through this problem several years ago, but it isn't really applicable the way we handle this certain thing now.

DR. BELSITO: I think it was the first time we dealt with pulegone, and didn't realize it'd be popping up in a lot of other botanical species. I don't know why; but it has; because, I mean, I think now we simply say, you know, chemicals of concern not reaching toxic levels.

DR. KLAASSEN: But how could it be toxic? It's a natural chemical.

(Laughter)

DR. LIEBLER: Revise the minutes -- insert laugh here.

DR. BELSITO: The oil is okay, and we want manufacture and impurities for the other constituents; and if significantly different from the oil; then what?

DR. ANSELL: Then they aren't added.

DR. BELSITO: But they're already in the report, Jay, the original report.

DR. ANSELL: Right. The original one; but if the residuals of the --

DR. BELSITO: If we don't get them or they're significantly different, then I'm not going to be comfortable, you know, saying that the original conclusion was correct and would reopen it to amend the conclusion.

DR. HELDRETH: Yeah; I think our traditional no-brainer rule was to alleviate the burden of just adding things in and making a case more difficult. So, just reopening and having no reason but to add these ingredients, and they're not no-brainers, I can see doing it; but since there seems to be a cause to relook at the ingredients that are already in the report, I don't think the no-brainer clause (inaudible).

DR. BELSITO: No; that's what I'm saying. The other ingredients really belong in here if our conclusion is correct. They don't really differ; that would be a no-brainer; but I'm wondering if our original conclusion was correct and would like to reopen it to get a look at that data.

DR. ANSELL: Right; I absolutely agree with that. It's the part with the add-ons, that if they're significantly different, the answer is not to develop a whole other report to support them; it's that they don't belong in this report.

DR. BELSITO: Right.

DR. LIEBLER: I think the no-brainer thinking works with a series of structures where you've got an extra methylene or ethyl, you know, something like that. Here, with botanicals, it's a different world, different rules; there are no botanical no-brainers.

DR. SNYDER: So, on the use table, Wilbur, under Table 3, so under the peppermint oil, the range we have at the bottom is --

MR. JOHNSON: There's a mistake in there. It should be at 40 percent of leaf water and not, I think, 0.1, yeah. That would be correct.

DR. SNYDER: Okay; because the leaf water -- it says up to 15 percent for dermal, which is quite a bit higher than percent of the oil, but, okay. So, that's going to be even higher? It's going to be 40 percent?

MR. JOHNSON: Yes; mm-hmm.

DR. SNYDER: Okay. Yes, we had use concentrations that are much higher for the other constituents than (inaudible).

DR. BELSITO: And then I have a question as to whether we need reproductive and genotoxicity for the leaf; because that would only be if they significantly differ.

DR. SNYDER: Well, I think the first step would be absorption; you know, we need absorption data or a 28-day dermal; and then if it's absorbed, or there's absorption in the 28-day dermal, then we go to (inaudible).

DR. BELSITO: Okay; so, the oil is okay; we need manufacturing impurities and composition for the other constituents; if significantly different, then other data needs would be absorption and 28-day dermal -- and if absorbed, and genotox.

DR. SNYDER: I guess that would be based on our compositions if there's something in there that we have some structure we'd be worried about.

DR. LIEBLER: Well, I think we need genotox anyway.

DR. SNYDER: Okay.

DR. LIEBLER: There are enough interesting organics in this so that --

DR. SNYDER: Yeah.

DR. BELSITO: Okay. Since it takes me forever to talk, the other ingredients are the leaf, the flower --

DR. SNYDER: Flower, leaf, and stem extract?

DR. BELSITO: Leaf, flower, stem, right; so, on the meristem?

DR. SNYDER: Okay.

DR. BELSITO: Okay; so, the other ingredients are they the leaf, the flower, the stem, and the meristem?

DR. SNYDER: Okay; so, now sensitization.

DR. BELSITO: Yeah. I think we're okay with sensitization, now. We waved two.

DR. SNYDER: I thought that was at 20 percent; there's no waive; yeah, that was 20 and 50 percent, but wasn't there one at 22 percent? Or am I thinking of a previous report. I thought I saw one here that was.

MR. JOHNSON: Yeah; there was a 48-hour single application patch test on the cleansing gel -- 50 percent.

DR. BELSITO: (Inaudible)

DR. SNYDER: That was negative, though; leaf water, percent gel?

MR. JOHNSON: Well, there was mild erythema in one subject.

DR. BELSITO: Peppermint leaf water is being used up to 15 percent in (inaudible). It's a concentration; and what page are we on here; because I have nothing about sensitization -- on irritation sensitization; leaf water, negative skin irritation sensitization data on a product containing leaf water in Table 4.

DR. SNYDER: Because there was this case report -- there are several case reports -- at 2 percent.

DR. BELSITO: Yeah, but those are patch tests.

DR. SNYDER: Tests; you're right; okay.

DR. BELSITO: People are already sensitized.

DR. SNYDER: Yeah.

DR. BELSITO: Okay; anything else?

MR. JOHNSON: What are the concerns, skin penetration enhancement?

DR. BELSITO: Yeah; I have that in my discussion. There's going to be a pulegone restriction; a botanical boilerplate, depending upon what we find out about this pennyroyal.

DR. SNYDER: Aerosol.

DR. BELSITO: There are lots of terpenes, so formulated to be non-sensitizing; add penetration enhancement to the discussion. So, those will be the big things so far. The botanical boilerplate, the inhalation and penetration enhancement in the discussion; and then, well, obviously, I have to see the other data that we asked for.

MR. JOHNSON: For the genotoxicity data request, did you want bacterial and mammalian cells, or --

DR. SNYDER: Yes.

MR. JOHNSON: -- both?

DR. BELSITO: And then, the last point I had -- I knew there was something else -- we have an ingredient with no concentrations. This is on page 34; it's just labelled peppermint. There's no definition in the dictionary; there are no reported VCR uses in 2017. There were two -- I'm not sure why that's there. VCRP has no reports; we have no reported concentration; and there was no definition in the dictionary as to what peppermint is. So, why is that in the table?

MR. JOHNSON: I think it was in the published report, peppermint.

DR. BELSITO: But can we get rid of it in the current reports?

MR. JOHNSON: We can.

DR. BELSITO: And say it's not a cosmetic ingredient and no one's saying that they're using it.

DR. HELDRETH: Did it change names or just get deleted?

DR. BELSITO: What?

MR. JOHNSON: It was just there.

DR. HELDRETH: Okay. I was just asking if it changed names or was it simply deleted.

MR. JOHNSON: It was in the old database, but it's not in the current one; so, I guess that's what happened.

DR. BELSITO: And then I guess the only other question I had since we're going through everything is so that this irritation with the peppermint leaf, water at 50 percent in a cleansing gel when the highest reported use we have is 15 percent in a wash-off; so, I was (a) confused about that and, you know, again, it's a cleansing gel, it's a surfactant, it's going to be irritating; but I just wanted to pose the question, do we think we need to say formulated to be non-irritating? I didn't think so, but that data is there.

MR. JOHNSON: I'm sorry, I had made a mistake in the table; actually, the mentha piperita peppermint leaf water is used in face and neck products, not spray products at concentrations up to 40 percent.

DR. BELSITO: So, it's used in leave-on products up to 40 percent?

MR. JOHNSON: That's correct.

DR. BELSITO: Okay; can you tell me where in the table that is?

MR. JOHNSON: Well, actually, it's not in the table. That table needs to be corrected; but if you go to page 60, PDF, page 60, in that table they use concentration data.

DR. BELSITO: 40 percent for the leaf water; face and neck products, not spray; okay. So, it's used in leave-ons up to 40 percent.

MR. JOHNSON: Right.

DR. BELSITO: So, then we have this data on the bath gel at 50 percent that's irritating; so, then, the question becomes, do we need a -- if and when we get around to say --

DR. SNYDER: Not the irritation (inaudible); okay.

DR. BELSITO: -- I mean, I think the irritation is probably more from the gel. Okay.

DR. SNYDER: Did we change the tables? Did the totals used to be at the bottom instead of the top?

MR. JOHNSON: Not on this.

DR. SNYDER: Is that common to all documents? I thought they were always

at the bottom.

MR. JOHNSON: It used to be, but years ago; but it was changed, though.

DR. SNYDER: Because I kept getting confused. I always go to the bottom to see what the ranges are, and then go up; and this -- it was like you supposed to start at the top.

DR. LIEBLER: Is it writer dependent?

DR. SNYDER: That's what I'm asking because I thought we still had some at the bottom.

MR. JOHNSON: I think I was the last holdout on that.

MS. BURNETT: Yeah, they supposed to be at the top.

DR. SNYDER: So, I'm not going crazy. It used to be at the bottom, but we changed it at the top.

DR. LIEBLER: You are crazy.

DR. SNYDER: I am crazy; that's all right.

DR. BELSITO: Okay; anything else on peppermint? Okay.

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Next is peppermint. Wilbur, you're up again. Let me see. Also known as menthe piperita I guess is how you pronounce the botanical name. And it is, so, in the expert panel previously had reviewed the oil, the leaf extract, the leaf, and the leaf water. And the conclusion was that these were safe as long as the concentration of pulegone. Am I saying that correct? Should not exceed 1%. This is a re-review of these ingredients. So one of the questions was do we add six more ingredients to the four ingredients which were found to be safe. So, I guess the first question would be, Tom, and Ron, and Ron, should we re-open this? Yes or no? And if we do re-open, do we add the six ingredients? And then lastly, what are the needs if we do that? So, first question, re-open or not?

DR. SHANK: Yes.

DR. MARKS: Okay. Move to re-open. Add the six ingredients?

DR. SHANK: No. Just two. I think we can add the leaf cell extract and the leaf juice. Because these are covered by the leaf extract data. Now, leaf water was tested for skin sensitization at 20%. Apparently it's used up to 40% in face and neck products. So we would require more data for that. And then we don't have any data for flowered leaf stem extract and water. Mera stem cell culture extract. So I wouldn't add those.

DR. MARKS: So, yeah, one of the questions. So that would be one approach. And that's sort of the re-open no-brainer. Or do we add the six ingredients and then issue an insufficient data announcement? And I wanted to get as much as possible info on the six additional ingredients. But Ron and Tom, which approach do you prefer? Do you want to just add the two? As no-brainers?

DR. SLAGA: Yes. It's obviously re-open and add the two because that, leave it with the same conclusion.

DR. HILL: Which two are we adding then?

DR. SHANK: Leaf cell extract and leaf juice.

DR. HILL: Okay. But not leaf extract per say?

DR. MARKS: We have the leaf extract on the original. Approved. Yes.

DR. HILL: Okay. And we don't think we need sensitization on that? Or do we have it formulated to be not sensitizing clause?

DR. MARKS: I have, well

DR. HILL: And I was also wondering about phototox, or photosensitization at least.

DR. MARKS: I have that irritation and sensitization is okay for the oil and leaf water. Let me see my notes here. In wave two. Yeah, we have the oil at 20% as you mentioned.

DR. HILL: So if we got oil at 20% then we feel good about the leaf extract?

DR. SHANK: It's not oil, it's water.

DR. MARKS: No. Yeah, the leaf water. We have an HRIPT at 20%. And the question is, can you carry that over to the other leaf ingredients?

DR. HILL: No. Because if you remember how these leaf waters are made, you don't get much. I don't know if we have full method of manufacture.

DR. MARKS: Then we'd be going back to the original report, of course. Because we approved it before as safe for the leaf extract and the leaf water. And the only thing we had was the sensitization for the leaf water. If I interpret the data correctly.

DR. HILL: That's a good reason to re-open for me.

DR. MARKS: Okay. I agree. So, re-open. At least at this point, add two ingredients, the leaf cell extract and leaf juice. So noted that you would want more sensitization data, particularly on the leaf extract. One presumes if we got the leaf extract. Now the question is, seems like if we want more data, we should do an insufficient data announcement. But

DR. SHANK: Then you can add them all.

DR. MARKS: Exactly. That's where I began, with adding all six and get as much possible on the six additional ingredients. Plus, I'm gonna put, Ron Hill, based on your comments, I think, six additional ingredients and leaf extract. Even though it was in the original report found to be safe. Sensitization. Does that? One of the things that I wanted to hear comments about, the cancer hepatotoxic and nephrotoxic issues that Wilbur brought up in his memo. That's

the second paragraph of his March 17th memo, where he says leaf extract hepatotoxicity, the leaf nephrotoxicity, and then obviously, I presume, we'll put the same limitation on pulegone in this one. But the hepatotoxicity and nephrotoxicity. Wilbur, do you want to clarify that in your memo?

MR. JOHNSON: Which memo are you talking about?

DR. MARKS: I'm looking at the March 17th memo. So the one that, and then I'm looking at the second paragraph, towards the end of that paragraph. The re-review document contains the following data for the panel's consideration. And then you put leaf extract and in there you have skin penetration enhancement, hepatotoxicity. And then under the peppermint, next you say the leaf. And you have in parentheses nephrotoxicity. So it's hard for me to ignore your memo and say we can't address those two.

MR. JOHNSON: Yeah, those data are included in the safety assessment.

DR. MARKS: Yeah, so they're fine. Yeah, okay. So the way the memo read, I was concerned was there nephro and hepatotoxicity. Okay. And I assume we're going to need the botanical and pesticide heavy metal boilerplates once we get to the final document. So, tomorrow I'm going to move to re-open, add the six new ingredients, and we will have a insufficient data announcement to get as much as possible information on the six additional ingredients. And particularly the leaf extract sensitization. But, you know, if we do the way we do the other botanicals, we're gonna in the end say, formulate to be non-irritating and non-sensitizing. Maybe we can move on to that now. But I like the idea of an insufficient data announcement. Tom, Ron, Ron, what do you think? We'll see how it plays out tomorrow. But, does that sound reasonable now? I know you, Ron Shank, were gonna move right to the leafs were gonna be safe.

DR. SHANK: Well, I'll go with insufficient.

DR. MARKS: Okay. Let's see where it goes. Any other? Wilbur.

MR. JOHNSON: Just a correction for Table 3. The leaf water is used in concentrations up to 40% in leave-on products. So the table will be corrected.

DR. MARKS: Up to 40%?

MR. JOHNSON: It's up to 40%. Yes.

DR. MARKS: So that's twice as much as we have the HRIPT. I'm glad we're getting as much possible. Not only on the six additional, but on the ones already reviewed. On all the ingredients now. So 40% of the peppermint leaf water.

MR. JOHNSON: Right.

DR. MARKS: Is the number of uses, 15, still the same?

MR. JOHNSON: Yes. This just relates to use concentration, data provided by industry.

DR. MARKS: Right.

MR. JOHNSON: So those uses will be the same.

DR. MARKS: Good.

MR. JOHNSON: Dr. Marks, will you just indicate the data needs associated with the IDA?

DR. MARKS: Well clearly, sensitization.

DR. SLAGA: Irritation.

DR. MARKS: Yeah, irritation, sensitization. Do we need anything other than that? Tom? Ron? Ron?

DR. HILL: I wanted to query Ron Shank specifically. Because I was reading the comments in the memo originally. And the issue was the validity of the oral safety studies. I guess it would be the peppermint oil that I was most interested in. Because there was discussion about absorption from the GI tract versus systemic circulation penetration. And I'm always thinking in terms of, first pass effect, which you specifically mentioned, because that was raised by Dr. Schroeter I believe at the time. And Dr. Bailey was in on that discussion. So, your response was something to the effect that, first pass was taken into account. But I wondered how that was even done, if you weren't looking for specific amounts absorbed. Or maybe they were.

DR. SHANK: So where is this?

DR. HILL: The discussion is on page 12 and 13, way back when, let's see where you were quoted. You were quoted on page 14 actually, near the top.

DR. MARKS: Ron Shank was?

DR. HILL: Yes. That's why I'm asking. The substance of Dr. Shank's

explanation will be included in the report discussion, which I guess there was something in there. Right now, if we're going insufficient, I want to just leave that hanging, unless you want to respond. For closer scrutiny. And the reason I'm bringing it up in part is because when you give a subchronic tox, you're giving relatively lower doses. So if you do acute, you give a high dose in general in the dose ranging. You give a high dose and you're saturating everything in sight, like efflux transporters and metabolizing enzymes and liver and so forth. But longer, slower studies, lower percentages in diet for example, orally, in rodents in particular, which are pretty aggressive first pass metabolizers. I'm not sure we're picking everything up. In that context, if you had the oil in a leave-on at 5%, given the mode of use. I mean, I know we've done in the past a conservative estimate, this percentage of the body is exposed this often to this particular chemical heavily. I mean, I eat peppermint with reckless abandon. I remember smearing the leaf juice on my body numerous times as a kid.

DR. BERGFELD: You're going to die.

DR. HILL: Am I? Well yes, I'm likely going to die.

(laughter)

DR. SHANK: I'd have to go back. That's 19 years ago.

(laughter)

DR. SHANK: Out of context, so

DR. MARKS: Out of context, I like it. So, well this is going to go, tomorrow we're going at least, no matter what the final will of the expert panel is, we're going to be seeing it again. So I'll let Ron Hill, your short term memory will bring it up again. And Ron, your long term memory will clarify it. So tomorrow I'm going to move to re-open, add the six new ingredients, and we'll issue an insufficient data notice to get irritation, sensitization on the six additional ingredients, and from the original report, leaf water. It's now being used at 40% with an HRIPT which is negative at 20%. So we don't have sensitization data at the present use concentration of leaf water. And then also leaf extract, which we have no sensitization data on. And Ron Hill, I agree, that's an important point. Does that sound good?

MR. JOHNSON: Just one more thing, Dr. Marks.

DR. MARKS: Uh oh, Wilbur. That could generate another ten minutes of discussion.

MR. JOHNSON: On page 23, are there any concerns relating to genotoxicity?

DR. MARKS: Tom?

DR. SLAGA: I don't have any.

MR. JOHNSON: Is that positive results?

DR. SLAGA: Well they're both positive and negative.

Day 2 of the April 10-11, 2017 CIR Expert Panel Meeting – Full Panel

DR. MARKS: So, this is looking at a potential amended Safety Assessment of mentha peperita. That is peppermint. The ingredients are derived ingredients. So this is a re-review. In 2001, the peppermint oil leaf extract, leaf and leaf water, were evaluated. And a conclusion indicated that these were safe. But limit the concentration of pulegone in these ingredients should not exceed one percent. Our team moves that we re-open this group of ingredients and that we add six new ingredients. We would put out an insufficient data notice to get irritation and sensitization on the six additional ingredients. And the leaf water, which was in the original report, 40 percent use we have now. HRIPT in the previous report was 20 percent. So I'd like to see something closer to the present use concentration. And then also, the leaf extract in the original report. So, re-open. Add the six ingredients. Insufficient data notice for irritation and sensitization.

DR. BERGFELD: Belsito. Comment.

DR. BELSITO: Yeah. We agree with re-opening. We had an interesting discussion, because we didn't think it was a no brainer to open the -- to add the additional ingredients. However, I felt that we really didn't have sufficient data on parts of the plant in the prior report, to say that they were safe. And so I wanted to re-open it to take a look at whether we were correct with the original Safety Assessment. And in that regard, had no problems with adding all of the other ingredients in re-opening.

DR. MARKS: Yeah. And the only other comment, I agree obviously Don, with what you said. And we'll address that at the next time. But, in the 2001 report, the pulegone was not to exceed one percent, partly based on a maximum leave on concentration of 0.2 percent in the original report. Now the oil concentration is five percent.

DR. BELSITO: Yeah.

DR. MARKS: So now, we have issues with that.

DR. BERGFELD: So, basically, we'll re-open and send out an insufficient data announcement, adding all these things about the six new ingredients. A review of the old ingredient. Anything else?

DR. LIEBLER: Just to clarify, for the new ingredients, in particular the method of manufacture --

DR. MARKS: Mm-hmm.

DR. LIEBLER: -- composition and impurities.

DR. BELSITO: Yes.

DR. BERGFELD: Okay.

DR. SNYDER: That also goes to the old report.

DR. BERGFELD: Yes.

DR. SNYDER: Because we have a lot more experience now with how to deal with botanicals. And so bring it up for botanical standards I think.

DR. BERGFELD: Any other additions to the request? Seeing none, we'll move on then and make that insufficient data announcement.

DR. BELSITO: Tom, you had a comment?

MR. JOHNSON: Vote. Do we have to vote?

DR. BERGFELD: I don't think on the re-reviews.

MR. JOHNSON: So the data request is on all of the ingredients except for the oil, is that correct?

DR. BELSITO: No.

DR. MARKS: No.

DR. BELSITO: We need new data on the oil because of the increased concentration of use. Right Jim?

DR. MARKS: Yes.

DR. BELSITO: Data on everything they've got.

DR. BERGFELD: Yeah.

Day 1 of the September 11-12, 2017 Panel Meeting – Dr. Belsito's Team

DR. BELSITO: Okay. Peppermint. So at the April meeting we agreed to reopen and to add six additional peppermint derived ingredients, and then went on to issue an insufficient data announcement. And so what were we asking for?

MR. JOHNSON: Skin irritation and the --

DR. BELSITO: The sensitization --

MR. JOHNSON: -- sensitization data.

DR. BELSITO: -- yeah.

MR. JOHNSON: And a data on opposition, method of manufacturer and impurities.

DR. BELSITO: Right. Okay. Okay, peppermint.

DR. SNYDER: We're seeing everything --

DR. BELSITO: All right.

DR. SNYDER: -- but the stem and the flower.

DR. BELSITO: Pardon?

DR. SNYDER: We received data on the leaf extract, and nothing on the stem or the flower.

DR. BELSITO: Right. Or the meristem.

DR. LIEBLER: I think what we received on the leaf extract, leaf water is really inadequate. It doesn't really describe. There are a couple of verbal descriptions, I mean, a couple sentences, right?

DR. SNYDER: You mean as far as the composition and impurities?

DR. LIEBLER: Yeah. I mean, the oil we've got nice data on.

SPEAKER: Yeah.

DR. LIEBLER: And the idea would be, if we can describe these with respect to the oil, then we could use a lot of the oil data to clear the others. I mean, for the leaf water, for example, which is one of the most widely used, I believe, there's hardly any -- and we also don't have impurities data for the oil, actually.

DR. SNYDER: Well, the oil was already reported in 2001. And we added all these (inaudible).

DR. BELSITO: I know, but if we find that the data is now insufficient, it doesn't mean we can't correct that report. I'm just pointing it out. I'm not concerned. While we're on page 31, under non-cosmetic use, you have leaf juice. Is that the same as leaf water? It's the second paragraph under non-cosmetic PDH31.

MR. JOHNSON: I'm not sure (inaudible).

DR. BELSITO: Okay.

MR. JOHNSON: I'll have to check on the juice.

DR. BELSITO: And then, Paul, are you -- we probably discussed this before but --

MR. JOHNSON: Oh, I'm sorry. Yeah, that was one of the additional six ingredients that was added to the --

DR. BELSITO: The leaf juice isn't one of our ingredients.

DR. SNYDER: It is.

MR. JOHNSON: Yes.

DR. BELSITO: It is?

MR. JOHNSON: Yes.

DR. BELSITO: Oh, okay.

MR. JOHNSON: (Inaudible 0:18:36.) The bladder tumors in the female rats.

DR. BELSITO: Page 36 of the PDF, that there appears to be a dose response for the malignant lymphomas in mice. It's from the old report.

DR. SNYDER: Oh, yeah, yeah, yeah. That's (inaudible).

DR. BELSITO: Is that a mouse strain or --

DR. SNYDER: Yeah, it's very high incident so the historical controls, even though they (inaudible) current controls were a little low, the historical incidence is very high, so

--

DR. BELSITO: Okay.

DR. SNYDER: That's okay. Yeah, so I thought, following up on what Dan said at the beginning, that it was insufficient for composition for all except the oil.

SPEAKER: Right, and I think that --

DR. BELSITO: And then depending upon composition, other toxicity endpoints may be needed. And also insufficient for sensitization and irritation for all except the oil and the leaf water. And the oil is safe when formulated to be non-sensitizing.

DR. SNYDER: We got HRT on the leaf extract.

DR. BELSITO: I know. I know. I said, insufficient for all except leaf water.

DR. SNYDER: I have a leaf extract (inaudible).

DR. BELSITO: I have leaf water. Okay, so where are we here?

MR. JOHNSON: Well, we have skin irritations added on --

DR. SNYDER: Leaf extract.

MR. JOHNSON: -- leaf extract.

DR. SNYDER: (Inaudible 0:20:10.)

MR. JOHNSON: Water, ethanol, leaf extract.

DR. BELSITO: We have just irritation on leaf extract?

MR. JOHNSON: Well, this is peppermint leaf extract.

DR. SNYDER: 10%, 100%.

MR. JOHNSON: Yeah, 10% and 100%, but it's extracted with water/ethanol.

DR. SNYDER: Okay. And then we got H (inaudible) HRIPT --

MR. JOHNSON: That's (inaudible).

DR. SNYDER: -- at 100% on a leaf extract.

MR. JOHNSON: And those are (inaudible).

DR. BELSITO: Are undiluted leaf --

MR. JOHNSON: (Inaudible 0:20:34.)

DR. BELSITO: -- extract. Well, I guess I interpreted that as leaf water. (Inaudible) leaf water, oil. Okay. So we have it for leaf water, leaf oil, and leaf extract.

DR. LIEBLER: In the leaf extract we have measurements of some chemical constituents, as reported on PDF 29. But we don't have data that allow us to say that the leaf extract, how similar it is to the oil for which we've got a lot of analyses for. We don't have most of the constituents of concern enumerated in the leaf extract. Either they are not detectable. The closest we come is flavonoids. You know? Just flavonoids. That's --

DR. BELSITO: Mm-hmm.

DR. LIEBLER: That's not really informative because there are a lot of flavonoids, some of which are problematic and many of which are not. And then we get to leaf extract powder. It just says, contains tannin and terpenoid. Okay? That doesn't get us very far. And then pretty much ditto for the leaf. So the leaf, the leaf extract, and the leaf extract powder, you know, if we had good data on any of them, I think that would clear all of them. But we don't really have good data on any of them.

DR. BELSITO: Mm-hmm.

DR. LIEBLER: The leaf water is also very minimal. It just says it contains essential oil. Well, duh. That's how you make it. Right?

DR. BELSITO: Yeah.

DR. LIEBLER: Steam, distill the leaves. So I think those are still problematic in terms of composition.

DR. BELSITO: Yeah, (inaudible).

DR. LIEBLER: We actually have more data on impurities than on composition.

DR. BELSITO: Mm-hmm. Yeah, so insufficient for all except the oil for composition.

DR. LIEBLER: Yeah.

DR. BELSITO: And insufficient for sensitization and irritation, depending upon the composition for all except the oil, the leaf extract, and the leaf water.

DR. LIEBLER: Yeah. And I think if, you know, even if you could say, if you didn't have to quantify all these constituents of concern but you could say, detectable or not

detectable in these, that would allow us to map this back to the oil. And the oil is, you know, pretty well-documented.

DR. BELSITO: Right.

DR. LIEBLER: And so if we can map these back to the oil and provide some reasonable evidence of similarity, then I think we can clear the whole group. It's just we're just not in the reasonable evidence of similarity place right now.

DR. BELSITO: Right.

MR. JOHNSON: Dr. Belsito, will you go over those data needs again so I can --

DR. BELSITO: So composition for all except the oil. And depending upon composition, sensitization and irritation for all except the oil, the leaf extract, and the leaf water. That's it.

MR. JOHNSON: And, Dr. Belsito, with respect to the skin irritation data and sensitization data on PDF page 72, we did not receive complete summaries, just results in the (inaudible). So are those data going to be sufficient with that in mind?

DR. BELSITO: Yeah. I mean, I, well, yeah, I didn't have a problem with that information. I mean, we didn't actually have the -- 72 is concentration of use. What page are you on, Wilbur? I'm sorry.

MR. JOHNSON: I thought it was on page 72.

DR. BELSITO: PDF 72 is Beth's memo on concentration of use.

MR. JOHNSON: Oh, okay.

DR. BELSITO: And we actually had experimental data, as I recall, on all of those.

MR. JOHNSON: I'm sorry, it was PDF 75.

DR. BELSITO: And then that was fine, particularly when coupled with reports that we actually had data on. I didn't have problems with that.

MR. JOHNSON: Okay.

DR. BELSITO: And the GENE-TOX data on page 35, I presume, none of us had problems with that data.

DR. SNYDER: So we're going to continue to reopen the process then? Because this is kind of getting into a --

DR. BELSITO: Well, yeah, I think --

DR. SNYDER: -- (inaudible).

DR. BELSITO: -- we have to reopen because we originally approved, along with peppermint oil, the leave water and we assumed the compositions were going to be the -- I don't know what we assumed back then when we did it, but we approved more than just the oil.

DR. SNYDER: I was surprised because in the original 2001 report, I didn't catch this, but it had some leaf extract and stuff in there.

DR. BELSITO: Mm-hmm. No, I know.

DR. SNYDER: But the report title said only oil.

DR. BELSITO: Right. But so we said they were safe to use. Now we're saying we don't have the information to say that. So I think we have to continue with reopening it because that could change --

SPEAKER: Plus --

DR. BELSITO: -- our entire conclusion. Remember we --

SPEAKER: (Inaudible 0:27:01.)

DR. BELSITO: -- opened -- what?

SPEAKER: It's the reformed (inaudible) in the original (inaudible).

DR. BELSITO: Yeah.

SPEAKER: Yeah.

DR. BELSITO: Because, yes, we're continuing to --

SPEAKER: That's surprising (inaudible).

DR. BELSITO: reopen.

SPEAKER: Yeah, okay.

DR. BELSITO: So again, my question about the GENE- TOX from (inaudible) aberration on the first paragraph on page 35, I'm assuming that's okay.

DR. LIEBLER: Yeah, I didn't have a problem with it. I think that some of these

tests were complicated and did by cytotoxicity of the test agent.

DR. BELSITO: Do we comment? Should we comment that on -- in a discussion or just ignore it?

DR. LIEBLER: We typically haven't, unless there were really, you know, significantly conflicting data.

DR. SNYDER: Well, a lot of times we won't accept it if they don't have the control data to show that there's cytotoxicity occurred here. We have it. So --

DR. BELSITO: Okay. (Inaudible) that.

DR. LIEBLER: I'd actually like to hear what Tom thinks about it, but I think that, you know, in the results or in describing the studies that we do have notations of where, you know, there's evidence of toxicity or inhibition. You know, like, high concentration. For example, the last agent described or last paragraph. I'm sorry, the second paragraph of PDF 35, the last sentence there, and then in the paragraph right above it, the last sentence of that one, too, describes the toxicity of the high doses.

DR. BELSITO: Okay.

DR. LIEBLER: So that's fine. I don't think it needs to be anything special.

DR. BELSITO: Okay. And then, Wilbur, just one other point. In the document on page -- PDF page 3, the second paragraph, you say, regarding the statement and the final report conclusion that the concentration of pulegone and these ingredients should not exceed 1%. The panel considered whether in hindsight their concerns should have been addressed using a non-sensitizing qualification approach. I'm not sure what you meant by that, because our concern with (inaudible) was carcinogenicity, not sensitization.

MR. JOHNSON: Well, that can be revised.

DR. BELSITO: Yeah, I think it --

MR. JOHNSON: But deleted, yes.

DR. BELSITO: I don't even know why that sentence is there. And then that statement should continue to be present in the discussion in the current report that goes forward, because of the presence of pulegone in some fashion.

JAY ANSELL: Right. But we don't think the 1% limit, based on carcinogenicity should continue through because that cancer study had been reviewed and has been -- the conclusions now being attributed to --

DR. LIEBLER: Methanol (inaudible).

JAY ANSELL: -- one artifact of the fixation method (inaudible).

DR. BELSITO: Fine. But it needs to be moved to the discussion and still brought out --

JAY ANSELL: Yeah.

DR. BELSITO: -- number one. Number two, the issue is carcinogenicity, not sensitization.

MR. JOHNSON: So, Dr. Belsito, what --

SPEAKER: Fixation, cause (inaudible).

MR. JOHNSON: -- statement should be included --

SPEAKER: (Inaudible 0:30:38.)

MR. JOHNSON: -- in the discussion, you know, relating to --

SPEAKER: (Inaudible 0:30:42.)

MR. JOHNSON: -- pulegone?

DR. BELSITO: Well, I mean, I think that, you know, there were concerns about its --

SPEAKER: This is (inaudible).

DR. BELSITO: -- carcinogenicity and then --

SPEAKER: (Inaudible).

DR. BELSITO: -- the findings of the recent --

SPEAKER: (Inaudible 0:30:51.)

DR. BELSITO: This was an anti-pea study? I don't remember the pulegone study that was done. It was a two year --

MR. JOHNSON: (Inaudible) NTP. NTP carcinos study.

DR. BELSITO: Right. The findings were that it was due to -- Paul, help me out.

DR. SNYDER: Well, it said that the bladder cancer was cytotoxicity.

DR. BELSITO: Cytotoxicity.

DR. SNYDER: Yeah.

DR. BELSITO: Yeah. So it --

DR. SNYDER: (Inaudible 0:31:12.)

DR. BELSITO: -- it wasn't --

DR. SNYDER: Yeah.

DR. BELSITO: -- a carcinogenic --

DR. SNYDER: No.

DR. BELSITO: -- factor, it was cytotoxic (inaudible).

DR. SNYDER: Yeah.

MR. JOHNSON: Oh, well, in the NTP report, it indicated that a female rat had increased incidences of urinary bladder tumors.

DR. SNYDER: That were due to cytotoxicity. So it wasn't a --

MR. JOHNSON: And what about the benign and malignant tumors of the liver?

DR. BELSITO: Again, hepatotoxicity.

MR. JOHNSON: Oh, okay.

DR. SNYDER: Yeah, there's no cancer in the liver, just some hyperplasias. A liver (inaudible) male/female.

MR. JOHNSON: Because I know the conclusion, NTP's conclusion, stated that the pulegone caused cancer of the urinary bladder in female rats, and cancer of the liver in male and female mice.

DR. BELSITO: Well, it may cause it, but then the question is, what is the mechanism (inaudible).

DR. SNYDER: That's in the second paragraph there, the subsequent --

DR. BELSITO: Is it a genotoxic --

DR. SNYDER: -- (inaudible).

DR. BELSITO: -- mechanism --

SPEAKER: No.

DR. BELSITO: -- or is it a cytotoxic?

DR. SNYDER: It's regenerative self-(inaudible) mode of action. There on the second paragraph.

DR. BELSITO: Okay.

DR. SNYDER: Yeah. And I guess --

DR. KLAASSEN: This has nothing to do with histopathology.

DR. BELSITO: Right.

DR. KLAASSEN: Artifacts.

DR. BELSITO: Yeah. And I guess I just want to raise one issue because, you know, I sort of had forgotten all the parts of the presentation, the botanical presentation, so I had to go back and look at exactly what meristem was. So meristem is when they harvest the small cells from the plant that are capable of dividing indefinitely and giving rise to the similar plants to the south. So the question came in my mind then is, if you're using material from a meristem cell culture, are the potential components of concern present -- could they be present, in higher amounts than what's being reported when you look at simply flower, leaf, stem, twig, bark?

SPEAKER: Sure.

DR. LIEBLER: They could be, but there would be just as plausible an argument that they could be present in lower amounts. And the problem with these cell cultures is they might be partially dedifferentiated to the extent that they don't produce the same repertoire of natural products. And I think our problem with the meristems is we never had any data on these things.

DR. BELSITO: Right. So I mean that's, that's the issue. So --

DR. LIEBLER: Yeah.

DR. BELSITO: -- I mean, I think meristem is something in particular where we

need composition and we can't infer it from composition of the other parts of the plant.

DR. LIEBLER: No.

MS. FIUME: I have to look back at the presentation as well, but I thought meristem was one that you said is specifically on its own, that you can't --

DR. BELSITO: Right.

MS. FIUME: -- use any of the other information in the report.

SPEAKER: Nope.

DR. BELSITO: Good. Well, I --

MS. FIUME: (Inaudible) stuff.

DR. BELSITO: -- was hoping we said that, because when thinking about it, you know, it's basically a cell culture of this pluripotent cell and God knows what comes out of it.

DR. LIEBLER: Right.

MS. FIUME: Yeah.

DR. BELSITO: It's like stem cell research.

DR. LIEBLER: Is there anybody producing that stuff now? Or is it, you know, a great pitch that lasted for a little while and left some ingredients in the dictionary and that's it?

MS. LORETZ: No idea.

SPEAKER: I don't know.

DR. LIEBLER: Okay.

DR. BELSITO: Okay.

DR. LIEBLER: I think I have my answer.

DR. BELSITO: Anything more on --

SPEAKER: You should stop --

DR. BELSITO: -- peppermint?

SPEAKER: -- thinking (inaudible).

DR. BELSITO: Products? Okay. So basically, Wilbur, the oil is fine.

MR. JOHNSON: Mm-hmm.

DR. BELSITO: All the others are insufficient for composition.

SPEAKER: Yep.

DR. BELSITO: And all of the others, except for the oil, the leaf extract, and the leaf water, are insufficient for sensitization and irritation.

SPEAKER: Okay.

SPEAKER: Mm-hmm.

DR. BELSITO: Okay? Okay dokey.

MR. JOHNSON: That statement relating to carcinos and SD, I see it in the summary but it's not in the discussion relating to the mechanism.

DR. BELSITO: Okay. Well, it should be in the discussion. Paul, would you craft some language or Dan?

DR. LIEBLER: Yeah, we can.

DR. BELSITO: Curt?

DR. SNYDER: I did pull up the original report to check out that liver comment, because I don't see that liver was an issue, but Bob will look at that.

DR. BELSITO: So then I guess it should, at some point, there should be a sentence in the panel, you know, at some point about constituents of concern. You talk about the sensitizing constituents, and probably between that and the heavy metals, talk about the pulegone. So just draft some language to put in there.

SPEAKER: Yep.

DR. BELSITO: And then point out the results of the most recent study and, you know, this is cytotoxicity given the concentration or the use concentrations for these peppermint derived ingredients. We're not concerned that this is not genotoxic. It's clearly a cytotoxic dose response relationship.

DR. KLAASSEN: This liver, (inaudible) has to be explained.

DR. SNYDER: Yeah, but it's probably got the high dose, 150 milligrams. They want it pretty high in this study. I'm trying to get to the data on the report here.

DR. KLAASSEN: And I think we're okay with --

MR. JOHNSON: That was --

DR. KLAASSEN: -- (inaudible).

MR. JOHNSON: That was for the mice.

SPEAKER: Mice, yeah.

MR. JOHNSON: For the rats it was up to 75.

SPEAKER: Yeah.

DR. BELSITO: Okay. Anything else for the peppermint? Okay doke. Okay.

Day 1 of the September 11-12, 2017 Panel Meeting – Dr. Marks' Team

DR. MARKS: Any other comments? If not, let's move on to peppermint. So Wilbur, in the April 2017 meeting, the panel reopened the peppermint document to add six derived ingredients and those are list in your memo of extract, flower leaf stem extract et cetera. That meeting the panel issued an insufficient data announcement and requested skin irritation and sensitization data. A compensation method of manufacturing impurities and then there -- was data received?

MR. JOHNSON: Yes.

DR. MARKS: And um.

MR. JOHNSON: We received a use concentration data on the additional six ingredients that were added to the safety assessment. We received method of manufacture, composition and impurities data on the leaf extract. That's the butylene glycol water extract. Another leaf extract which is the water ethanol extract, leaf powder and peppermint leaf water. We also received an invitro skin irritation data on the leaf extract, that's the water ethanol extract. At concentrations of 10 percent and 100 percent. And an HRIPP on the leaf extract, that's the water ethanol extract at a concentration of 100 percent.

DR. MARKS: Okay. So now let's move on. What do we need for this tentative report -- amended report?

DR. EISENMANN: One thing about the original report, you had a limit of pulegone of percent.

DR. MARKS: Yes.

DR. EISENMANN: Um, the basis of that, I think was this brain lesions. There's one more study on that that looked at in another 28-day study determined the brain lesions were a histological effect. So they did a fixing -- it was an error in fixation. So they did fixation using two different methods and they didn't see the brain lesions. So that 1 percent pulegone limit -- I'm not sure where you want to go with that. If you notice the iso standard generally the pulegone is up to 3 percent in peppermint oil. The extracts apparently, they're all within the less than the 1 percent so those don't matter.

MR. JOHNSON: And I might add that adverse effects on the liver are, you know, are noted in that same study by Mulk.

MS. EISENMANN: Right. Generally, it's liver effects not brain effects that have concern for pulegone.

DR. MARKS: So, yeah in 2001, four ingredients are safe with pulegone not exceed 1 percent limit. And that was presumably for the cancer end point. And now we have 5 percent pulegone in the oil. So that and then the other --

MS. EISENMANN: It's up to 3 percent in the oil.

DR. MARKS: Three? I don't know where I got five then.

MR. JOHNSON: That's for sources outside of the United States. For sources within the U.S. it's 2.5 percent.

DR. MARKS: So it's higher than the 1 percent limit that was before. So should we start with that or should we start under needs. Why don't we start with that, Tom, do you think there should be a limit on the pulegone at this point?

DR. SLAGA: (Inaudible).

DR. MARKS: I thought it was but she said it was 2.5., so it's higher than the 1 percent, both sources. I thought it was -- I don't know where I got the 5 percent, page 25. Let me see what it says there. I thought it was five but...

MR. JOHNSON: I know table 3 says, up to 3 percent. DR. MARKS: Pulegone.

MR. JOHNSON: Right. In peppermint oil.

DR. MARKS: Okay. It's still more.

DR. SLAGA: Do any of you have data to support that? Do we have data to support this (inaudible).

DR. JOHNSON: I don't know. I know in the NTP study it reported the doses that were administered.

DR. SLAGA: Yeah.

DR. JOHNSON: But no concentration.

DR. SLAGA: Well, we usually like to have some data to (inaudible) well beyond the 1 percent. Unless -- if we don't I'll just leave it at 1 percent.

SPEAKER: Right.

DR. EISENMANN: But you know now what the 1 percent was based on with these brain lesions and the 28-day study. And there's this -- they did it on the same group.

DR. SLAGA: (Inaudible) there was a problem with fixation, right?

DR. EISENMANN: Correct. I mean, the title of that study is, Lack of Histological Cerebellar Changes in wistar rats given pulegone for 28-days comparison of emersion and profusion tissue fixation, that's the title of the study.

DR. SHANK: The lesions with one of the fixation and not the other?

DR. EISENMANN: That's what I presume, yes. I didn't read the study, I only read what EMEA said about EMEA said about it.

DR. SHANK: Because if they didn't get an even 1 then there's a problem.

SPEAKER: Definitely a problem.

DR. EISENMANN: And this was from the same group that did the original studies.

DR. SHANK: (Inaudible).

DR. MARKS: So you don't want to make a decision yet about the pulegone until you've reviewed those?

DR. SHANK: That's correct.

SPEAKER: I agree.

DR. SHANK: And there's still insufficiency for separations other than the oil.

DR. MARKS: Yeah, I had methods of manufacturer impurities for the extract. I don't know how. I didn't see that data, did I miss that?

DR. SHANK: No.

DR. MARKS: And then I'm sure you'll have more Ron. So that was where I wanted. Now, I figure if we got it for the extract then we're probably no for the rest of it. Because the extracts the total plant. And then I wanted sensitization on the leaf water at 40 percent. The question of that 20 percent leaf water was okay. The leaf extract which I assume would include what's in the leaf water was okay at 100 percent for sensitization.

So I was questioning whether we really needed the leaf water at 40 percent. I could overlook that since we have the leave extract at 100 percent. The extract itself of the whole plant is 7.9 percent and we don't have any sensitization on that. We have again as I mentioned leaf extract, we have an oil, we have the letter but we don't have the whole thing together. So probably the biggest sensitization for me is sensitization for the extract. Does that sound reasonable team?

DR. SLAGA: Yes.

DR. SHANK: Now, there's more than one kind of extract, is it not?

DR. MARKS: Oh yeah, and that's -- and when I said the extract that was -- it was the add-ons and I used the add-on the extract at 7.9 percent. But there was not I'm not talking about the leaf extract and I'm not talking about the leaf cell extract. I'm talking about the extract which I assume is the whole. That's why I want a method of manufacturing also. I kind of used the extract as the sentinel ingredient. And then maybe I missed it but in this spread sheet I didn't see the 6th added ingredients on there. There's not much data on the 6th extra ingredients. Factors little if anything other than method of composition of the flower leaf stem extract.

DR. SLAGA: So that's only sensitization data (inaudible).

DR. MARKS: Yeah, basically, -- well I wanted to have the method of manufacturing of purities for the extract. Because I think we should know what's in the extract assuming that's everything. And then the sensitization for the extract and those were the two I needed. And then we need to clarify the pulegone.

DR. SHANK: Pulegone, yeah.

DR. MARKS: And it's kind of interesting because I say I like data, but with botanicals we always put formulate to be non-sensitizing. So one could say if you have that as your conclusion do you really need this. I would like to see it.

DR. SHANK: But would that apply to pulegone?

DR. MARKS: No. So tomorrow I'm going to move -- shall we --

DR. SHANK: Insufficient.

DR. MARKS: Um-hum. Insufficient data announcement and we want the sensitization. And then how do you want the pulegone? Clarify the pulegone studies or review it or we can just -- that's really not a need.

DR. SHANK: I'll email it to them (inaudible).

SPEAKER: (Inaudible).

DR. MARKS: So I'll put in there pulegone limit question mark 1 percent that's mentioned that. Okay.

DR. SHANK: That's been in several reports is it not? The pulegone (inaudible).

SPEAKER: (Inaudible).

MS. FIUME: Yes.

SPEAKER: They change it.

DR. SHANK: But you can't base it on something that's not real.

DR. SLAG: That's not right.

DR. MARKS: Okay. Any other comments? So on insufficient data announcement I'll move tomorrow. We want the extract method of manufacturing and impurities and sensitization data. And then we want to clarify the pulegone limit of 1 percent.

SPEAKER: Alright.

MR. JOHNSON: Excuse me. Doctor Marks, this will be a tentative report with an insufficient data conclusion. Because an insufficient data announcement was issued at the April meeting. So this would be the tentative report.

DR. MARKS: Thank you.

MR. JOHNSON: You're welcome.

DR. MARKS: I had that before, tentative report.

DR. EISENMANN: Now, should a more comprehensive search be done on pulegone, rather than just um, just a question. I'm not sure you used pulegone as a search term Wilbur.

MR. JOHNSON: No, I did not. No, I did not. I just spoke -- I was aware of the NTP carcinogenicity study. And that's why I included those data in the safety assessment.

DR. EISENMANN: And maybe you don't need necessarily go back. I mean, well except this one study is older. I was just going to say maybe since the NTP bio essay might be helpful to look at if anything new on pulegone.

SPEAKER: When was that study done, the NTP.

MR. JOHNSON: And there's a brief summary of that in the safety assessment.

DR. EISENMANN: It's not too old.

MR. JOHNSON: That was done in 2011.

DR. MARKS: So are we going to -- if we're issuing a tentative report then we're saying it's safe for the other -- what is it nine. Are there ten ingredients total? The original four and then we added six. So safe for nine ingredients and an insufficient for the extract, does that sound reasonable?

DR. SHANK: Yes.

DR. MARKS: And then clarify the pulegone limit. Okay, does that sound -- I see you thinking Ron Shank.

DR. SHANK: Well, I was just wondering should a search be done, specifically for pulegone or with the NTP study always has preliminary data in it so that they can find the dose. For the carcinogenicity study. they always do preliminary short term studies. If that's available that should help us. Because if we find out 1 percent --

SPEAKER: They don't usually like to release it separate --

DR. SLAGA: They did a two week.

SPEAKER: -- because want to look at totality of all of the studies before they -- I don't if the...

DR. EISENMANN: I was just wondering since the NTP bio essay was done if you want to for like, mechanistic type data that might be useful. Rather than the post (inaudible) I was just going to suggest since the NTP bio acid came out that might be helpful.

MR. JOHNSON: They did do a two study and a three- month study prior to the two- year study.

DR. MARKS: And those were oral?

MR. JOHNSON: Oral studies.

DR. MARKS: Um-hum. Any other comments? Okay.

Day 2 of the September 11-12, 2017 Panel Meeting – Full Panel

Moving on to the next ingredient in this group, and that's peppermint, Dr. Marks presenting.

DR. MARKS: So at the April 2017 meeting, the panel agreed to open the peppermint document from 2001 to add six more peppermint-derived ingredients. We issued an insufficient data notice, or announcement, for skin irritation, sensitization, composition, method of manufacture, and impurities.

We did receive some data and our team moves that a tentative report be issued "safe for nine ingredients when formulated to be non-sensitizing" and insufficient for the extract. We wanted the method of manufacture and impurities for the extract. And despite the conclusion to "formulate to be non-sensitizing," we also wanted to see sensitization data on the extract at its use concentration of 7.9 percent. In the discussion, we clarify that a pulegone limit of 1 percent, which was in the original document from 2001.

DR. BERGFELD: And that's a motion?

DR. MARKS: Yes.

DR. BERGFELD: Is there a second, or a discussion?

DR. BELSITO: Yeah. So we looked at this and we were shocked that we actually had allowed the other non-oil ingredients to be found safe as used back then because we have absolutely no composition for any of them except the oil. We have no clue what's in any of them.

So we found it was insufficient for composition for all except the oil. And depending upon the composition, other toxicologic endpoints may be needed. And we thought it was insufficient for sensitization and irritation for all except the oil, the leaf extract, and the leaf water which we had data on.

DR. BERGFELD: Comment.

DR. MARKS: What were that sensitization again? Which ones did you say we have?

DR. BELSITO: We had sensitization and irritation for oil, leaf extract, and leaf water. So those three are fine. But all of the other ingredients in the report -- so the flower leaf stem, flower leaf extract, flower leaf stem water, leaf cell extract, leaf juice, and the meristem cell culture.

DR. MARKS: Yeah. I guess my reasoning was if we have sensitization data on the extract, that would include components from the whole plant, assuming that that's what the extract is. And if we had sensitization data on that which was -- would be --

DR. BELSITO: Well, we have data for the leaf extract and the leaf water.

DR. MARKS: Right.

DR. BELSITO: We don't have data on the stem and --

DR. MARKS: Oh, I agree. We can ask for all that. I would just be reassured if I had the extract that that would include all the other components of the plant and if we had that, we could --

DR. LIEBLER: Depending on whether that's actually true.

DR. MARKS: Exactly. Well, that's why --

DR. LIEBLER: And it's a circular problem. We don't know what the method of manufacture is, we don't know what's in it to make that assumption.

DR. MARKS: That's correct. That's why we asked for that specifically -- method of manufacture. But sure, I think we can go out with your team's request and --

DR. BERGFELD: Can you restate the motion, then?

DR. BELSITO: So it's "insufficient for composition for all except the oil," and depending upon the composition, we got "other toxicologic endpoints may be needed." And "insufficient for sensitization and irritation" for all except the oil, the leaf extract, and the leaf

water.

DR. BERGFELD: So, if this is going to have to go out, it's a draft tentative amended report, so how will we send this out? As an insufficient data report?

DR. HELDRETH: We could send it out as an IPS.

DR. BERGFELD: Okay.

DR. SLAGA: (Inaudible) for insufficient data.

DR. BERGFELD: Yeah. It's -- yeah. Okay. All right. Is there a second on Don's motion? Okay. And Wilbur?

MR. JOHNSON: What are we saying about the prior 1 percent concentration limit on pulegone?

DR. BELSITO: Jim addressed that. We're keeping that in the discussion.

MR. JOHNSON: Okay. Now, I know that was based upon -- in the original report, it was based upon concerns about the regions in the brain and the effect on the liver. Should those concerns be addressed in the discussion?

DR. BELSITO: I mean, it's -- yeah. I mean, those can be addressed, sure.

MR. JOHNSON: What should we be saying about those? And the carcinogenicity of the pulegone?

DR. BELSITO: Right. I mean that, you know, it's been the --

DR. SNYDER: Yeah. (Inaudible) in the rat. And so we can address that in the discussion.

MR. JOHNSON: What should we --

DR. BELSITO: Wilbur's asking specifically the wording for the --

DR. SNYDER: Well, that the endpoints evaluated in the NTP study were attributed to renal toxicity and the liver toxicity. Okay.

DR. SLAGA: Just for the liver, now.

DR. SNYDER: Yeah.

DR. BERGFELD: What did you say, Tom?

DR. SLAGA: Just for the liver. We don't need the brain stuff because of that complication.

MR. JOHNSON: So the cerebellum region should not be mentioned at all in the discussion?

DR. SLAGA: I don't think it's needed.

DR. BERGFELD: Any other comments about the cerebellum brain region? No it's not needed.

So we want to call the question, then, if there are no other comments on an insufficient data report.

All those in favor, indicate by raising your hands. And the list of needs have been stated, I believe.

(The motion passed unanimously.) Okay.

Day 1 of the December 4-5, 2017 CIR Expert Panel Meeting – Dr. Belsito's Team

DR. BELSITO: Peppermint derived ingredients. So at the September meeting, we issued a tentative amended report with the conclusion that peppermint oil is safe in cosmetics in the present practice of use in concentration described in the safety assessment when formulated to be non-sensitizing, but the available data were insufficient for the nine other peppermint derived ingredients. We wanted composition data on all the ingredients. We wanted, depending upon the concentration data, other toxicologic end points. We got data in both Wave 1 and Wave 2 -- or we got new data that was submitted before we got these and then a bunch of data that came to us and Wave 2, so I'm not going to go through all that data. But in looking at the report, does that direct data that we received since our last meeting meet our needs?

DR. LIEBLER: I thought we're okay on peppermint extract and the leaf stem stuff, but anything with the flower in it we don't have, nor the meristem, of course, which we never get. But the flower-containing ingredients, I don't know if we can interpolate from the leaf stem to flower, so I felt that our needs had not been met for the flower -- the two flower containing ingredients.

DR. BELSITO: I think I agreed with you. I'm trying -- on page 57, yes. Dan, you said looking at the ingredients that were in the report, we're saying that it's insufficient for the flower leaf stem extract, the flower leaf stem water, and the meristem cell culture?

DR. LIEBLER: Yes.

DR. BELSITO: So that's what I said as well that all of the leaf extracts and the whole plant -- or the peppermint extract were safe, so you included the peppermint extract?

DR. LIEBLER: Yes, I did.

DR. BELSITO: The data that we still needed were composition method of manufacturing physical properties. Depending upon that additional toxicity data for the flower leaf stem extract, the flower leaf stem water, and the meristem cell culture?

DR. LIEBLER: Right, same place.

DR. BELSITO: Curt, Paul?

DR. SNYDER: That's fine.

DR. BELSITO: So in the introduction, Wilbur, the second paragraph, it says that the panel noted that much of the data on this ingredient, that being the oil, considered relevant to the entire group. Well, clearly that wasn't the case, because we eliminated it. So that sentence needs to be deleted, that's PDF page 41. That's it, that's the only other comment I had. In the discussion formulated to be non-sensitizing, the specific -- some names of some specific individual composition materials that could give issues in peppermint oil and leaf, that's already here, penetration enhancement to heavy metals, usual -- I guess the discussion is pretty -- already very robust, so really just a change in the conclusion.

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DR. MARKS: Okay. Any other comments? If not, we'll move on to our next ingredient which is peppermint, mentha piperita. Okay. So this is a draft final amended report. At the September meeting this year, the panel issued a tentative amended report with a conclusion that insufficient to make a determination of safety for nine and safe for the tenth. Let me see -- We have new data on an extract, a leaf extract. We don't know if the extract contains a whole plant.

DR. SHANK: Yes, it said it was the whole plant.

MR. JOHNSON: The leaf extract data received on the leaf extract and the whole plant extract.

DR. MARKS: So the extract is the whole plant. Okay. Well I changed some of the things around. Like we could increase the number that are safe. The question is can we do them all.

DR. SHANK: I think we can.

DR. MARKS: So I had we could extend to all the leaf ingredients we could say are safe. How about the flower, leaf stem and what are the flower, leaf, stem extract and the meristem cell culture?

DR. SLAGA: That's part of the whole plant.

DR. MARKS: Yeah, and that's the whole plant so that's where I was looking at Don's comments. The last time I hadn't realized we now had the extract -- that the extract is the whole plant because we know the extract is safe. So the final conclusion would be safe when formulated to be non-sensitizing?

DR. HILL: I have a question about that though because the extract is in use up to 7.9 percent if that's actually accurate. But they did, for example, the genotoxicity and there was something else where they used a 10 percent dilution of that one and then there was 2.5 percent extract but it was tested at 10 percent dilution so then you're at 25 percent. So when you look down more carefully at what they did and what concentration ranges they've actually covered I think we're really not covered.

DR. SHANK: Well the leaf water is used up to 40 percent.

DR. HILL: But leaf water is -- and I'm a little unclear about that. I don't remember which definition they used in here because I thought leaf water was you put the plant in a sauna and then pass vapor through and keep collecting that vapor. But now I'm wondering is it steam distillation happens and then the aqueous is the leaf water? Is that --

DR. EISENMAN: Yes.

DR. HILL: Okay. So the oil goes one way and the water that's left is the water.

DR. EISENMANN: Yes.

DR. HILL: Okay.

DR. EISENMANN: And that's how it's supposed to be, yes.

DR. SHANK: And that could be covered by just saying formulated -- it is a botanical so formulated to be non-sensitizing.

DR. MARKS: That's what we put on all the botanicals now and actually it gets into some of these others because if sensitization is the only significant issue remaining that we just -- this is nice because we actually have a fair amount of sensitization data on here at significant concentrations:

percent, the leaf extract at 100 percent so -- I think we still, though, the reasoning with non-sensitizing in the conclusion is that if you take several botanicals, put them together and the component which is sensitizing (inaudible) is present in all the botanicals you potentially could get above the threshold of sensitization. So tomorrow we'll second a -- this has got to be another tentative amended report, because we're shifting a number -- we're making them all safe now, correct?

MR. JOHNSON: Correct.

DR. MARKS: I don't see how we can do this and not send it out again but I don't know. Give me your -- that to me seems like a significant change that we go from insufficient to safe on nine of the ingredients.

DR. SLAGA: Well we got all that new data.

DR. MARKS: Yeah, it's accurate. But I think procedurally we need to - -

DR. SLAGA: It has to go out again?

DR. MARKS: Yeah. Do we need to send it out again or not?

DR. EISENMANN: Up to you.

DR. HELDRETH: Technically it's less restricted so your hand isn't forced, but if you feel like it's a really significant change and you would like - -

DR. EISENMANN: Or because the data is in separate - - I mean it came in so it couldn't be in your version and if you want us to read the whole report with the new information, that might be a reason to.

DR. SHANK: Is it more restrictive now?

DR. HILL: No.

DR. SHANK: No, it's less restrictive but it's a significant change in the conclusion from insufficient to now sufficient and safe. I would think it would have to go out again.

DR. HELDRETH: It's a significant amount of new data that's going to need to be moderated and explained and especially why that data - -

DR. MARKS: Yeah, that's what I had in mine. We had to issue another tentative amended report and the conclusion would be all these ingredients are safe when formulated to be non-sensitizing. And then I think it's going to be really important that, and again, the extract is used to support some of these others like the mixture of the flower, leaf, and stem and the meristem. Okay, second another tentative amended report, all safe when formulated to be non-sensitizing. Hopefully the Belsito team will feel similarly and if not, we will reconcile the differences between the house and the senate.

DR. HILL: Good luck with that.

DR. MARKS: Wilbur, you wanted to make a comment I thought, at least I read that non-verbal communication.

MR. JOHNSON: So basically you're saying that the new data on the whole plant extract are sufficient for evaluating the safety of all the ingredients for which data were not received.

DR. MARKS: Yeah and actually that's now at this point very little, because it was really in my mind only the stem - - what was it? The flower, leaf, stem, those two ingredients and the meri cells were the only which we didn't have good irritation or sensitization, but now that we can read across so to speak with the extract, I'm fine.

DR. SHANK: The only argument would be concentration in leaf water at 40 percent.

DR. MARKS: Yeah but the leaf water at 40 percent - -

DR. HILL: That's very little, if anything.

DR. MARKS: Yes and we have an HRIPT which of course percent is half the concentration but still it was clean.

And we had the extract, the leaf extract which I would think had everything in it not diluted with a water and 100 percent is okay in the HRIPT.

DR. SHANK: Okay, I understand.

DR. HILL: Well okay. This whole extract thing is really bugging me because if you look at - - I don't know if you can get to page 43 quickly. I'm looking at the leaf extract.

DR. MARKS: But is it in this or - -

DR. HILL: NO.

DR. EISENMANN: If it's the CO2 with the extract - -

DR. HILL: No, this is not CO2. This is where they used multiple solvents like petroleum, dichloromethane,

(inaudible) and butanol and water, I presume they combined all of that. So then what they say is they've got eriocitrin at 383 milligram per gram of extract. And rosmarinic acid at 381 milligram per gram of extract so basically out of the one gram of extract, 700 milligrams is - - more than 700, 750 milligrams is these two things. But then when you look at the testing of all of them, it's always diluted so that's the whole issue. Some of the extracts are 50 percent are butylene glycol water - -

DR. MARKS: But there had to be one because I had - - undiluted.

DR. HILL: But when they say extract undiluted, but you don't know what the concentration of that extract is, that's the problem. They're not testing that clearly because that would be a solid. And some of these other ones they tested at 10 percent dilution so it's a little scruff. I mean, I still think everything's fine. But there's a lot of places in here where there's complete lack of clarity in terms of how much substance we actually have because there's nothing that came to standardization.

DR. MARKS: The clear part is the conclusion formulated not to be sensitizing.

DR. HILL: As long as you're not worried about genotoxicity.

DR. SHANK: Yeah.

DR. HILL: That's what I'm - -

DR. MARKS: Oh, now you're talking about genotoxicity.

DR. HILL: Well because of the way they did that testing on some of these,
that's - -

DR. SHANK: Are you the whole plant extract this testing now?

DR. HILL: I'm not concerned, I just - - again, the lack of clarity is bothersome.

DR. MARKS: Is peppermint grass?

DR. EISENMANN: Yes.

DR. MARKS: That's what I thought.

DR. HILL: And what I was pointing out in part is that the composition of the
leaf extract is very different from the composition of the oil. And I think there was some
discussion that these were - -

DR. EISENMANN: And to know what you're extracting with because the CO2
extract is - -

DR. HILL: Yeah. But that's because of - -

MR. JOHNSON: Dr. Marks, the concern about sensitization potential was
based upon constituents of concern before, and not really a concern about the sensitization
potential of the individual ingredients. But now that we have sensitization data on the ingredients,
should anything be mentioned in the discussion about the sensitization potential of the individual
ingredients?

DR. MARKS: Don't we have a standard way of expressing that? I thought in
the other botanicals there's a paragraph where we have a standard way of saying when multiple
botanicals are mixed together potentially - -

MR. JOHNSON: Right, it's in the safety assessment.

DR. MARKS: That's what I thought so I think that answers it, Wilbur.

MR. JOHNSON: Okay.

DR. MARKS: Okay. Any other comments? So tomorrow I'll second a move that another
tentative amended report be issued that all these ingredients are safe when formulated to be
non-sensitizing. Okay. I don't think the team needs specific comments from you.

Day 2 of the December 4-5, 2017 CIR Expert Panel Meeting – Full Panel

So moving on to the next ingredient, which is peppermint. Dr. Belsito.

DR. BELSITO: Yes. So at the September 2017 meeting we issued a tentative amended report for public comment with conclusion that peppermint oil was safe in cosmetics in the present practice of use and concentration described in the safety assessment formulated to be non sensitizing. However, the available data for the other materials in this report were insufficient. I won't read all the various insufficiencies that we listed.

Prior to receiving these documents we received quite a bit of data and then subsequently we received more data in wave two. And based upon that data our group felt that we could go with the safest use for the oil, the leaf extract, the leaf water, the extract, the leaf cell extract and the leaf juice when formulated to be non sensitizing. And the others those would be the flower, leaf, stem extract. The flower leaf stem water and the meristem cell culture would remain insufficient for composition sensitization and irritation.

DR. BERGFELD: Is that a motion?

DR. BELSITO: That's a motion.

DR. BERGFELD: Jim, do you want to comment on it?

DR. MARKS: Our team similarly agree with the increased number of ingredients were safe. However, we felt since we had the data on the extract and we know the extract is composed of the whole plant that we could move forward saying all are safe when formulated to be non sensitizing. We didn't think that the flower stem water or the flower leaf stem extract and water and the cell meristem cell culture would be a concern. And as far as needing sensitization data we have in the conclusion formulated to be non sensitizing. So that was our reasoning to go ahead and declare all safe.

DR. BELSITO: Dan, you want to comment?

DR. LIEBLER: Yea, so my concern had to do with the flower which arguably, is going to have a different composition and I thought about the approach that you just described using the peppermint extract sort of not really carefully defined as a sort of overall extract. But the problem is I don't know that this extract is prepared from flowering plants, for example. And I just didn't feel like the flower was accounted for. And then the meristem is our usually meristem black hole where we have no idea in culturing these cells to differentiate, dedifferentiate god only knows what they are producing relative to the plant. Two different reasons for me view. Perhaps a little overly cautious on the flower, peppermint flower, but you know, so that's -- put that out there for your consideration.

DR. MARKS: Okay. Second the motion?

DR. BERGFELD: Do you second the motion? And so would you redefine those that are unsafe or restate them -- insufficient excuse me.

DR. BELSITO: The flower, leaf, stem extract. The flower, leaf, stem water. Flower, leaf, stem extract. Flower, leaf, stem water and the meristem cell culture.

DR. BERGFELD: Thank you. Anything else that needs to be added at this time before I call for the vote? Any other comments? Seeing none, I'll call the vote. All those in favor of this conclusion thank you unanimous.

Amended Safety Assessment of *Mentha piperita* (Peppermint)-derived Ingredients as Used in Cosmetics

Status: Draft Final Amended Report for Panel Review
Release Date: February 9, 2018
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The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst.

ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of *Mentha piperita* (peppermint)-derived ingredients; most of the ingredients function as fragrance ingredients and/or skin conditioning agents in cosmetic products. Because final product formulations may contain multiple botanicals, each containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should continue to use good manufacturing practices to limit impurities that could be present in botanical ingredients. The Panel reviewed data relevant to the safety of these ingredients and concluded that *Mentha Piperita* (Peppermint) Oil, Leaf, and leaf-derived ingredients are safe in cosmetics in the present practices of use and concentration, when formulated to be non-sensitizing, and that the available data are insufficient to make a determination that the remaining 3 ingredients are safe under the intended conditions of use in cosmetic formulations.

INTRODUCTION

The safety of four of the cosmetic ingredients named in this safety assessment has been previously reviewed by the Panel; in 2001, the Panel issued a final report with a conclusion stating that *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water are safe as used in cosmetic formulations.¹ The conclusion also stated that the concentration of pulegone, a constituent of these botanical ingredients, should not exceed 1%. In accordance with its Procedures, CIR evaluates the conclusions of previously-issued reports every 15 years, and therefore a re-review was initiated. The new conclusion reached in this re-review supersedes the original conclusion. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI Dictionary), most of these ingredients are reported to function as fragrance ingredients and/or skin conditioning agents in cosmetic products.²

In addition to the four *Mentha piperita* (peppermint)-derived ingredients that are mentioned above, this re-review included 6 related previously unreviewed ingredients. The complete list of ingredients included in this assessment is:

<i>Mentha Piperita</i> (Peppermint) Extract	Mentha Piperita (Peppermint) Leaf Extract
<i>Mentha Piperita</i> (Peppermint) Flower/Leaf/Stem Extract	<i>Mentha Piperita</i> (Peppermint) Leaf Juice
<i>Mentha Piperita</i> (Peppermint) Flower/Leaf/Stem Water	Mentha Piperita (Peppermint) Leaf Water
Mentha Piperita (Peppermint) Leaf*	<i>Mentha Piperita</i> (Peppermint) Meristem Cell Culture
<i>Mentha Piperita</i> (Peppermint) Leaf Cell Extract	Mentha Piperita (Peppermint) Oil

*Previously reviewed ingredients are indicated in [blue](#).

The cosmetic ingredient names, according to the *Dictionary*, are written as above, i.e., capitalized, without italics, and unabbreviated. When referring to the plant from which these ingredients are derived, the standard scientific practice of using italics to identify genus and species will be followed (e.g., *Mentha piperita*).

This safety assessment includes relevant published and unpublished data for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A list of the typical search engines and websites used, sources explored, and endpoints that CIR evaluates, is available on the CIR website (<https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites>; <https://www.cir-safety.org/supplementaldoc/cir-report-format-outline>). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Safety test data that have been found in the published literature or those provided by the Personal Care Products Council (Council) as unpublished data since the final report was issued, are included. Some safety test data on menthol, menthone, and pulegone are also included in the original published final report; these data and additional data are presented in Table 1. Considering that a limitation on pulegone is mentioned in the original conclusion, it should be noted that a National Toxicology Program (NTP) oral carcinogenicity study with positive results on pulegone was published in 2011³ and that a 1998 publication on the absence of test substance-related

histological cerebellar changes in Wistar rats dosed orally with pulegone is available.⁴ These studies are also presented in Table 1.

Excerpts from the 2001 safety assessment on the previously reviewed ingredients are disseminated throughout the text of this re-review document, as appropriate, and are identified by italicized text. For complete and detailed information, please refer to the original report, which is available on the CIR website (<https://www.cir-safety.org/ingredients>).

CHEMISTRY

Definition and General Characterization

Collectively, the botanical ingredients reviewed in this safety assessment are derivatives of the leaf, stem root, and whole *Mentha piperita* herb. The definitions of *Mentha piperita* (peppermint)-derived ingredients are stated in Table 2.²

Chemical and Physical Properties

Properties/specifications relating to *Mentha piperita* (peppermint)-derived ingredients are presented in Table 3.^{1, 5, 6, 7}

Method of Manufacture

Mentha Piperita (Peppermint) Oil

European and American peppermint oil is distilled with steam from the fresh, above-ground parts of the flowering plant Mentha piperita Linne, rectified by distillation and not dementholized.¹ It has been reported that the menthone content decreases while the menthol content increases in peppermint leaves upon storage for 1 to 2 months, at 22°C to 24°C. However, the relative menthone to menthol proportion remained practically constant during the total storage time.

According to one source, Mentha Piperita (Peppermint) Oil has been extracted (distilled; water solvent) from the leaves of *Mentha piperita* harvested (first in July and second harvest in September) in Washington state.⁸

Mentha Piperita (Peppermint) Extract

According to one source, the main steps in the process of manufacturing a trade name mixture defined as an aqueous solution containing 7.5% Mentha Piperita (Peppermint) Extract are: solubilization of *Mentha piperita* in water, separation of soluble and insoluble phases, and filtration and sterilizing filtration.⁹

Mentha Piperita (Peppermint) Leaf Extract

The following method relates to preparation of the butylene glycol/water extract of Mentha Piperita (Peppermint) Leaf Extract.¹⁰ Dried raw material is extracted with 50 vol% 1,3-butylene glycolic solution. After extraction, the additional steps in the production process include: filtrate → sedimentation → filtrate → adjustment → packaging.

In another method, the preparation of a water/ethanol extract is described.¹¹ Dried raw material is extracted with 30 vol% ethanol solution. After extraction, the additional steps in the production process include: filtrate → concentration → adjustment → sedimentation → filtrate → adjustment → packaging.

The production method described herein relates to preparation of Mentha Piperita (Peppermint) Leaf Extract (powder form).¹⁰ According to the method of production, dried raw material is extracted with 30 vol% ethanol solution. After extraction, the additional steps in the production process include: filtrate → concentration → add exsiccated sodium sulfate as vehicle → drying → packaging.

In the supercritical fluid extraction with natural carbon dioxide production method for *Mentha Piperita* (Peppermint) Leaf Extract from the dried leaves of *Mentha piperita*, neither additives nor other technical adjuncts are introduced during the production process.¹²

Mentha Piperita (Peppermint) Leaf Water

In the preparation of *Mentha Piperita* (Peppermint) Leaf Water, dried raw material is subjected to steam distillation.¹⁰ After distillation, the remaining steps in the production process are: water soluble fraction obtained → adjustment → filtrate → packaging.

Composition and Impurities

Pulegone is found in young peppermint leaves, and is metabolized to menthol as the leaves mature. It has also been reported that pulegone is found only in Mentha Piperita (Peppermint) Oil from young plants and in trace amounts in “inferior” oils; pulegone is absent from “good quality” Mentha Piperita (Peppermint) Oil.¹ However, a supplier of Mentha Piperita (Peppermint) Oil reported pulegone concentrations of 1% to 4%, depending on the origin of the oil. Published studies that have investigated the pulegone content of Mentha Piperita (Peppermint) Oil also reported a range of <1% to 4% for Mentha Piperita (Peppermint) Oils of a North American origin.

Mentha Piperita (Peppermint) Oil

The major constituents of *Mentha Piperita* (Peppermint) Oil include the terpenes: (-)-menthol (30 - 55%), (-)-menthone (14 - 32%), (+)-isomenthone (1.5 - 10%), (-)-menthyl acetate (2.8-10%), (+)-menthofuran (1.0 - 9.0%), and 1,8-cineol (3.5 - 14%).¹³

Certain trends were observed between oil extracted from first and second harvest leaves, and oil extracted from fresh leaves versus dried leaves.⁸ When compared to the second harvest, oils from the first harvest were generally higher in (Z)-3-hexenol, 1,8-cineol, α -pinene, β -pinene, sabinene hydrate, isomenthone, menthofuran, pulegone, β -caryophyllene, and germacrene d, but lower in limonene, menthol, and menthone. When compared to oils from dried leaves, oils from fresh leaves were higher in 1,8-cineol, α -pinene, limonene, isomenthone, menthofuran, menthone, and pulegone and lower in β -caryophyllene, germacrene d, and menthol. Menthyl formate was found in all of the *Mentha piperita* (peppermint) oils (from leaf extraction) that were analyzed.

Major components of the essential oil of *Mentha piperita* adult plants from Poland include menthone, menthol, menthyl acetate, carvone, piperitone, 1,8-cineol, and pulegone.¹⁴

According to the United States Pharmacopeial Convention's (USP) Food Ingredients Expert Committee, the acceptance criteria for *Mentha Piperita* (Peppermint) Oil include not less than 5% total esters (calculated as menthyl acetate) and not less than 50% menthol.⁷

The international standard for *Mentha Piperita* (Peppermint) Oil, published by the International Organization for Standardization, contains the chromatographic profile for this ingredient that is presented in Table 4.¹⁵ Pulegone and menthofuran were among the chemicals detected. A public statement from the European Medicines Agency on the use of herbal medicinal products containing pulegone and menthofuran indicated that *Mentha Piperita* (Peppermint) Oil contains a maximum of 4% pulegone and between 1% and 9% menthofuran.¹⁶ It was also noted that the Scientific Committee on Food (SCF) has concluded that pulegone is mainly metabolized through pathways involving menthofuran and that these two substances show similar toxicity.

Mentha Piperita (Peppermint) Extract

Mentha Piperita (Peppermint) Extract (combined in a trade name mixture) is an aqueous solution composed of 7.5% (maximum percentage) *Mentha Piperita* (Peppermint) Extract, with < 40 ppm pulegone and < 50 ppm menthol.⁹ The following statement relating to composition was also provided: “Our active can be divided in sugars (47%), mineral ashes (38%), proteins (13%), and polyphenols (2%).” Composition data on this trade name mixture also include the following impurities: alkaloids (< 0.05 g/l; assay of alkaloids performed with Dragendorff reagent), copper (0.23 ppm), iron (3.76 ppm), manganese (21 ppm), nickel (0.19 ppm), and zinc (3.14 ppm). In an assay of

allergens, no allergens were detected in this aqueous solution (i.e., the concentrations were less than the sensitivity of the method (< 1 ppm)). There also was no trace of pesticides in this aqueous solution.

The following information relating to impurities is included in a certificate of analysis for a trade name mixture containing 2.5% *Mentha Piperita* (Peppermint) Extract: lead (< 10 ppm), arsenic (< 3 ppm), mercury (< 1 ppm), and pesticide residues (meets USP specification).⁶

***Mentha Piperita* (Peppermint) Leaf**

The major monoterpene constituents of *Mentha Piperita* (Peppermint) Leaf are: (-)-limonene; 1,8-cineol; (+)-pulegone; (-)-menthone; (+)-isomenthone; (+)-menthofuran; (-)-menthol; and (+)-neomenthol.²⁰ *Mentha Piperita* (Peppermint) Leaf also contains caffeic acid, rosmarinic acid, and the following flavonoids: apigene-, diosmetin-, and luteolin glycosides, and free lipophile methoxylized flavones such as xanthomicrol and gardenine D.²¹

The following elemental contaminants have been detected in *Mentha piperita* herbal tea (tea leaves) samples (n = 3) from Serbia: manganese (111.97 mg/kg dry weight), iron (443.90 mg/kg), copper (17.15 mg/kg), and zinc (26.86 mg/kg), molybdenum (2.695 mg/kg), cobalt (0.161 mg/kg), nickel (1.882 mg/kg), selenium (0.107 mg/kg), aluminum (554 mg/kg), and tin (3.66 mg/kg).²²

It is possible that pesticide residues may be present as impurities in the leaves of *Mentha piperita*. In a study in which *Mentha Piperita* (Peppermint) Leaves were soaked in pesticides, the dissipation rate of pesticide residues during the drying process was said to have been satisfactory, except for the pirimiphos-ethyl pesticide, because of its high octanol-water partition coefficient and low vapor pressure.²³

***Mentha Piperita* (Peppermint) Leaf Extract**

An analysis of *Mentha Piperita* (Peppermint) Leaf Extract indicated that the leaves principally contained cinnamic acid, caffeic acid, rosmarinic acid, and various flavonoids (flavones and flavanones).¹⁷ The following solvents were used to extract the peppermint leaves: light petroleum, dichloromethane, acetonitrile, ethyl acetate, methanol, *n*-butanol, and water. Eriocitrin (383.3 ± 2.2 mg/g extract) and rosmarinic acid (381.2 ± 1.9 mg/g extract) were the most abundant components identified within the leaves, while naringenin-7-*O*-glucoside (0.8 ± 0.01 mg/g extract) was the least abundant component identified. Kynurenic acid (3.82 ± 0.46 µg/g) has also been detected in *Mentha Piperita* (Peppermint) Leaf Extract.¹⁸ It should be noted that kynurenic acid is a constituent of human synovial fluid.

Composition data provided by the Council indicate that *Mentha Piperita* (Peppermint) Leaf Extract (butylene glycol/water extract) contains tannin and terpenoid (which contains 2.8 ppm pulegone) and that *Mentha Piperita* (Peppermint) Leaf Extract (water/ethanol extract) contains essential oil, tannin and terpenoid.¹⁰

Data on impurities provided by the Council indicate that *Mentha Piperita* (Peppermint) Leaf Extract (butylene glycol/water extract) contains not more than 10 ppm heavy metals and not more than 2 ppm arsenic.¹⁰ *Mentha Piperita* (Peppermint) Leaf Extract (water/ethanol extract) contains not more than 10 ppm heavy metals and not more than 1 ppm arsenic.

The supercritical fluid extraction with natural carbon dioxide production method for *Mentha piperita* (Peppermint) Leaf Extract from the dried leaves of *Mentha piperita* results in no solvent residues, no inorganic salts, no heavy metals, and no reproducible microorganisms.¹²

According to data provided by the Council, *Mentha piperita* (peppermint) leaf extract powder contains not more than 10 ppm heavy metals and not more than 2 ppm arsenic.¹⁰

A chromatographic profile for *Mentha Piperita* (Peppermint) Leaf Extract (CO₂ extract) is presented in Table 4.^{5,19}

Mentha Piperita (Peppermint) Leaf Water

Composition data provided by the Council indicate that *Mentha Piperita* (Peppermint) Leaf Water contains essential oil (menthol).¹⁰ The data also included specifications that indicate that *Mentha Piperita* (Peppermint) Leaf Water contains not more than 10 ppm heavy metals and not more than 1 ppm arsenic.¹

USE Cosmetic

The safety of *Mentha piperita* (peppermint)-derived ingredients is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.²⁴ Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Council, of maximum reported use concentrations by product category.²⁵

According to 2017 VCRP data, the greatest use frequency is being reported for *Mentha Piperita* (Peppermint) Oil, which is being used in 827 cosmetic products (433 leave-on products + 360 rinse-off products + 34 products diluted for bath use).²⁴ The results of a concentration of use survey conducted in 2016 indicate that *Mentha Piperita* (Peppermint) Leaf Water is being used at concentrations up to 40% in leave-on products (face and neck products [not spray]), which is the greatest use concentration that is being reported for *Mentha piperita* (peppermint)-derived ingredients reviewed in this safety assessment.²⁵ Current and historical use frequency and concentration of use data are presented in Table 5. These data indicate that the highest maximum use concentration of *Mentha Piperita* (Peppermint) Oil in cosmetics increased from 3% in 1997 to 5% in 2017. Because 1997 use concentration data on the remaining 3 ingredients that were reviewed in the original safety assessment, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint), were not provided, a comparison of 1997 versus 2017 use concentration data cannot be made.

According to VCRP and Council survey data, the following *Mentha piperita* (peppermint)-derived ingredients are not being used in cosmetic products: *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Water, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Leaf Cell Extract, *Mentha Piperita* (Peppermint) Leaf Juice, and *Mentha Piperita* (Peppermint) Meristem Cell Culture.

Cosmetic products containing *Mentha piperita* (peppermint)-derived ingredients may be applied to the skin and hair or, incidentally, may come in contact with the eyes (at maximum use concentrations up to 0.0018 % *Mentha Piperita* (Peppermint) Leaf Extract in eye lotions) and mucous membranes (at maximum use concentrations up to 3.9% *Mentha Piperita* (Peppermint) Oil in bath oils, tablets, and salts). Additionally, use in lipstick products (at maximum use concentrations up to 2.9% *Mentha Piperita* (Peppermint) Oil) is being reported, the application of which may result in incidental ingestion. Products containing *Mentha piperita* (peppermint)-derived ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Mentha Piperita (Peppermint) Oil is being used in both pump hair sprays (maximum use concentrations up to 0.02%) and aerosol hair sprays (maximum use concentrations up to 0.017%) which may result in incidental inhalation exposure. Additionally, use of this ingredient in foot sprays at maximum use concentrations up to 0.5% is being reported. *Mentha Piperita* (Peppermint) Leaf Extract is also being used in pump and aerosol hair sprays, but at lower maximum use concentrations, and in face and neck/body and hand spray products at maximum use concentrations up to 0.001%. *Mentha Piperita* (Peppermint) Extract is being used in face and neck sprays at a highest maximum use concentration of 1.3%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.^{26,27,28,29} Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.^{26,27}

Mentha Piperita (Peppermint) Oil is being used in foot powders at maximum use concentrations up to 1%, and Mentha Piperita (Peppermint) Leaf Extract is being used in face powders at maximum use concentrations up to 0.0018%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.^{30,31,32}

Noncosmetic

Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract, and Mentha Piperita (Peppermint) Leaf Juice

Mentha Piperita (Peppermint) Oil is a generally recognized as safe (GRAS) ingredient according to the US FDA for use in dietary supplements.¹ It is described as a naturally occurring carminative that relaxes gastrointestinal smooth muscle. A final ruling by the FDA labeled Mentha Piperita (Peppermint) Oil as safe and effective as an antitussive (topical/inhalant). Final rulings cautioned that Mentha Piperita (Peppermint) Oil is not safe and effective for use as an expectorant in either topical/inhalant or lozenge form, or for use as a nasal decongestant, mouthwash, or digestive aid.

Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract, and Mentha Piperita (Peppermint) Leaf Juice are generally recognized as safe (GRAS) by the US FDA for use in food for human consumption (21CFR182.20). Mentha Piperita (Peppermint) Oil is an inactive ingredient in drug products that have been approved by the U.S. FDA.³³ A number of active ingredients, Mentha Piperita (Peppermint) Oil included, have been present in over-the-counter (OTC) drug products for various uses.³⁴ However, the FDA has determined that, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of Mentha Piperita (Peppermint) Oil as an active ingredient in the following drug products: nasal decongestant drug products, digestive aid drug products, insect bite and sting drug products, and astringent drug products.

Mentha Piperita (Peppermint) Oil is on the U.S. Environmental Protection Agency (EPA) list of active ingredients eligible for minimum risk pesticide products.³⁵

Mentha Piperita (Peppermint) Oil

According to the European Medicines Agency Committee on Herbal Medicinal Products (HMPC) community herbal monograph on *Mentha x piperita* L., aetheroleum (i.e., peppermint oil; aetheroleum is a term used to describe a preparation made from the above-ground parts of a plant), this herbal medicine is administered orally for the symptomatic relief of minor spasms of the gastrointestinal tract, flatulence, and abdominal pain, especially in patients with irritable bowel syndrome.³⁶ It is also an herbal medicine that is administered cutaneously for the symptomatic relief of mild tension-type headache. These uses have been identified as well-established uses by the HMPC. The highest recommended daily dose in the European Union is 1.2 ml peppermint oil (i.e. 1,080 mg peppermint oil, which contains maximum 140 mg pulegone + menthofuran). For a 60 kg person, this would correspond to a daily intake of 2.3 mg/kg body weight.

TOXICOKINETIC STUDIES

Dermal Penetration

Mentha Piperita (Peppermint) Oil

Eserine in a Mentha Piperita (Peppermint) Oil vehicle was applied to a 2.2 cm² shaved area on the abdomen of mice. The absorption rate for Mentha Piperita (Peppermint) Oil was measured as the latent period between application and appearance of eserine-induced signs.¹ Mentha Piperita (Peppermint) Oil had a latent period of 58 minutes.

Penetration Enhancement

Mentha Piperita (Peppermint) Leaf Extract

The skin penetration enhancement potential of Mentha Piperita (Peppermint) Leaf Extract (aqueous ethanol extract) was evaluated using dorsal porcine skin (dermatomed to thickness of 500 μm).³⁷ A square section of skin was cut to provide a dose area of 1 cm^2 and placed in a flow-through diffusion cell. [^{14}C]-Caffeine (hydrophilic) or [^{14}C]-salicylic acid (hydrophobic) was applied topically with 10% Mentha Piperita (Peppermint) Leaf Extract to porcine skin. The receptor fluid for the diffusion cell was “a Krebs-Ringer bicarbonate buffer spiked with dextrose and BSA (acronym not defined)”. Ethanol alone served as the control. When compared to [^{14}C]-caffeine in the presence of ethanol (control), the dermal absorption of [^{14}C]-caffeine was significantly greater ($p > 0.05$; flux and permeability of caffeine increased by over 3-fold) in the presence of Mentha Piperita (Peppermint) Leaf Extract. However, this was not true for [^{14}C]-salicylic acid.

Penetration Inhibition

Mentha Piperita (Peppermint) Oil

Mentha Piperita (Peppermint) Oil and ring-UL-[^{14}C]benzoic acid were applied to full-thickness human skin (breast or abdominal) samples in a static diffusion cell.³⁸ The receptor fluid for the diffusion cell was “0.9% sodium chloride and 1% Tween in water”. As the concentration of Mentha Piperita (Peppermint) Oil increased from zero to 5% in the donor phase, the maximal flux of benzoic acid decreased. The differences were significant at 1.0% and 5.0% Mentha Piperita (Peppermint) Oil, where the maximal fluxes were reduced to 81% and 52% of the control, respectively.

Absorption, Distribution, Metabolism, and Excretion

Oral

Mentha Piperita (Peppermint) Oil

The rate of Mentha Piperita (Peppermint) Oil absorption and excretion following oral administration was determined by measuring urinary menthol glucuronide.¹ Four male volunteers ingested 180 mg of an enteric-coated Mentha Piperita (Peppermint) Oil-coated capsule following a 16-h fast. Menthol was liberated from its glucuronide metabolite by treating the urine with β -D-glucuronidase. It was estimated that between 37 and 116 mg of menthol corresponding to an average 40% recovery of the administered menthol dose was excreted by each panelist within 14 h.

Mentha Piperita (Peppermint) Oil is relatively rapidly absorbed after oral administration and eliminated mainly via the bile.¹³ The major biliary metabolite is menthol glucuronide, which undergoes enterohepatic circulation. The urinary metabolites result from hydroxylation at the C-7 methyl group at C-8 and C-9 of the isopropyl moiety, forming a series of mono- and dihydroxymenthols and carboxylic acids, some of which are excreted, in part, as glucuronic acid conjugates. Studies with tritiated L-menthol in rats indicated approximately equal excretion in the feces and urine. The main metabolite identified was menthol-glucuronide. Additional metabolites are mono- or di-hydroxylated menthol derivatives.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Mentha Piperita (Peppermint) Oil

Mentha Piperita (Peppermint) Oil had a 24-h oral LD₅₀ of 4441 mg/kg in fasted Wistar rats; the 48-h LD₅₀ was 2426 mg/kg.¹ In a study involving fasted mice, an LD₅₀ of 2410 mg/kg was reported for *Mentha Piperita* (Peppermint) Oil diluted in olive oil.

Short-Term Toxicity Studies

Oral

Mentha Piperita (Peppermint) Oil

In 3 of 4 short-term oral toxicity studies (28-day or 5-week studies) involving 20 to 28 rats per group, brain lesions (specifically, cyst-like spaces in the cerebellum) were observed at *Mentha Piperita* (Peppermint) Oil doses up to 100 mg/kg/day.¹ In the remaining study (12 rats per group), these lesions were not observed in rats dosed with *Mentha Piperita* (Peppermint) Oil at doses of 20, 150, or 500 mg/kg/day for 5 weeks.

Subchronic Toxicity Studies

Oral

Mentha Piperita (Peppermint) Oil

Groups of 28 Wistar rats were given oral doses of 10, 40, and 100 mg/kg *Mentha Piperita* (Peppermint) Oil (diluted with soybean oil) daily for 90 days.¹ All hematological and biochemical parameters were within normal range, and there were no significant differences in absolute and relative organ weights. Brain lesions (specifically, cyst-like spaces in the cerebellum) were observed in all dose groups, but these results were classified as significant only for animals of the 100 mg/kg/day dose group. No other lesions of encephalopathy were observed. Nephropathy (hyaline droplet formation) was observed only in male rats of the 100 mg/kg/day dose group, and there was no evidence of epithelial degeneration. The no-observed-adverse-effect level (NOAEL) for *Mentha Piperita* (Peppermint) Oil was 40 mg/kg/day in this study.

GENOTOXICITY STUDIES

In Vitro

Mentha Piperita (Peppermint) Oil

The mutagenic potential of *Mentha Piperita* (Peppermint) Oil was investigated using the *Salmonella*/mammalian microsome test.¹ The following *Salmonella typhimurium* strains were used: TA1535, TA100, TA1537, and TA98. The sample tested contained 38.1% menthol, 33.7% menthone, and 1.7% pulegone; the remaining components were not identified. *Mentha Piperita* (Peppermint) Oil, tested at doses of 6.4, 32, and 160 µg/plate, produced the same number of revertants as the negative control. Toxicity was noted at the next (and maximum) dose of 800 µg/plate. Metabolic activation appeared to have made the oil less toxic to the bacteria. *Mentha Piperita* (Peppermint) Oil was not genotoxic.

In an in vitro chromosomal aberration test using a Chinese hamster fibroblast cell line, *Mentha Piperita* (Peppermint) Oil, at a maximum concentration of 0.25 mg/ml (in ethanol), produced polyploidism in 3% of the cells and structural aberrations in 7% of the cells at 48 h after treatment. The results were considered equivocal, as scores of either ≥ 10% or ≤ 4.9% were necessary for classification as either positive or negative, respectively. The results for *Mentha Piperita* (Peppermint) Oil (150 µg/ml) were negative in a mouse lymphoma L5178Y TK +/- cell mutagenesis assay. Results were also negative for this ingredient (at 155 µg) in an unscheduled DNA synthesis assay using rat hepatocytes.¹

The genotoxicity of *Mentha Piperita* (Peppermint) Oil was evaluated in a chromosome aberration test using human peripheral blood lymphocytes.³⁹ Lymphocyte cultures were incubated for 24 h with test substance concentrations up to 0.30 µl/ml. When chromosome aberrations (chromatid breaks, chromatid exchanges, chromosome breaks, and chromosome exchanges) were scored, not less than 100 metaphases per culture were

analyzed. *Mentha Piperita* (Peppermint) Oil was the most clastogenic at a concentration of 0.20 µl/ml (8-fold increase over acetone solvent control); the number of aberrant cells decreased at higher concentrations. The authors noted that the dose-response curve for *Mentha Piperita* (Peppermint) Oil was complicated, with a clear peak response at a concentration of 0.20 µl/ml.

Mentha Piperita (Peppermint) Oil was tested at concentrations up to 0.30 µl/ml in the sister chromatid exchange (SCE) test involving human lymphocytes.³⁹ The test conditions were essentially the same as those in the preceding chromosome aberration test, with the exception that 5-bromo-2'-deoxyuridine was added (10 µg/ml) to cultures initially. To determine the replicative index, 200 cells were scored. *Mentha Piperita* (Peppermint) Oil induced SCEs in a dose-independent manner. The authors noted that, seemingly, the saturation of SCE-inducing capacity occurred at high concentrations of *Mentha Piperita* (Peppermint) Oil. Results also indicated that *Mentha Piperita* (Peppermint) Oil inhibited cell replicative kinetics, some signs of which were observed at a concentration of 0.15 µl/ml. At concentrations $\geq$ 0.20 µl/ml, statistically significant inhibition of cell replicative kinetics was evident.

***Mentha Piperita* (Peppermint) Extract**

The genotoxicity of a trade name mixture containing 2.5% *Mentha Piperita* (Peppermint) Extract was evaluated in the Ames test using the following *Salmonella typhimurium* strains, with and without metabolic activation: TA97a, TA98, TA100, TA102, and TA1535.⁴⁰ The test substance was diluted with sterile distilled water to a concentration of 10% (effective concentration of extract = 0.25%) prior to testing each strain. Sterile deionized water served as the solvent control and positive controls (not stated) were also used. The test substance was not cytotoxic to the test system and was not genotoxic to any of the strains tested, either with or without metabolic activation. The bacterial strains tested were sensitive to the positive control mutagens and had a spontaneous reversion rate that was well within the accepted values for each strain.

ANTIGENOTOXICITY STUDIES

***Mentha Piperita* (Peppermint) Leaf Extract**

Oral pretreatment with *Mentha Piperita* (Peppermint) Leaf Extract (aqueous extract) (1 g/kg/day for 3 consecutive days) before exposure to gamma radiation was found to be effective in protecting against chromosomal damage in the bone marrow of Swiss albino mice (number tested not stated).⁴¹ The exposure of mice to 8 gray (Gy) ionizing radiation (1 Gy is equivalent to the absorption of one joule of radiation energy per kilogram of matter) only resulted in chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, and acentric fragments. In mice pretreated with *Mentha Piperita* (Peppermint) Leaf Extract, there was a significant decrease in the frequency of aberrant cells when compared to the irradiated control. A significant increase in the percentage of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, acentric fragments, total aberrations, and aberrations/damaged cell was observed at 12 h post-irradiation necropsy time in control animals. However, a significant decrease in the percentage of aberrations of this type was observed in mice pretreated with *Mentha Piperita* (Peppermint) Leaf Extract.

The modulatory effects of *Mentha Piperita* (Peppermint) Leaf Extract (aqueous extract) on genotoxicity and lung tumor incidence were evaluated using 4 groups of 30 to 76 Swiss albino mice.⁴² Beginning at 3 weeks of age (weaning), the mice received a single subcutaneous injection of benzo[a]pyrene and were then dosed orally (by gavage) with either water (group of 53 mice) or *Mentha Piperita* (Peppermint) Leaf Extract (1 g/kg; group of 76 mice). The remaining 2 groups of mice in the study were identified as no benzo(a)pyrene or *Mentha Piperita* (Peppermint) Leaf Extract dosing (30 mice) and *Mentha Piperita* (Peppermint) Leaf Extract alone (30 mice). When compared to mice in the benzo(a)pyrene only group, *Mentha Piperita* (Peppermint) Leaf Extract reduced the frequency of chromosomal aberrations and micronuclei in bone marrow cells. *Mentha Piperita* (Peppermint) Leaf Extract had an antigenotoxic effect in this study. Results relating to the modulatory effect of *Mentha Piperita* (Peppermint) Leaf Extract on lung tumor formation are included in the Anticarcinogenicity section of the report text.

CARCINOGENICITY STUDIES

Oral

Mentha Piperita (Peppermint) Oil

In a carcinogenicity study of toothpaste and its components, groups of 52 male pathogen-free CFLP (ICI-redefined) mice were dosed by gavage with 4 or 16 mg Mentha Piperita (Peppermint) Oil/kg/day, 6 days per week for 80 weeks. Treatment was followed by a 16- to 24-week observation period. An untreated group of 52 male mice and a vehicle control group of 260 male mice that received the toothpaste base (which did not contain chloroform, eucalyptol, or Mentha Piperita (Peppermint) Oil) were maintained as controls. At least one neoplasm at any site was observed in 73%, 69%, 65%, and 71% of mice of the low-dose, high-dose, untreated control, and vehicle control groups, respectively. The incidence of neoplasms of the lungs and kidneys was comparable among mice of the treated and nontreated groups. Hepatic cell tumor incidence for Mentha Piperita (Peppermint) Oil-dosed mice (25%) was comparable to the incidence for mice of the vehicle control group (27%); the incidence for the untreated group was 19%. Malignant lymphoma was found in 25%, 21%, 10%, and 14% of mice of the low-dose, high-dose, untreated, and vehicle control groups, respectively. The researchers did not discuss whether the differences in tumor incidence were significant.¹

ANTICARCINOGENICITY STUDIES

Mentha Piperita (Peppermint) Leaf Extract

The modulatory effects of Mentha Piperita (Peppermint) Leaf Extract (aqueous extract) on genotoxicity and lung tumor incidence were evaluated using 4 groups of 30 to 76 Swiss albino mice.⁴² The dosing procedure (including groups tested) was identical to that stated for Swiss mice in the second study of the Antigenotoxicity section in this report. The mice were killed at 9 weeks of age and evaluated for lung tumor incidence. Dosing with Mentha Piperita (Peppermint) Leaf Extract caused a significant reduction in the number of lung adenomas from an incidence of 67.92% in mice given benzo[a]pyrene only to 26.31%, which amounted to 61.26% inhibition. Tumor multiplicity was 1.22 in the benzo[a]pyrene only group and 1.15 in the benzo[a]pyrene + Mentha Piperita (Peppermint) Leaf Extract group. Mentha Piperita (Peppermint) Leaf Extract had an inhibitory effect on lung tumor formation in this study. Results relating to the modulation of genotoxicity are included in the Antigenotoxicity section of the report text.

The anticancer potential of Mentha Piperita (Peppermint) Leaf Extract (double-distilled; water extract) was studied using Swiss albino mice (number not stated).⁴³ Two stage mouse skin carcinogenesis was initiated by 7,12-dimethyl-benz[a]anthracene (DMBA). Two weeks later, croton oil (promoter) was applied 3 times per week for 14 weeks. The mice were dosed orally with Mentha Piperita (Peppermint) Leaf Extract (800 mg/kg/day) for the same period. At the end of the dosing period, average latent period, tumor incidence, size, burden, weight and cumulative number of papillomas were assessed. Dosing with Mentha Piperita (Peppermint) Leaf Extract caused inhibition of skin papilloma formation induced by DMBA and the application of croton oil, in terms of a significant decrease in the cumulative number of papillomas, tumor burden, and tumor incidence. In the control group, the tumor incidence was 100 percent. However, after dosing with the test substance for 15 days, the tumor incidence was reduced to 64%. There was a significant increase in the latency period for the appearance of papillomas in test animals (11 weeks in control group; 13 weeks in test group).

The possible molecular mechanisms underlying the cytotoxicity and anticarcinogenic potential of Mentha Piperita (Peppermint) Leaf Extract (petroleum ether, benzene, chloroform, ethyl acetate, methanol, or water extract) on 6 human cancer (HeLa, MCF-7, Jurkat, T24, HT-29, MIAPaCa-2) and normal (IMR-90, HEK-293) cell lines were evaluated.⁴⁴ In the human cancer cell lines tested with doses of 1 µg/ml, 10 µg/ml, and 100 µg/ml for 6 h, the number of apoptotic cells was incremental with an increase in the dose of *Mentha piperita* extracts. However, of all the extracts tested, the chloroform and ethyl acetate extracts resulted in a significantly higher apoptotic index after 6 hours, and the results were dose-dependent. When compared to the cancer cell lines, no significant changes were observed in normal cells. Similarly, of all of the extracts tested, the chloroform and ethyl acetate extracts of *Mentha piperita* had significant dose- and time-dependent anticarcinogenic activity, leading to G1 cell cycle arrest and mitochondrial-mediated apoptosis, perturbation of oxidative balance, upregulation of Bax gene, elevated expression

of p53 and p21 in the treated cells, and acquisition of senescence phenotype, while inducing pro-inflammatory cytokines response.

A study was performed to evaluate the antitumor activity of *Mentha Piperita* (Peppermint) Leaf Extract (methanol extract), using SW-480 human colon adenocarcinoma cells in a relevant cell anti-proliferation assay.⁴⁵ Statistically significant ($\alpha = 0.05$) growth inhibition was observed at a concentration of 31 $\mu\text{g/ml}$. An IC_{50} (concentration required for 50% inhibition, $\mu\text{g/ml}$) of 92.3 $\mu\text{g/ml}$ was reported for *Mentha Piperita* (Peppermint) Leaf Extract.

OTHER RELEVANT STUDIES

Cytotoxicity

***Mentha Piperita* (Peppermint) Oil**

The cytotoxicity of *Mentha Piperita* (Peppermint) Oil was evaluated in the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using 2 human cancer cell lines, MCF-7 and LNCaP.⁴⁶ *Mentha Piperita* (Peppermint) Oil from plants that were harvested during the summer and winter was tested. The following IC_{50} values ($\mu\text{g/ml}$) were reported: MCF-7 cell line (75.2 ± 2.9 [summer]; 80.8 ± 3.2 [winter]) and LNCaP cell line (90.4 ± 3.7 [summer]; 95.7 ± 4.5 [winter]). IC_{50} values in the 10 to 100 $\mu\text{g/ml}$ range represented a potentially toxic chemical, and IC_{50} values $< 10 \mu\text{g/ml}$ represented a potentially very toxic chemical.

In another study, essential oil was extracted from the leaves of *Mentha piperita*.⁴⁷ The oil essential oil was found to be cytotoxic in the following 4 human cancer cell lines: human lung carcinoma SPC-A1 cells ($\text{IC}_{50} = 10.89 \mu\text{g/ml}$), human leukemia K562 cells ($\text{IC}_{50} = 16.16 \mu\text{g/ml}$) and human gastric cancer SGC-7901 cells ($\text{IC}_{50} = 38.76 \mu\text{g/ml}$). The essential oil was inactive against human hepatocellular carcinoma BEL-7402 cells.

Mentha piperita

The inhibitory effect of polysaccharides extracted from *Mentha piperita* on A549 non-small cell lung adenocarcinoma cells was investigated using the MTT assay.⁴⁸ The results indicated that polysaccharides extracted from *Mentha piperita* had a moderate toxic effect on the A549 cell line ($\text{IC}_{50} = 879.52 \pm 22.55 \mu\text{g/ml}$). The growth of A549 cells was inhibited by *Mentha piperita* in a dose-dependent manner. The inhibitory rate was $54.54\% \pm 1.38\%$ at the highest concentration tested (1 mg/ml).

Hepatotoxicity

***Mentha Piperita* (Peppermint) Leaf Extract**

Mentha Piperita (Peppermint) Leaf Extract (methanol extract) and other botanical extracts were tested on both human (HepG2/C3A) and rat (MH1C1) hepatoma cells, using a battery of toxicity endpoints.⁴⁹ The extract was dissolved in dimethyl sulfoxide (DMSO) and then diluted in culture medium to a final concentration of 1000 $\mu\text{g/ml}$. The following 8 endpoints covering a variety of biological activities relevant to hepatotoxicity were used for hepatotoxicity evaluation: oxidative stress, mitochondrial membrane permeability, cellular neutral and polar lipid accumulation, CYP1A, 2B, 3A activities, albumin excretion, and total DNA content. Cluster analysis was used to group the phenolics into 4 clusters for each cell type. Two of the clusters were cluster 1 (compounds clustering with the solvent control (DMSO) and cluster 2 (compounds with reported in vivo liver toxicity). Overall and individual liver activity of the phenolics on both human and rat hepatoma cell lines were compared. For HepG2/C3A cells, 100% of the observations for *Mentha Piperita* (Peppermint) Leaf Extract and thyme extract, 92% for cinnamon extract, and 89% for juniper berry extract were assigned to cluster 1 (control group). For rat MH1C1 cells, 100% of the juniper berry extract and *Mentha Piperita* (Peppermint) Leaf Extract observations were assigned to cluster 1. The authors noted that because there are currently no reports of liver toxicity associated with peppermint, *Mentha Piperita* (Peppermint) Leaf Extract is useful as a negative control.

Nephrotoxicity

Mentha Piperita (Peppermint) Leaf Extract

The effects of Mentha Piperita (Peppermint) Leaf Extract (“*Mentha piperita* tea” (i.e., aqueous extract)) on rat kidney tissue were evaluated. The tea (prepared daily) was made by pouring 250 ml of boiling water over one heaped teaspoon (5 g) of the dried leaves of *Mentha piperita* L (grown in Turkey) and steeping for 5 to 10 minutes. Groups of 12 male Wistar albino rats were used. Test animals received *Mentha piperita* tea (20 g/l) in drinking water for 30 days. Control rats were given commercial drinking water during the study. The following histopathological changes, described as slight, were reported for the group dosed with *Mentha piperita* tea: hydropic degeneration of tubular epithelial cells, epithelial cells with pyknotic nuclei and eosinophilic cytoplasm, tubular dilatation and enlargements in Bowman capsules. In conclusion, the results indicate that *Mentha piperita* is not nephrotoxic to rats.⁵⁰

Effect on Histamine Release

Mentha Piperita (Peppermint) Leaf Extract

The 50% ethanol extract of peppermint leaves and stems significantly inhibited histamine release from rat peritoneal mast cells that was induced by compound 48/80 (polymer produced by the condensation of *N*-methyl-*p*-methoxyphenethylamine with formaldehyde) *in vitro*.⁵¹

Mentha Piperita

In a study involving human basophil cell suspensions, obtained from workers who were exposed to an additive containing penicillin, the cell suspensions were incubated with 10⁻¹ to 10⁻³ mg/ml Peppermint (dry aroma). A dose-dependent increase in histamine release was noted, and it was concluded that this release was due to nonimmunological mechanisms.¹

Immune System Effects

Mentha Piperita (Peppermint) Oil

The results of a host-resistance assay involving groups of 20 mice that had been dosed orally with Mentha Piperita (Peppermint) Oil (up to 1250 mg/kg/day for 5 days) suggested immunosuppression and/or increased susceptibility to bacterial-induced mortality. The results of a plaque-forming assay involving groups of 10 mice that received the same oral doses were negative.¹

Effect on Hair Growth

In a study involving C57BL/6 mice, the data suggest that 3% Mentha Piperita (Peppermint) Oil (diluted in jojoba oil) facilitates hair growth by promoting the conservation of vascularization of hair dermal papilla, which may contribute to the induction of early anagen stage.⁵²

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies are summarized in Table 6.

Irritation

In Vitro

Mentha Piperita (Peppermint) Leaf Extract

The skin irritation potential of *Mentha Piperita* (Peppermint) Leaf Extract (water/ethanol extract) at concentrations of 10% and 100% was evaluated using the in vitro reconstructed human epidermis test method, and results were negative.

Animal

***Mentha Piperita* (Peppermint) Oil**

*Hairless sites on 5 white rabbits were injected intradermally with 0.05 ml Mentha Piperita (Peppermint) Oil. Gross examinations were performed at 24 h and 48 h, at 1 and 2 weeks, and, in some cases, at 1 month after dosing. Dosing was repeated between 5 and 10 times. At microscopic examination of skin samples, moderate reactions characterized by polymorphonuclear leucocytes, lymphocytes, and plasma cells (without necrosis) were observed in 3 rabbits. Severe reactions, which were marked by the above as well as necrosis, were observed in the other 2 rabbits.*¹

***Mentha Piperita* (Peppermint) Extract**

The skin irritation potential of a trade name mixture containing 7.5% *Mentha Piperita* (Peppermint) Extract was evaluated using 3 rabbits (strain not stated).⁹ No cutaneous reactions were observed, and the authors concluded that the mixture was a non-irritant.

Human

***Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, and *Mentha Piperita* (Peppermint) Leaf Water**

The skin irritation potential of a lipstick containing 0.2961% *Mentha Piperita* (Peppermint) Leaf Extract was evaluated in a 48-h occlusive patch test using the following group of 50 subjects: normal (25), with eczema (4 subjects), with allergy (4 subjects) and with sensitive skin (17 subjects). Results were classified as negative.⁵³ Slight erythema was observed in 1 of 50 subjects after repeated applications of a cleaning gel containing 50% *Mentha Piperita* (Peppermint) Leaf Water in a product use study.⁵⁴ Mild and moderate erythema were observed in 12 and 6 subjects, respectively, patch tested with 50% *Mentha Piperita* (Peppermint) Leaf Water (10% aqueous solution dilution; effective concentration = 5% *Mentha Piperita* (Peppermint) Leaf Water).⁵⁵ In one of the skin sensitization studies on 20% *Mentha Piperita* (Peppermint) Oil that is summarized in the following section, it was reported that there was no evidence of skin irritation in the 104 subjects tested.⁵⁶

Sensitization

Animal

***Mentha Piperita* (Peppermint) Extract**

The skin sensitization potential of a trade name material containing 7.5% *Mentha Piperita* (Peppermint) Extract was evaluated in the maximization test using 10 albino guinea pigs.⁹ No macroscopic cutaneous reactions attributable to allergy were associated with application of the trade name material. There also were no cutaneous intolerance reactions in animals of the negative control group (further details not provided).

Human

***Mentha Piperita* (Peppermint) Oil**

*In the maximization test, 25 healthy male panelists received five 48-h occlusive induction patch (containing 8% Mentha Piperita (Peppermint) Oil) applications. Pre-treatment was for 24 h with an occlusive patch containing 5% sodium lauryl sulfate (SLS) prior to each exposure. After a 10-day non-treatment period, the subjects were challenged on the back with a 48-h patch (also preceded by SLS treatment). No evidence of sensitization was found.*¹

Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaf Extract, and Mentha Piperita (Peppermint) Leaf Water

In a human repeated insult patch test (HRIPT) on 20% Mentha Piperita (Peppermint) Oil involving 104 subjects, results were negative for skin irritation and sensitization.⁵⁶ Skin sensitization also was not observed in another HRIPT on 20% Mentha Piperita (Peppermint) Oil involving 101 subjects.⁵⁷ An HRIPT on a trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract was performed using 52 male and female subjects, and results were negative for dermal irritation and allergic contact sensitization.⁵⁸ Results were also negative in an HRIPT evaluating the cumulative irritation and/or allergic contact sensitization potential of a cosmetic product containing 0.00554% Mentha Piperita (Peppermint) Extract in 51 subjects.⁵⁹ In a maximization test on a cosmetic product containing 0.00554% Mentha Piperita (Peppermint) Extract involving 26 subjects, there was no evidence of contact allergy.⁶⁰ HRIPT results for undiluted Mentha Piperita (Peppermint) Leaf Extract (water/ethanol extract) in 52 subjects were also negative.¹¹ A face cream containing 20% Mentha Piperita (Peppermint) Leaf Water did not induce cumulative skin irritation or sensitization in an HRIPT involving 107 subjects.⁶¹

Photosensitization/Phototoxicity

Animal

Mentha Piperita (Peppermint) Oil

Undiluted Mentha Piperita (Peppermint) Oil was applied to the backs of 6 Skh:hairless mice. Thirty minutes later, the mice were irradiated for either 1 h with light from a fluorescent blacklight at an integrated UVA of 3 W/m², or for 40 minutes with light from a Xenon lamp at a weighted erythema energy of 0.1667 W/m². The mice were examined at 4 h, 24 h, 48 h, 72 h, and 96 h after radiation treatment. No effects were noted. In a second experiment, using 2 miniature swine and following the same protocol, no effect was produced by 100% Mentha Piperita (Peppermint) Oil.¹

OCULAR IRRITATION STUDIES

In Vitro

Mentha Piperita (Peppermint) Extract

The ocular irritation potential of a trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract was evaluated using an in vitro toxicity testing system consisting of normal, human-derived epidermal keratinocytes.⁶³ The cells had been cultured to form a stratified squamous epithelium that is similar to that found in the cornea. The procedure utilized a tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT)) that is reduced by succinate dehydrogenase (in viable mitochondria of viable cells) to a formazan derivative. The amount of MTT that is reduced by a culture is proportional to the number of viable cells. The trade name mixture, at a concentration of 10% in corn oil (effective concentration of extract = 0.25%) and a volume of 100 µl, was added to cell cultures; the incubation periods were 1 h, 4 h, and 24 h. Corn oil served as the negative control. An ET₅₀ (time of exposure needed for a test material to reduce the viability of treated tissues to 50% of control tissues) was calculated. Values for % viability were: 108% (at 1 h), 100% (at 4 h), and 34% (at 24 h). Results indicated that the trade name mixture at a concentration of 10% (ET₅₀ = 15.5 h (non-irritating, minimal)) had an ocular irritation potential that was somewhat less than sodium dodecyl sulfate at a concentration of 0.3% (ET₅₀ = 740 minutes (12.3 h)).

Animal

Mentha Piperita (Peppermint) Extract

A trade name mixture containing 7.5% Mentha Piperita (Peppermint) Extract was instilled (0.1 ml) into 1 eye of each of 3 New Zealand rabbits.⁹ Slight conjunctival redness was observed in 2 animals and lacrimation was observed in 1 animal. The trade name mixture was classified as a slight ocular irritant.

Human

Mentha Piperita (Peppermint) Leaf Water

The ocular irritation potential of a cleansing gel containing 50% Mentha Piperita (Peppermint) Leaf Water was studied using 50 subjects.⁵⁴ The subjects applied the product twice per day for 4 weeks, and were instructed to record any signs felt or observed during product use. Product use did not cause any signs of ocular or palpebral irritation.

CLINICAL STUDIES

Multicenter Studies

Mentha Piperita (Peppermint) Oil

Data from multicenter studies evaluating the skin irritation/sensitization potential of Mentha Piperita (Peppermint) Oil in patients are summarized in Table 6.^{62,64}

A multicenter study involving 13,398 patients was performed by the US/Canadian North American Contact Dermatitis Group (NACDG), whereby 71 patients were patch tested with Mentha Piperita (Peppermint) Oil (2% in petrolatum). A positive reaction prevalence rate of 0.53% was reported for this ingredient.⁶⁴ In another multicenter study, neither irritant nor allergic reactions were observed in 73 patients patch tested with Mentha Piperita (Peppermint) Oil according to International Contact Dermatitis Research Group (ICDRG) patch test procedures.⁶²

Case Reports

Case reports are summarized in Table 6.

Positive patch test reactions to Mentha Piperita (Peppermint) Oil and Mentha Piperita were reported in 8 of 9 case reports on patients with diseased skin.^{65,66,67,68,69,70,71} Though positive patch test results were reported in one of the studies on Mentha Piperita (Peppermint) Oil, prick test results in that study were negative. In patients without skin disease, but with peppermint sensitivity, positive prick test reactions to Mentha Piperita (Peppermint) Leaf, Mentha Piperita (Peppermint) Leaf Extract, and Mentha Piperita were reported.^{72,73}

Other Clinical Reports

Mentha Piperita (Peppermint) Oil

Positive reactions were observed in 7 of 450 dermatitic patients who were patch tested with 2% Mentha Piperita (Peppermint) Oil in yellow soft paraffin.¹ In another study, positive reactions to 2% Mentha Piperita (Peppermint) Oil were observed in 6 of 86 dermatitic patients. A patch containing 1% Mentha Piperita (Peppermint) Oil (vehicle unknown) was applied to the backs of 56 patients with chronic urticaria. No reactions were noted after a 1-h or 48-h exposure.

No reactions were observed in 25 spice factory workers who were patch tested with 2% Mentha Piperita (Peppermint) Oil in petrolatum. It has been reported that the patch testing of individual components of Mentha Piperita (Peppermint) Oil using 3 patients with allergic contact dermatitis established that the allergens were menthol and trace components such as piperitone or pulegone.¹

Dermal

A triple-blind clinical trial involved 96 randomly selected subjects (47 cases and 49 controls; all pregnant women) with a diagnosis of pruritus gravidarum.⁷⁴ The case and control subjects were instructed to apply 60 ml of peppermint oil (0.5% in sesame oil) and identical placebos, respectively, twice per day for 2 weeks. Applications

were made to the area of the itch. The authors noted that Mentha Piperita (Peppermint) Oil did not cause any special side effects in any of the subjects tested. They also stated that, based on these results, peppermint oil can be consumed for symptomatic treatment of skin itching on pregnant women.

Oral

Each of 6 pediatric patients with irritable bowel syndrome received a single oral dose of Mentha Piperita (Peppermint) Oil (187 mg).⁷⁵ Each capsule contained 83 mg of menthol as a constituent of Mentha Piperita (Peppermint) Oil. Each patient drank 125 ml of water after ingestion of the capsule. No adverse events were reported. The delayed appearance of menthol in the plasma was reported; a substantial lag time (range 1 h to 4 h) was observed in all subjects. Thus, an apparent prolonged absorption time was demonstrated. The authors noted that reasons for the delayed time of peak (T_{max}) likely related to formulation-specific factors (i.e., delayed release) and, potentially, enterohepatic recirculation.

Exposure Assessment

Dermal

Mentha Piperita (Peppermint) Oil

The FDA calculated an estimated human exposure from cosmetic use based on the concentration of use information supplied by industry.¹ Using a body splash product containing 0.2% Mentha Piperita (Peppermint Oil) and assuming 100% absorption over a body surface of 17,000 cm² and a daily application of 1 mg/cm² (~17 ml of the product), the FDA estimated an exposure of 34 mg/day. For a 60-kg person, this amounted to an estimated daily dose of 0.6 mg/kg/day.

Oral

Mentha Piperita (Peppermint) Oil

In the European Union, the highest recommended daily dose of Mentha Piperita (Peppermint) Oil is 1.2 ml, i.e., 1080 mg Mentha Piperita (Peppermint) Oil (contains a maximum of 140 mg pulegone + menthofuran).¹⁶ For a 60 kg person, this would correspond to a daily intake of 2.3 mg/kg body weight. This recommended daily dose of Mentha Piperita (Peppermint) Oil in medicinal products results in pulegone/menthofuran that exceeds the tolerated daily intake (TDI) (0.1 mg/kg) that was established for food by the Committee of Experts on Flavoring Substances (CEFS).

Risk Assessment

Dermal

Mentha Piperita (Peppermint) Oil

A maximum dermal use level of 5.4% has been recommended for Mentha Piperita (Peppermint) Oil.⁷⁶ This dermal restriction is based on 8% menthofuran (pulegone metabolite) and 3% pulegone content, with limits of 0.5% for menthofuran and of 1.2% for pulegone. The authors also recommended that Mentha Piperita (Peppermint) Oil, due to menthol content, should be avoided altogether in cases of cardiac fibrillation and in individuals with a glucose-6-phosphate dehydrogenase deficiency. No further information relating to these recommendations is provided.

Oral

Mentha Piperita (Peppermint) Oil and Pulegone (a component of Mentha Piperita (Peppermint) Oil)

The authors of a book entitled *Essential Oil Safety* have recommended a maximum daily oral dose of 152 mg Mentha Piperita (Peppermint) Oil.⁷⁶ This oral restriction is based on 8% menthofuran and 3% pulegone content, with limits of 0.2 mg/kg/day for menthofuran and 0.5 mg/kg/day for pulegone.

Mentha piperita

A study was performed to characterize data on dietary botanical supplement (DBSs) associated with adverse event reports submitted to the FDA Center for Food Safety and Applied Nutrition's Adverse Event Reporting System (CAERS).⁷⁷ FDA obtained CAERS data from 1999 to 2003 involving adverse effects associated with the 6 most frequently used DBSs, including peppermint. No adverse events were reported for single-ingredient peppermint supplements during the study period.

SUMMARY

The safety of the following ingredients in cosmetics was previously reviewed by the Panel, and a final report with a conclusion stating that these ingredients are safe as used in cosmetic formulations was published in 2001: *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water. The conclusion also stated that the concentration of pulegone, a constituent of these botanical ingredients, should not exceed 1%. The current safety assessment is, in part, a re-review of the 4 *Mentha piperita* (peppermint)-derived ingredients, and is inclusive of safety test data that have become available since the final report was issued.

The current safety assessment is also an first review of the following 6 *Mentha piperita* (peppermint)-derived ingredients: *Mentha Piperita* (Peppermint) Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Water, *Mentha Piperita* (Peppermint) Leaf Cell Extract, *Mentha Piperita* (Peppermint) Leaf Juice, and *Mentha Piperita* (Peppermint) Meristem Cell Culture.

According to 2017 VCRP data, the greatest use frequency is being reported for *Mentha Piperita* (Peppermint) Oil, which is being used in 827 cosmetic products, mostly leave-on products. The results of a concentration of use survey provided in 2016 indicate that *Mentha Piperita* (Peppermint) Leaf Water is being used at a concentration up to 40% in leave-on products, which is the greatest use concentration that is being reported for *Mentha piperita* (peppermint)-derived ingredients reviewed in this safety assessment.

In the U.S., *Mentha Piperita* (Peppermint) Oil is GRAS for use in food for human consumption. It is also an inactive ingredient in drug products that have been approved by the FDA, and is on the EPA list of active ingredients eligible for minimum risk pesticide products.

Mentha Piperita (Peppermint) Leaf Extract (10% aqueous ethanol extract) caused a statistically significant increase in the penetration of caffeine, but not salicylic acid, through porcine skin. *Mentha Piperita* (Peppermint) Oil inhibited the penetration of benzoic acid through human skin.

Following oral administration, *Mentha Piperita* (Peppermint) Oil is relatively rapidly absorbed and eliminated mainly via the bile. The major biliary metabolite is menthol glucuronide. Additional metabolites are mono- or di-hydroxylated menthol derivatives.

Mentha Piperita (Peppermint) Leaf Extract (aqueous extract) was not nephrotoxic to rats when administered (20 g/l) in drinking water daily for 30 days.

No adverse events were reported for single-ingredient peppermint supplements in a study that was performed to characterize data on dietary botanical supplement (DBSs) associated with adverse event reports submitted to the FDA Center for Food Safety and Applied Nutrition's Adverse Event Reporting System (CAERS).

Mentha Piperita (Peppermint) Oil was clastogenic in a chromosome aberration test involving peripheral blood lymphocytes. In a genotoxicity assay involving human lymphocytes, *Mentha Piperita* (Peppermint) Oil induced sister chromatid exchanges in a dose-dependent manner. A trade name mixture containing 2.5% *Mentha*

Piperita (Peppermint) Extract (diluted to a concentration of 10% (effective concentration of extract = 0.25%)) was not cytotoxic or genotoxic in the Ames test, with or without metabolic activation.

Oral pretreatment with *Mentha Piperita* (Peppermint) Leaf Extract (aqueous extract) before exposure to gamma radiation was found to be effective in protecting against chromosomal damage in the bone marrow of Swiss albino mice. In another study, the oral administration of *Mentha Piperita* (Peppermint) Leaf extract had an antigenotoxic (i.e., reduced the frequency of chromosomal aberrations and micronuclei in bone marrow cells) in Swiss albino mice intraperitoneally injected with benzo(a)pyrene.

Oral dosing with *Mentha Piperita* (Peppermint) Leaf Extract caused a significant reduction in the number of lung adenomas from an incidence of 67.92% in Swiss albino mice intraperitoneally injected with benzo[a]pyrene to 26.31%. Oral dosing with *Mentha Piperita* (Peppermint) Leaf Extract also caused inhibition of skin papilloma formation induced by DMBA and the application of croton oil, in terms of a significant decrease in the cumulative number of papillomas, tumor burden, and tumor incidence.

In the human cancer cell lines tested with *Mentha Piperita* (Peppermint) Leaf Extract (various extractants used), the number of apoptotic cells was incremental with an increase in the dose of *Mentha piperita* extracts. *Mentha Piperita* (Peppermint) Leaf Extract (only from chloroform and ethyl acetate extractants) had significant dose- and time-dependent anticarcinogenic activity, leading to G1 cell cycle arrest and mitochondrial-mediated apoptosis, perturbation of oxidative balance, upregulation of Bax gene, and elevated expression of p53 and p21 in the treated cells. In a study that was performed to evaluate the antitumor activity of *Mentha Piperita* (Peppermint) Leaf Extract (methanol extract) in an anti-proliferation assay involving SW-480 human colon adenocarcinoma cells, statistically significant growth inhibition was observed.

Results were positive for *Mentha Piperita* (Peppermint) Oil (from plants harvested during seasons of the year) in cytotoxicity assays involving human cancer cell lines: The following IC₅₀ values (µg/ml) were reported: MCF-7 cell line (75.2 ± 2.9 [summer]; 80.8 ± 3.2 [winter]) and LNCaP cell line (90.4 ± 3.7 [summer]; 95.7 ± 4.5 [winter]). In another study, *Mentha Piperita* (Peppermint) Oil was cytotoxic to the following human cancer cell lines: human lung carcinoma SPC-A1 cells (IC₅₀ = 10.89 µg/ml), human leukemia K562 cells (IC₅₀ = 16.16 µg/ml) and human gastric cancer SGC-7901 cells (IC₅₀ = 38.76 µg/ml). The essential oil was inactive against human hepatocellular carcinoma BEL-7402 cells.

Mentha Piperita (Peppermint) Leaf Extract (1000 µg/ml, methanol extract) did not induce hepatotoxicity in *in vitro* assays involving human (HepG2/C3A) and rat (MH1C1) hepatoma cells.

The 50% ethanol extract of peppermint leaves and stems significantly inhibited compound 48/80-induced histamine release from rat peritoneal mast cells *in vitro*.

In a study involving C57BL/6 mice, it was concluded that 3% *Mentha Piperita* (Peppermint) Oil (diluted in jojoba oil) facilitated hair growth by promoting the conservation of vascularization of hair dermal papilla.

A trade name mixture containing 7.5% *Mentha Piperita* (Peppermint) Extract was non-irritating to the skin of 3 rabbits. No macroscopic cutaneous reactions attributable to allergy were observed in a maximization test in which 10 albino guinea pigs were patch tested with a trade name material containing 7.5% *Mentha Piperita* (Peppermint) Extract during induction and challenged with the undiluted material and the material at a concentration of 50% (effective concentration of extract = 3.75%)

In a 48-h occlusive patch test, a lipstick product containing 0.2961% *Mentha Piperita* (Peppermint) Leaf Extract did not cause skin irritation in any of the 50 subjects tested. In an *in vitro* skin irritation study on *Mentha Piperita* (Peppermint) Leaf Extract (water/ethanol extract) involving reconstructed human epidermis, results were negative (MTT > 50%).

There was no evidence of dermal irritation or allergic contact sensitization in an HRIPT in which 52 male and female subjects were patch tested with a 10% dilution of a trade name mixture containing 2.5% *Mentha Piperita* (Peppermint) Extract (effective concentration of extract = 0.25%). A cosmetic product containing 0.00554% *Mentha Piperita* (Peppermint) Extract did not cause dermal irritation or allergic contact dermatitis in 51 male and

female subjects patch tested with a cosmetic product (an off-white cream) containing 0.00554% *Mentha Piperita* (Peppermint) Extract. In the maximization test, a cosmetic product (off-white cream) containing 0.00554% *Mentha Piperita* (Peppermint) did not induce contact sensitization in the 26 male and female subjects tested.

A face cream containing 20% *Mentha Piperita* (Peppermint) Leaf Water did not induce cumulative skin irritation or sensitization in an HRIPT involving 107 subjects. Negative results were also reported for a lipstick product containing 0.2961% *Mentha Piperita* (Peppermint) Leaf Extract in a 48-h occlusive patch test (skin irritation test) involving 50 subjects. Slight erythema was observed in 1 of 50 subjects who applied a cleansing gel containing 50% *Mentha Piperita* (Peppermint) Leaf Water twice per day for 4 weeks; there were no signs of ocular or palpebral irritation in any of the subjects. In a 48-h, single-application patch test, the skin irritation potential of a cleansing gel containing 50% *Mentha Piperita* (Peppermint) Leaf Water (10% aqueous dilution [effective concentration = 5%]) was evaluated using 52 subjects. A score of 1 (mild erythema) was reported for 12 subjects on day 2 and for 18 subjects on day 3. A score of 2 (moderate erythema) was reported for 2 subjects on day 2 and for 3 subjects on day 3. On day 4, 46 subjects had a score of 0 and 6 had a score of 1. The authors concluded that the skin compatibility of the diluted product was considered good.

The skin irritation and sensitization potential of 20% *Mentha Piperita* (Peppermint) Oil was evaluated in an HRIPT involving 104 subjects and results were negative. Similarly, there was no evidence of sensitization to 20% *Mentha Piperita* (Peppermint) Oil in an HRIPT involving 101 subjects. HRIPT results for undiluted *Mentha Piperita* (Peppermint) Leaf Extract (water/ethanol extract) in 52 subjects were also negative.

A trade name mixture containing 2.5% *Mentha Piperita* (Peppermint) Extract was classified as non-irritating in an in vitro toxicity testing system, consisting of normal, human-derived epidermal keratinocytes, for evaluating ocular irritation potential. Slight ocular irritation was observed in a study in which 3 rabbits were tested with a trade name mixture containing 7.5% *Mentha Piperita* (Peppermint) Extract.

In a multicenter study, neither irritant nor allergic reactions were observed in 73 patients patch tested with *Mentha Piperita* (Peppermint) Oil according to International Contact Dermatitis Research Group (ICDRG) patch test procedures. Another multicenter study involving 13,398 patients was performed by the US/Canadian North American Contact Dermatitis Group (NACDG), whereby 71 patients were tested with *Piperita* (Peppermint) Oil (2% in petrolatum). The prevalence rate for this ingredient was 0.53 %.

Mostly positive patch test reactions to *Mentha Piperita* (Peppermint) Oil (2% in petrolatum) and *Mentha Piperita* were reported in case reports on patients with diseased skin. In patients without skin disease, but with peppermint sensitivity, positive prick test reactions to *Mentha Piperita* (Peppermint) Leaf, *Mentha Piperita* (Peppermint) Leaf Extract, and *Mentha Piperita* were reported. In other clinical reports, oral dosing with *Mentha Piperita* (Peppermint) Oil (0.5% in sesame oil, 60 ml) did not cause any side effects in 47 pregnant female patients. A single oral dose of *Mentha Piperita* (Peppermint) Oil did not cause any adverse events in 6 pediatric patients.

DISCUSSION

The safety of the following cosmetic ingredients was reviewed previously by the Panel, and a final report with a conclusion stating that these ingredients are safe as used in cosmetic formulations was published in 2001: *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water. The conclusion also states that the concentration of pulegone, a constituent of these botanical ingredients, should not exceed 1%. The current safety assessment is a re-review of the safety of these 4 ingredients, and is also an initial safety evaluation of the following 6 related ingredients that were not listed as cosmetic ingredients prior to development of the published safety assessment: *Mentha Piperita* (Peppermint) Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Water, *Mentha Piperita* (Peppermint) Leaf Cell Extract, *Mentha Piperita* (Peppermint) Leaf Juice, and *Mentha Piperita* (Peppermint) Meristem Cell Culture.

In the 2001 published final safety assessment on *Mentha piperita* (peppermint)-derived ingredients, the Panel expressed concern that rat oral-dosing studies on *Mentha Piperita* (Peppermint) Oil and pulegone reported cyst-like spaces in the cerebellum that were attributed to pulegone. Therefore, the Panel established a 1%

concentration limit on pulegone in *Mentha piperita* (peppermint)-derived ingredients due to toxicity concerns. In these studies, brain sections were fixed by immersion using 4% neutral buffered formaldehyde. Because immersion fixation of nervous tissue might cause artifacts observed as vacuolar retraction spaces around neurons, a study involving rats was performed, using both immersion and perfusion tissue fixation methods, to determine whether the cerebellar lesions observed in earlier studies were caused by dosing with pulegone. Study results for rats dosed with pulegone did not reveal the occurrence of test substance-related, cyst-like spaces in the white matter of the cerebellum using either perfusion or immersion tissue fixation techniques. A possible explanation for the observed dose-dependent cyst-like spaces seen in previous studies could have been due to an interaction between impurities in the test substance and the fixation agent used therein. Given this possibility, the Panel agreed that the brain lesions may have been an artifact of the fixation method, and that the 1% limitation on pulegone is no longer warranted.

The Panel also considered the positive effects that were observed in female rats, and in male and female mice, dosed with pulegone in the 2011 National Toxicology Program (NTP) oral carcinogenicity study. However, the Panel did not express concern over these findings relative to pulegone as a component of *Mentha Piperita* (Peppermint) Oil in cosmetic products, based on the understanding that the cytotoxic dose-response relationship (renal and liver toxicity) that was associated with cancer development would not be relevant to pulegone exposure from a cosmetic product containing *Mentha piperita* (Peppermint) Oil at current use concentrations.

Because final product formulations may contain multiple botanicals, each possibly containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *Mentha piperita* (peppermint)-derived ingredients, the Panel is concerned about the presence of terpenes (e.g., limonene) and terpenoids (e.g., menthol) in cosmetics, which could result in sensitization.

The Panel expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

Additionally, the Panel recognized that *Mentha Piperita* (Peppermint) Leaf Extract can enhance the penetration of other ingredients through the skin. The Panel cautioned that care should be taken in formulating cosmetic products that may contain *Mentha piperita* (peppermint)-derived ingredients in combination with any ingredients whose safety was based on their lack of dermal absorption data, or when dermal absorption was a concern.

The issue of incidental inhalation exposure was discussed by the Panel, as *Mentha piperita* (peppermint)-derived ingredients are being used in products that could possibly be inhaled. For example, *Mentha Piperita* (Peppermint) Leaf Extract is used in face and neck sprays at maximum use concentrations up to 1.3%. Another example relating to inhalation exposure from products that are sprayed is the use of *Mentha Piperita* (Peppermint) Oil in both pump hair sprays (maximum use concentrations up to 0.02%) and aerosol hair sprays (maximum use concentrations up to 0.017%) which may result in incidental inhalation exposure. The use of *Mentha Piperita* (Peppermint) Leaf Extract at maximum use concentrations up to 0.0018% in face powders may also result in incidental inhalation exposure. Additionally, the Panel noted that droplets/particles from spray cosmetic products would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at <http://www.cir-safety.org/cir-findings>.

Finally, the Panel agreed that the available composition data on seven of the ingredients are sufficient, but the data relating to composition of *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem/Water, and *Mentha Piperita* (Peppermint) Meristem Cell Culture are insufficient. Also, after considering the available skin irritation and sensitization data, the Panel determined that skin sensitization data on these three ingredients also are insufficient. Thus, the Panel determined that the following additional data are needed in order to evaluate the safety :

- Composition data on *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem/Water and *Mentha Piperita* (Peppermint) Meristem Cell Culture.
 - Depending on the composition data that are received, other toxicological endpoints may be needed.
- Skin irritation and sensitization data on *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract, *Mentha Piperita* (Peppermint) Flower/Leaf/Stem/Water and *Mentha Piperita* (Peppermint) Meristem Cell Culture.

The Panel also agreed that the available data are sufficient for determining that *Mentha Piperita* (Peppermint) Oil, Leaf, and leaf-derived ingredients are safe in cosmetics in the present practices of use and concentration, when formulated to be non-sensitizing. The non-sensitizing caveat relates to the avoidance of a cumulative effect of multiple botanicals, in a single formulation, that share a constituent in common.

CONCLUSION

The CIR Expert Panel concluded that the following 7 *Mentha piperita* (peppermint)-derived ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be non-sensitizing:

- *Mentha Piperita* (Peppermint) Oil
- *Mentha Piperita* (Peppermint) Extract
- *Mentha Piperita* (Peppermint) Leaf
- *Mentha Piperita* (Peppermint) Leaf Cell Extract*
- *Mentha Piperita* (Peppermint) Leaf Extract
- *Mentha Piperita* (Peppermint) Leaf Juice*
- *Mentha Piperita* (Peppermint) Leaf Water

**Not reported to be in use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

The CIR Expert Panel also concluded that the available data are insufficient to make a determination that the following 3 *Mentha piperita* (peppermint)-derived ingredients are safe under the intended conditions of use in cosmetic formulations:

- *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Extract**
- *Mentha Piperita* (Peppermint) Flower/Leaf/Stem Water**
- *Mentha Piperita* (Peppermint) Meristem Cell Culture**

***Not reported to be in use.*

TABLES

Table 1. Toxicity Data on Components of *Mentha Piperita* (Peppermint)–Derived Ingredients

Study Type/Protocol	Results
<u>Short-Term Oral Toxicity</u>	
Menthone: 28-day study on groups of 20 rats. Doses up to 800 mg/kg/day.	Brain lesions (cyst-like spaces in the cerebellum). ¹
Menthol: 28-day study on groups of 20 rats. Doses up to 800 mg/kg/day	No brain lesions. ¹
Pulegone: 28-day study on groups of 20 rats. Doses up to 160 mg/kg/day	Brain lesions (cyst-like spaces in the cerebellum). ¹
Pulegone: 28-day study on groups of 28 female rats (CrL: (WI)BR, SPF strain). Doses (in soybean oil) of 160 mg/kg/day. Untreated control.	Alopecia. Statistically significant, test substance-related decrease in body weight and food consumption ($p < 0.001$) observed throughout study. Statistically significant reduction in plasma creatinine. Statistically significant increase in plasma alkaline phosphatase and non-statistically significant increase in alanine aminotransferase. Increased plasma concentrations of alkaline phosphatase and alanine aminotransferase taken together with increased absolute liver weight was non- statistically significant, but increased relative liver weight ($p < 0.05$) was indicative of adverse effect on the liver. However, no significant histopathology of liver observed. Number and severity of cyst-like spaces in cerebellum considered comparable to observations normally present in historical control Wistar rats at laboratory where study was performed. Authors noted that dose-dependent cyst- like spaces in cerebellum observed in previous studies could have been due to interaction between impurities in test substance and fixation agent used. ⁴
<u>Developmental and Reproductive Toxicity</u>	
Menthol: Groups of 15 to 23 pregnant animals dosed with Brazilian menthol via oral intubation. Mice received doses up to 185 mg/kg/day on gestation days (GD) 6 to 15. Rats received doses up to 218 mg/kg on GDs 6 to 15. Hamsters received doses up to 405 mg/kg/day on GDs 6 to 10. Artificially inseminated rabbits received doses up to 425 mg/kg/day on GDs 6 to 18. Caesarean sections were performed on all dams.	No teratogenic effects. ¹
<u>Genotoxicity</u>	
Menthol: <i>Salmonella typhimurium</i> strains: TA1535, TA100, TA1537, and TA98. Doses of 6.4, 32, 160, and 800 µg/plate in Ames test with or without metabolic activation.	Toxicity at highest dosed; less toxicity with metabolic activation. Same number of revertants reported for test and control cultures exposed to lower doses. ¹
Pulegone: <i>Salmonella typhimurium</i> strains: TA1535, TA100, TA1537, and TA98. Doses of 6.4, 32, 160, and 800 µg/plate in Ames test with or without metabolic activation.	Toxicity at highest dose; less toxicity with metabolic activation. Same number of revertants reported for test and control culture exposed to lower doses. ¹
Menthone: <i>Salmonella typhimurium</i> strains: TA1535, TA100, TA1537, TA98, and TA97. Doses of 6.4, 32, 160, and 800 µg/plate in Ames test with or without metabolic activation.	Toxicity at highest dose. Statistically significant number of revertants in strain TA1537 at doses of 6.4 and 32 µg/plate without metabolic activation. Further testing with a more sensitive strain (TA98) yielded statistically significant increases in the number of revertants at all doses tested without metabolic activation; results were dose-related. ¹
Menthol (Brazilian): Cytogenetic assay (rats). Doses of 1.45, 14.5, and 145 mg/kg, and, in some instances, doses of 500, 1150, and 3000 mg/kg, or 5000 mg/kg.	Non-genotoxic. ¹
Menthol (Brazilian): Dominant lethal assay (rats). Doses of 1.45, 14.5, and 145 mg/kg, and, in some instances, doses of 500, 1150, and 3000 mg/kg, or 5000 mg/kg.	Non-genotoxic. ¹
Menthol (Brazilian): Host-mediated assay (mice). Doses of 1.45, 14.5, and 145 mg/kg, and, in some instances, doses of 500, 1150, and 3000 mg/kg, or 5000 mg/kg. <i>Salmonella typhimurium</i> strain TA1530 and <i>Saccharomyces</i> strain D3 tested <i>in vitro</i> .	Weakly positive but significant response at high dose in <i>Salmonella typhimurium</i> TA1530, and elevated recombinant frequencies noted in <i>Saccharomyces</i> strain D3. ¹
<u>Carcinogenicity</u>	
Menthol: National Cancer Institute (NCI) 2-year bioassay. <i>dl</i> -menthol administered orally to Fischer 344 rats (3750 ppm or 7500 ppm) or to B6C3F ₁ mice (2000 ppm or 4000 ppm).	Negative trend in fibroadenomas of the mammary gland observed in female rats (20 of 50 control; 10 of 49 low-dose; 7 of 49 high-dose). No evidence of carcinogenicity in rats or mice or either sex. ¹

Table 1. Toxicity Data on Components of *Mentha Piperita* (Peppermint)–Derived Ingredients

Study Type/Protocol	Results
<u>Carcinogenicity</u>	
Pulegone: National Toxicology Program (NTP) 2-year bioassay. Pulegone (in corn oil) administered to groups of 50 male and 50 female F344/N rats and groups of 50 male and 50 female B6C3F1 mice by gavage (5 days/week) for 105 weeks. Male rats received doses of 18.75, 37.5, or 75 mg/kg; female rats and male and female mice received doses of 37.5, 75, or 150 mg/kg.	Effects in the kidneys (hyaline glomerulopathy and nephropathy), liver (oval cell hyperplasia, bile duct hyperplasia, hypertrophy, hepatocyte necrosis, and portal fibrosis), nose (olfactory epithelium degeneration, inflammation, and metaplasia), and forestomach (inflammation, hyperplasia, and ulcer) reported. Increased incidences of liver neoplasms in male and female B6C3F1 mice in the study led to the conclusion that there was clear evidence of carcinogenic activity in mice. For female F344/N rats, it was concluded that there was some evidence of carcinogenicity based on an increased incidence of urinary bladder neoplasms. Five of 47 rats in 150 mg/kg female stop-exposure group (gavage with pulegone stopped at week 60 because of severely reduced body weights) diagnosed with papilloma and carcinoma, combined. Male rats did not show increased incidences of bladder tumors or neoplasms of other organs.³ The International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence in experimental animals for the carcinogenicity of pulegone, and that there is inadequate evidence in humans for the carcinogenicity of pulegone.⁷⁸ IARC's conclusions were based on positive oral carcinogenicity data in male and female mice and in female rats.
Pulegone: Female rats dosed orally (gavage) with 75 or 150 mg/kg for 4 and 6 weeks.	Results supported hypothesis that cytotoxicity followed by regenerative cell proliferation is mode-of-action for pulegone-induced urothelial tumors in female rats.⁷⁹
<u>Anticarcinogenicity</u>	
(-)-Menthol: Rats dosed orally with 1% or 0.5% (-)-menthol for 20 weeks. Dosing initiated 2 weeks prior to dimethylbenzanthracene (DMBA) tumor induction.	Significant inhibition ($p < 0.001$) of DMBA-induced rat mammary gland carcinogenesis following 20 weeks of oral dosing with 1% (-)-menthol. Chemopreventive effect noted when rats dosed with 0.5% menthol for 2 weeks prior to and 1 week after DMBA induction.¹
<u>Skin Irritation</u>	
Methanol: Two Tiger Balm formulations containing 8% and 10% menthol applied for 23 h, under occlusive patches, to abraded and intact sites on New Zealand white rabbits. Total number of patches applied was 21. A third group was treated with control wax (mixture of hard and soft waxes).	Dermal irritation observed in all treated animals, with the following severity scale: 8% menthol balm < control wax < 10% menthol balm. 8% menthol balm almost innocuous in male rabbits. Irritation observed was not progressive and tolerance developed within 10 days. No severe damage noted at microscopic examination of skin (increased hyperkeratosis noted at treated sites), and no evidence of systemic toxicity.¹
<u>Skin Sensitization</u>	
Menthol	Because menthol is a predominant component of <i>Mentha Piperita</i> (Peppermint) Oil and <i>Mentha Piperita</i> (Peppermint) Leaf Extract, it should be noted that a review article on the sensitization potential of menthol is available. After performing an extensive review of the reported cases of sensitization to menthol, it was concluded that the literature does not adequately document the clinical relevance of the patch test reactions nor the impact of irritant reactions.⁸⁰
<u>Other Clinical Reports</u>	
Menthol: 877 patients with primary contact, atopic, nummular, and stasis dermatitis and eczema were tested with 5% menthol in yellow paraffin	Reactions observed in 1% of the panelists within 96 h.¹

Table 2. Definitions and Functions of the Ingredients in this Safety Assessment.²

Ingredient CAS No.	Definition & Idealized Structures	Function
Mentha Piperita (Peppermint) Oil 8006-90-4 84082-70-2	Mentha Piperita (Peppermint) Oil is a volatile oil obtained from the whole plant <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous
Mentha Piperita (Peppermint) Leaf Extract 84082-70-2	Mentha Piperita (Peppermint) Leaf Extract is the extract of the leaves of the peppermint, <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous; Skin-Conditioning Agents - Occlusive
Mentha Piperita (Peppermint) Leaf	Mentha Piperita (Peppermint) Leaf is the dried leaves of <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Fragrance Ingredients
Mentha Piperita (Peppermint) Leaf Water 84082-70-2	Mentha Piperita (Peppermint) Leaf Water is an aqueous solution of the steam distillate obtained from the leaves of <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Flavoring Agents; Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous
Mentha Piperita (Peppermint) Extract 84082-70-2	Mentha Piperita (Peppermint) Extract is the extract of the whole plant, <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Skin-Conditioning Agents - Miscellaneous
Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract 84082-70-2	Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract is the extract of the flowers, leaves and stems of <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Flavoring Agents; Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous
Mentha Piperita (Peppermint) Flower/Leaf/Stem Water 84082-70-2	Mentha Piperita (Peppermint) Flower/Leaf/Stem Water is the aqueous solution of the steam distillates obtained from the flowers, leaves and stems of <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Fragrance Ingredients
Mentha Piperita (Peppermint) Leaf Cell Extract	Mentha Piperita (Peppermint) Leaf Cell Extract is the extract of a culture of the leaf cells of <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Antioxidants; Skin Protectants
Mentha Piperita (Peppermint) Leaf Juice 84082-70-2	Mentha Piperita (Peppermint) Leaf Juice is the juice expressed from the leaves of <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Skin-Conditioning Agents - Miscellaneous
Mentha Piperita (Peppermint) Meristem Cell Culture	Mentha Piperita (Peppermint) Meristem Cell Culture is a suspension of the cultured meristem cells of <i>Mentha piperita</i> . The accepted scientific name for <i>Mentha piperita</i> is <i>Mentha x piperita</i> .	Skin-Conditioning Agents - Miscellaneous

Table 3. Physical and Chemical Properties of Mentha Piperita (Peppermint)-Derived Ingredients

Property	Value	Reference
<u>Mentha Piperita (Peppermint) Oil</u>		
Form	Colorless or pale yellow liquid	1
Angular rotation (°C)	Between -18 and 32	1
Refractive index (at 20°C)	Between 1.459 and 1.465	1
Specific gravity	Between 0.896 and 0.908	1
Assay for total esters	Not less than 5% of esters, calculated as menthyl acetate	1
Assay for total menthol	Not less than 50% of menthol	1
Dimethyl sulfide	Passes test (rectified); fails test (natural)	1
Heavy metals (as Pb)	Passes test (limit of 0.004%)	1
Solubility	Soluble in alcohol: Passes test (1 volume dissolves in 3 volumes of 70% alcohol);	1
	Soluble in most vegetable oils; insoluble in propylene glycol	7
<u>Mentha Piperita (Peppermint) Leaf Extract</u>		
Form	Yellow to light brown, clear oil	5
Refractive index (at 20°C)	1459-1469	5
Density (at 20°C)	0.890-0.910	5
<u>Mentha Piperita (Peppermint) Extract (2.5% in tradename mixture)</u>		
Refractive index	1.4498	6
Specific gravity	1.031	6

Table 4. Chromatographic Profiles for *Mentha Piperita* (Peppermint) Oil¹⁵ and *Mentha Piperita* (Peppermint) Leaf Extract*.^{5,19}

Components	Origins Other Than U.S.		U.S. Type	
	Min. (%)	Max. (%)	Min. (%)	Max. (%)
<i>Mentha Piperita</i> (Peppermint) Oil				
3-Octanol	0.1	0.5	0.1	0.4
1,8-Cineol	3.0	8.0	4.0	6.0
Limonene ^a	1.0	3.0	1.0	2.5
<i>trans</i> -Sabinene Hydrate	0.5	2.0	0.5	2.3
Menthone	13.0	28.0	15.0	25.0
Isomenthone	2.0	8.0	2.0	4.5
Menthofuran	1.0	8.0	1.5	6.0
Neomenthol	2.0	6.0	2.5	4.5
Menthol	32.0	49.0	36.0	46.0
Pulegone	0.5	3.0	0.5	2.5
Menthyl Acetate ^b	2.0	8.0	3.0	6.5
β -Caryophyllene	1.0	3.5	1.0	2.5
Origin Not Stated				
<i>Mentha Piperita</i> (Peppermint) Leaf Extract				
Essential Oil ^c	80	90		
Essential Oil ^d		> 65		
Water		Not specified		
Volatile Compounds^e				
Limonene		< 2		
1,8-Cineol		not specified		
L-Menthone	20	40		
Menthofuran		< 2		
Isomenthone		Not specified		
Isomenthol		Not specified		
Neomenthol		Not specified		
L-Menthol	25	40		
Pulegone		< 3		
Menthyl Acetate	5	15		
beta Caryophyllene		Not specified		
Germacrene		Not specified		
Allergen Compounds^f				
Eugenol		< 0.05		
Linalool		< 0.3		
d-Limonene		< 1		

**Mentha Piperita* (Peppermint) Leaf Extract (CO₂ extract)^aThe limonene is regarded to be predominantly L-limonene based on physical tests. It is believed that there might be a small amount of D-limonene present, but the exact quantity is unknown^bThe menthyl acetate is regarded to be predominantly L-menthyl acetate based on the physical tests. It is believed that there might be a small amount of D-menthyl acetate present, but the exact quantity is unknown.^cGravimetric distillation detection method used^dVolumetric distillation detection method used^eGas chromatography-mass spectrometry detection method used^fAllergen compounds present are subject to declaration if the concentration exceeds 0.001% in leave-on and 0.01% in rinse-off products. However, values < 0.01% are not mentioned.

Table 5. Frequency and Concentration of Use of *Mentha piperita* (peppermint)-derived Ingredients According to Duration and Exposure. ^{1,24,25}

Table 3: Frequency and Concentration of Use of Mentha piperita (Peppermint) derived Ingredients According to Duration and Exposure.		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
		Mentha Piperita (Peppermint) Oil				Mentha Piperita (Peppermint) Leaf Extract			
		2017	1998	2017	1997	2017	1998	2017	1997
Totals*		827	102	0.0001-5	0.1-3	198	35	0.000075-0.5	NR
Duration of Use									
Leave-On	433	52	0.0006-5	0.2-2	118	10	0.000075-0.5	NR	
Rinse-Off	360	44	0.0001-1.9	0.1-3	79	24	0.0001-0.2	NR	
Diluted for (Bath) Use	34	6	0.018-3.9	NR	1	1	NR	NR	
Exposure Type									
Eye Area	4	NR	0.00094	NR	4	NR	0.0018	NR	
Incidental Ingestion	214	24	0.05-2.9	0.2-1.2	7	NR	0.00075-0.5	NR	
Incidental Inhalation-Spray	19;120 ^a	NR;23 ^a	0.017-1;0.002-1.1 ^a	NR	5;34 ^a	NR;2 ^a	0.00041-0.0046;0.00057-0.026 ^a	NR	
Incidental Inhalation-Powder	1	NR	0.01-1	NR	3	NR	0.0018	NR	
Dermal Contact	458	75	0.0006-5	0.1-2	139	22	0.000075-0.2	NR	
Deodorant (underarm)	2	NR	NR	NR	NR	NR	0.0018	NR	
Hair - Non-Coloring	148	1	0.0001-0.96	3	50	13	0.00018-0.2	NR	
Hair-Coloring	NR	NR	0.0024	NR	NR	NR	0.032	NR	
Nail	7	2	0.00064-1.5	NR	2	NR	NR	NR	
Mucous Membrane	317	30	0.0025-3.9	0.2-1.2	8	6	0.00075-0.5	NR	
Baby Products	1	NR	0.2	NR	NR	NR	NR	NR	
		Mentha Piperita (Peppermint) Leaf				Mentha Piperita (Peppermint) Leaf Water			
		2017	1998	2017	1997	2017	1998	2017	1997
Totals*		NR	NR	0.0002-1.6	NR	15	NR	0.00013-40	NR
Duration of Use									
Leave-On	NR	NR	0.001	NR	9	NR	0.00013-40	NR	
Rinse-Off	NR	NR	0.0002-1.6	NR	6	NR	0.00021-0.00067	NR	
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR	NR	NR	
Exposure Type									
Eye Area	NR	NR	NR	NR	2	NR	NR	NR	
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	
Incidental Inhalation-Spray	NR	NR	NR;0.001 ^a	NR	NR;4 ^a	NR	NR;0.00043-10 ^a	NR	
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR	
Dermal Contact	NR	NR	0.001-1.6	NR	13	NR	0.00013-40	NR	
Deodorant (underarm)	NR	NR	NR	NR	1	NR	15	NR	
Hair - Non-Coloring	NR	NR	0.0002-0.023	NR	NR	NR	NR	NR	
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR	
Nail	NR	NR	NR	NR	NR	NR	NR	NR	
Mucous Membrane	NR	NR	0.001-0.16	NR	NR	NR	NR	NR	
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	
		Mentha Piperita (Peppermint) Extract							
		2017		2017					
Totals*		71		0.00006-7.9					
Duration of Use									
Leave-On	43		0.00006-7.9						
Rinse-Off	27		0.0001-1						
Diluted for (Bath) Use	1		1						
Exposure Type									
Eye Area	NR		NR						
Incidental Ingestion	4		0.0099-3.4						
Incidental Inhalation-Spray	10; 21 ^a		1.3; 0.005-1 ^a						
Incidental Inhalation-Powder	NR		NR						
Dermal Contact	63		0.00006-7.9						
Deodorant (underarm)	1		1						
Hair - Non-Coloring	4		0.0004-1						
Hair-Coloring	NR		NR						
Nail	NR		NR						
Mucous Membrane	3		0.0099-3.4						
Baby Products	NR		NR						

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

^a It is possible these products are sprays, but it is not specified whether the reported uses are sprays..

NR - no reported use

Table 6. Dermal Irritation and Sensitization Data

Ingredient	Subjects/Cell Type	Protocol	Results
Irritation Studies			
<u>In Vitro</u>			
Mentha Piperita (Peppermint) Leaf Extract (water/ethanol extract) (10% and 100%)	Reconstructed human epidermis	<i>In vitro</i> reconstructed human epidermis test method. In this test, the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in test material-treated tissues is expressed as a percentage relative to negative control-treated cultures.	Negative (MTT > 50%). ¹¹
<u>Animal</u>			
Trade name mixture containing 7.5% Mentha Piperita (Peppermint) Extract	3 rabbits (strain not stated)	Mixture (0.5 ml) applied under semi-occlusive dressing to intact skin for 4 h. Area of application (cm ²) not stated.	No cutaneous reactions observed. Classified as non-irritant. ⁹
<u>Human</u>			
Lipstick containing 0.2961% Mentha Piperita (Peppermint) Leaf Extract	50 subjects, described as follows: normal (25), with eczema (4 subjects), with allergy (4 subjects) and with sensitive skin (17 subjects).	In occlusive patch test, product (~ 20 mg) applied in a square test chamber (8 mm x 8 mm) to the back of each subject for 48 h. Reactions scored 30 minutes after patch removal and at 72 h post-application. Sodium dodecyl sulfate (1% in water) and water served as the positive and negative controls, respectively.	Product and negative control did not cause skin irritation in any of the subjects tested and product was classified as harmless. Positive control caused positive reactions in 12 subjects. ⁵³
50% Mentha Piperita (Peppermint) Leaf Water in a cleansing gel	50 subjects	Subjects applied product twice per day for 4 weeks, and were instructed to record any signs felt or observed during product use.	Slight erythema was observed in one subject. ⁵⁴
50% Mentha Piperita (Peppermint) Leaf Water in a cleansing gel (10% aqueous dilution [effective concentration = 5%])	52 subjects	Diluted product applied, under occlusive patch, to the skin for 48 h. Reactions scored up to 48 ± 4 h after patch removal (day 4).	A score of 1 (mild erythema) was reported for 12 subjects on day 2 and for 18 subjects on day 3. A score of 2 (moderate erythema) was reported for 2 subjects on day 2 and for 3 subjects on day 3. On day 4, 46 subjects had a score of 0 and 6 had a score of 1. Skin compatibility of diluted product considered good. ⁵⁵
Sensitization Studies			
<u>Animal</u>			
Trade name material containing 7.5% Mentha Piperita (Peppermint) Extract	10 albino guinea pigs	Maximization test. 1 st induction: 2 intradermal injections of trade name material, 2 injections of Freund's complete adjuvant (FCA), and 2 injections of FCA and material mixture. 2 nd induction: topical application of material 24 h after brushing with 10% sodium lauryl sulfate (SLS). After 19-day non-treatment period, challenge phase involved topical applications of material (undiluted and at concentration of 50% (effective concentration of extract = 3.75%)) under an occlusive dressing for 24 h	No macroscopic cutaneous reactions attributable to allergy associated with application of material. No cutaneous intolerance reactions in animals of the negative control group (further details not provided). ⁹

Table 6. Dermal Irritation and Sensitization Data

Ingredient	Subjects/Cell Type	Protocol	Results
<u>Human</u>			
Trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract. Prior to application, mixture prepared as a 10% dilution using distilled water (effective concentration of extract = 0.25%).	52 male and female subjects.	Human repeated insult patch test (HRIPT). 1" x 1" semi-occlusive patch containing the diluted mixture (0.2 ml) applied for 24 h to upper back (between the scapulae) 3 times per week for total of 9 applications. After a 2-week non-treatment period, diluted mixture applied to new test site adjacent to original site. Reactions scored at 24 h and 72-h post-application.	No evidence of dermal irritation or allergic contact sensitization in any of the subjects tested. Also, no adverse events were identified during the study. ⁵⁸
Cosmetic product (off-white cream) containing 0.00554% Mentha Piperita (Peppermint) Extract	51 male and female subjects	HRIPT. Cream (~ 0.23 g) applied for 24 h to upper back (between the scapulae; area (cm ²) not stated). Application procedure (induction and challenge) same as reported in preceding study. Challenge reactions scored at same intervals.	No dermal irritation or allergic contact dermatitis in subjects tested. ⁵⁹
Cosmetic product (off-white cream) containing 0.00554% Mentha Piperita (Peppermint) Extract	26 male and female subjects	Maximization test. The test site (upper outer arm; area not stated) pre-treated with 0.25% aqueous SLS, applied under occlusive patch for 24 h. Product (0.05 ml) then applied, under occlusive patch, to same site for 48 h (or for 72 h, if placed over weekend). Product application followed by re-application of SLS patch for 24 h. Sequence repeated for total of 5 induction exposures. After 10-day non-treatment period, new test site (on opposite arm) pre-treated with SLS prior to application of challenge patch. At challenge, product (0.05 ml) applied for 48 h, under an occlusive patch, to same site. Reactions scored at time of patch removal and 48 h later.	No evidence of contact sensitization. ⁶⁰
100% Mentha Piperita (Peppermint) Leaf Extract (water/ethanol extract)	52 subjects	HRIPT (protocol details not included).	Negative results. ¹¹
20% Mentha Piperita (Peppermint) Leaf Water in a face cream	107 subjects (96 women, 11 men) with no history of atopy	In HRIPT, product applied (0.02 ml) to the back using occlusive patch (small Finn chamber), and 9 induction applications made over 3-week period. For 1st, 2nd, 5th, 7th, and 8th applications, duration of exposure was 48 ± 4 h. Duration of exposure was 72 ± 4 h for 3rd, 6th, and 9th applications. Challenge phase consisted of single application to new site and a previously treated site for 48 ± 4 h.	Product did not induce cumulative irritation or sensitization. ⁶¹

Table 6. Dermal Irritation and Sensitization Data

Ingredient	Subjects/Cell Type	Protocol	Results
20% Mentha Piperita (Peppermint) Oil	104 male and female subjects	In HRIPT, test substance applied (0.2 ml) for 24 h to upper back (between scapulae) using 3/4" x 3/4" semi-occlusive patch. Application repeated 3 times per week for total of 9 induction applications. 24-h challenge patch applied after 2-week non-treatment period, and reactions scored at 24 h and 72 h.	No evidence of irritation or sensitization. ⁵⁶
20% Mentha Piperita (Peppermint) Oil	101 male and female subjects	In HRIPT, nine 24-h applications of test substance (0.2 ml) made to back using semi-occlusive patches (dimensions not stated). 24-h challenge patch applied after 10- to 15-day non-treatment period.	No evidence of sensitization. ⁵⁷
Multicenter Studies			
Mentha Piperita (Peppermint) Oil (concentration not stated)	73 patients	Patients patch tested according to International Contact Dermatitis Research Group (ICDRG) patch test procedures during 1994 to 1998	Neither irritant nor allergic reactions reported. ⁶²
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	13,398 patients in study (71 patch tested with ingredient)	Patients patch tested during years 2009 to 2014 to determine frequency of positive patch test reactions to essential oils. Study used a retrospective analysis of patch test results and relevant demographical/clinical data that were collected electronically by the US/Canadian North American Contact Dermatitis Group (NACDG) and other networks.	Positive reaction prevalence rate of 0.53% reported for Mentha Piperita (Peppermint) Oil. ⁶⁴
Case Reports			
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	Male patient with orofacial granulomatosis mainly affecting lower lip	Patch test	Allergic positive reaction. ⁶⁵
Mentha Piperita (Peppermint) Oil (concentration not stated)	Female patient with lichenoid eruption on oral mucosa	Patch and prick tests	Positive patch test reaction, i.e., itching, erythema, and swelling, beginning at day 5; ++ reaction by day 7. Prick test results negative. ⁶⁶
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	Male patient with history of hand eczema and sensitization to tixocortol pivalate. Presented with severe eczematous contact dermatitis after repeated applications of a local action transcutaneous (LAT) patch for lumbar pain containing Mentha Piperita (Peppermint) Oil.	Patch test	Strong positive reactions to LAT patch and to Mentha Piperita (Peppermint) Oil (2% in petrolatum). ⁶⁷

Table 6. Dermal Irritation and Sensitization Data

Ingredient	Subjects/Cell Type	Protocol	Results
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	Female patient with allergic contact dermatitis after consuming herbal tea containing Mentha Piperita (Peppermint) Oil.	Patch test. Reactions evaluated at 2, 3 and 7 days according to ICDRG procedures.	Positive patch test reaction (+ reaction) observed on days 2 and 3. ⁶⁸
Mentha Piperita (Peppermint) Oil (concentration not stated)	4 patients with allergic contact cheilitis (lips and perioral skin), secondary to exposure to lip balm that contained Mentha Piperita (Peppermint) Oil	Patch test. Reactions evaluated at 48 h and 96 h.	Positive patch test reactions to lip balm and to Mentha Piperita (Peppermint) Oil. ⁶⁹
Mentha Piperita (Peppermint) Leaf and Peppermint (concentration not stated)	Male patient with IgE-mediated anaphylaxis to peppermint (<i>Mentha piperita</i>) after sucking on peppermint candy. 5 healthy controls	Skin prick and prick-to-prick tests	Patient had strongly positive prick test reaction to slurry of peppermint candy and fresh peppermint leaf. Prick testing of patient with saline slurry of peppermint candy caused wheal and flare, with largest diameters of 10 mm and 35 mm (W10/F25), respectively. Prick-to-prick test with fresh peppermint leaf revealed skin test response of W25/F50. All prick tests on 5 controls negative. ⁷²
Mentha Piperita (Peppermint) Leaf Extract (concentration not stated)	Female patient became symptomatic with dyspnea when near peppermint (<i>Mentha piperita</i>) scent	Skin prick test	Positive skin prick test reaction to commercial Mentha Piperita (Peppermint) Leaf Extract. ⁷³
Mentha Piperita (Peppermint) (concentration not stated)	Male patient with severe cheilitis	Patch test	Negative reaction.. ⁷⁰
Mentha Piperita (Peppermint) fragrance (1:50, 2% in petrolatum)	Female patient with recurrent irritant rash after applying a Mentha Piperita (Peppermint) foot spray	Patch test	+ reaction on days 2 and 4. ⁷¹

REFERENCES

1. Andersen, F. A. Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. *Int J Toxicol*. 2001;20(3):61-73.
2. Nikitakis, J. and Lange B. International Cosmetic Ingredient Dictionary and Handbook Online Version (wINCI). <http://webdictionary.personalcarecouncil.org/jsp/Home.jsp>. Washington, DC. Last Updated 2017. Date Accessed 3-6-2017.
3. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of pulegone (CAS No. 89-82-7) in F344/N rats and B6C3F1 mice (gavage studies). NTP TR 563. NIH Publication No. 11-5905. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr563.pdf. Last Updated 2011.
4. Molck, A.-M. Poulsen M. Lauridsen S. T. and Olsen P. Lack of histological cerebellar changes in Wistar rats given pulegone for 28 days. Comparison of immersion and perfusion tissue fixation. *Toxicology Letters*. 1998;95:117-122.
5. Flavex Naturextrakte GmbH. 2015. Analytical Specification Peppermint LeafC02-se extract.
6. Anonymous. 2017. Certificate of analysis trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract.
7. United States Pharmacopeial Convention. Food Chemicals Codex. Tenth ed. Rockville, MD: The United States Pharmacopeial Convention, 2016.
8. Chen, M. Z. Trinnaman L. Bardsley K. St. Hilaire C. J. and Da Costa N. C. Volatile compounds and sensory analysis of both harvests of double-cut Yakima Peppermint (*Mentha piperita* L.). *Journal of Food Science*. 2011;76(7):C1032-C1038.
9. Personal Care Products Council. 2017. Safety assessment of Mentha Piperita Derived Ingredients as Used in Cosmetics (information on Mentha Piperita (Peppermint) Extract).
10. Anonymous. Summary information on Mentha Piperita (Peppermint) Leaf Extract and Mentha Piperita (Peppermint) Leaf Water. Unpublished data submitted by the Personal Care Products Council on 6-22-2017. 2017. pp.1-2.
11. Anonymous. 2017. Summary information Mentha Piperita (Peppermint) Leaf Extract and Mentha Piperita (Peppermint) Leaf Water.
12. Flavex Naturextrakte GmbH. 2015. Peppermint LeafC02-se extract.
13. Grigoleit, H. G. and Grigoleit P. Pharmacology and preclinical pharmacokinetics of peppermint oil. *Phytomedicine*. 2005;12:612-616.
14. Bertoli, A. Leonardi M. Krzyzanowska J. Oleszek W. and Pistelli L. In vitro production of M. x piperita not containing pulegone and menthofuran. *Acta Biochemica Polonica*. 2012;59(3):417-423.
15. The International Organization for Standardization (ISO). Oil of peppermint (*Mentha x piperita* L.). International Standard ISO 856. Second edition, 2006-04-15. Reference number ISO 856:2006(E). 2006. pp.1-12. Geneva, Switzerland:
16. European Medicines Agency. Public statement on the use of herbal medicinal products containing pulegone and menthofuran. http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2014/12/WC500179556.pdf. London. Last Updated 2014.
17. Dorman, H. J. D. Kosar M. Baser K. H. and Hiltunen R. Phenolic profile and antioxidant evaluation of *Mentha piperita* L. (peppermint) extracts. *Natural Product Communications*. 2009;4(4):535-542.
18. Zgrajka, W. Turska M. Rajtar G. Majdan M. and Parada-Turska J. Kynurenic acid content in anti-rheumatic herbs. *Annals of Agricultural and Environmental Medicine*. 2013;20(4):800-802.
19. Flavex Naturextrakte GmbH. 2015. Allergen compounds according to Cosmetic EU Regulation 1223/2009: Peppermint Leaf C02-se extract.
20. Gershenzon, J. McConkey M. E. and Croteau R. B. Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiology*. 2000;122:205-213.
21. Gruenwald, J. Brendler T. and Jaenicke C. PDR for Herbal Medicines. Fourth ed. Montvale, NJ: Thomson Healthcare, Inc., 2017.

22. Mihaljev, Z. Zivkov-Balos M. Cupic Z and Jaksic S. Levels of some microelements and essential heavy metals in herbal teas in Serbia. *Acta Poloniae Pharmaceutica - Drug Research*. 2014;71(3):385-391.
23. Ozbey, A. and Uygun U. Effect of drying on organophosphorus pesticide residues in peppermint (*Mentha piperita* L.). *Bull. Environ., Contam. Toxicol.* 2006;77:638-642.
24. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2017. Washington, D.C.: FDA.
25. Personal Care Products Council. 10-16-2017. Concentration of Use by FDA Product Category: Peppermint-Derived Ingredients.
26. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett.* 2011;205(2):97-104. PM:21669261.
27. Bremmer HJ, Prud'homme de Lodder LCH, and van Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 20200. <http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf>. Date Accessed 8-24-2011. Report No. RIVM 320104001/2006. pp. 1-77.
28. Rothe H. Special aspects of cosmetic spray evaluation. Unpublished information presented to the 26 September CIR Expert Panel. Washington D.C. 2011.
29. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing*. 2004;14(11):24-27. <http://www.spraytechnology.com/index.mv?screen=backissues>.
30. Aylott RI, Byrne GA, Middleton, J, and Roberts ME. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci.* 1979;1(3):177-186. PM:19467066.
31. Russell RS, Merz RD, Sherman WT, and Sivertson JN. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol.* 1979;17(2):117-122. PM:478394.
32. CIR Science and Support Committee of the Personal Care Products Council (CIR SSC). 11-3-2015. Cosmetic Powder Exposure.
33. Food and Drug Administration (FDA). Inactive ingredient search for approved drug products. <http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm>. Last Updated 2017. Date Accessed 2-23-2017.
34. Food and Drug Administration (FDA). Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. 21 CFR 310.545. <http://www.accessdata.fda.gov/SCRIPTs/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=310.545>. Last Updated 2016. Date Accessed 2-27-2017.
35. United States Environmental Protection Agency (EPA). List of active ingredients eligible for minimum risk pesticide products. <https://www.epa.gov/sites/production/files/2015-12/documents/minrisk-active-ingredients-tolerances-2015-12-15.pdf>. Last Updated 2015. Date Accessed 2-23-2017.
36. European Medicines Agency Committee on Herbal Medicinal Products (HMPC). Community herbal monograph on *Mentha x piperita* L., aetheroleum. http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Community_herbal_monograph/2010/01/WC500059313.pdf. Last Updated 2007. Date Accessed 2-24-2017.
37. Muhammad, F. Wiley J. and Riviere J. E. Influence of some plant extracts on the transdermal absorption and penetration of marker penetrants. <http://dx.doi.org/10.3109/15569527.2016.1147456>. Cutaneous and Ocular Toxicology. Last Updated 2016. Date Accessed 5-16-2016.
38. Nielsen, J. B. Natural oils affect the human skin integrity and the percutaneous penetration of benzoic acid dose-dependently. *Basic Clin Pharmacol Toxicol.* 2006;98(6):575-581.
39. Lazutka, J. R., Mierauskiene, J, Slapsyte, G, and Dedonyte, V. Genotoxicity of dill (*Anethum graveolens* L.), peppermint (*Mentha x piperita* L.) and pine (*Pinus sylvestris* L.) essential oils in human lymphocytes and *Drosophila melanogaster*. *Food Chem Toxicol.* 2001;39(5):485-492.
40. Consumer Product Testing Co. 2017. Bacterial reverse mutation assay (trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract).
41. Samarth, R. M. and Kumar A. *Mentha piperita* (Linn.) leaf extract provides protection against radiation induced chromosomal damage in bone marrow of mice. *Indian Journal of Experimental Biology.* 2003;41:229-237.

42. Samarth, R. M. Panwar M. and Kumar A. Modulatory effects of *Mentha piperita* on lung tumor incidence, genotoxicity, and oxidative stress in benzo[a]pyrene-treated Swiss albino mice. *Environmental and Molecular Mutagenesis*. 2006;47:192-198.
43. Kumar, A. Samarth R. M. Yasmeen S. Sharma A. Sugahara T. Terado T. and Kimura H. Anticancer and radioprotective potentials of *Mentha piperita*. *BioFactors*. 2004;22:87-91.
44. Jain, D. Pathak N. Khan S. Raghuram G. V. Bhargava A. Samarth R. and Mishra P. K. Evaluation of cytotoxicity and anticarcinogenic potential of *Mentha* leaf extracts. *International Journal of Toxicology*. 2011;30(2):225-236.
45. Yi, W. and Wetzstein H. Y. Anti-tumorigenic activity of five culinary and medicinal herbs grown under greenhouse conditions and their combination effects. *J.Sci.Food Agric*. 2011;91:1849-1854.
46. Hussain, A. I. Anwar F. Nigam P. S. Ashraf M. and Gilanif A. H. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *J.Sci.Food Agric*. 2010;90:1827-1836.
47. Sun, Z. Wang H. Wang J. Zhou L. and Yang P. Chemical composition and anti-inflammatory, cytotoxic, and antioxidant activities of essential oil from leaves of *Mentha piperita* grown in China. *PLoS One*. 2014;9(12):e114767
48. Liu, X. Sun Z. L. Jia A. R. Shi Y. P. Li R. H. and Yang P. M. Extraction, preliminary characterization and evaluation of *in vitro* antitumor and antioxidant activities of polysaccharides from *Mentha piperita*. *Int.J.Mol.Sci*. 2014;15:16302-16319.
49. Liu, Y. Flynn T. J. Ferguson M. S. Hoagland E. M. and Yu L. Effects of dietary phenolics and botanical extracts on hepatotoxicity-related endpoints in human and rat hepatoma cells and statistical models for prediction of hepatotoxicity. *Food and Chemical Toxicology*. 2011;49:1820-1827.
50. Akdogan, M. Kilinc I. Oncu M. Karaoz E. and Delibas N. Investigation of biochemical and histopathological effects of *Mentha piperita* L. and *Mentha spicata* L. on kidney tissue in rats. *Human and Experimental Toxicology*. 2003;22:213-219.
51. Inoue, T. Sugimoto Y. Masuda H. and Kamei C. Effects of peppermint (*Mentha piperita* L.) extracts on experimental allergic rhinitis in rats. *Biol.Pharm.Bull*. 2001;24(1):92-95.
52. Oh, J. Y., Park, MA, and Kim, YC. Peppermint Oil Promotes Hair Growth without Toxic Signs. *Toxicol Res*. 2014;30(4):297-304.
53. Derma Consult GmbH. 2015. Human patch test of a lipstick containing 0.2961% *Mentha Piperita* (Peppermint) Leaf Extract.
54. Peritesco. Ocular and cutaneous acceptability study of a cleansing gel (containing 50% *Mentha Piperita* (Peppermint) Leaf Water) during 4 weeks under ophthalmological and dermatological control. Unpublished data submitted by the Personal Care Products Council on 3/1/2016. 2008. pp.1-42.
55. 4-Front Research. Single patch test of a cleansing gel containing 50% *Mentha Piperita* (Peppermint) Leaf water. Unpublished data submitted by the Personal Care Products Council on 3/1/2016. 2007. pp.1-23.
56. Consumer Product Testing Company. Repeated insult patch test on 20% *Mentha Piperita* (Peppermint) Oil. Unpublished data submitted by the Research Institute for Fragrance Materials (RIFM) on 3/13/2017. 2001. pp.1-16.
57. TKL Research, Inc. Repeated insult patch test on 20% *Mentha Piperita* (Peppermint) Oil. Unpublished data submitted by the Research Institute for Fragrance Materials (RIFM) on 3/13/2017. 2002. pp.1-20.
58. Consumer Product Testing Co. 2017. Repeated insult patch of a trade name mixture containing 2.5% *Mentha Piperita* (Peppermint) Extract.
59. Consumer Product Testing Co. 2012. Executive summary of a repeated insult patch test of cosmetic product (off-white cream containing 0.00554% *Mentha Piperita* (Peppermint) Extract).
60. Ivy Laboratories (KGL Inc). 2011. Executive summary of a human maximization test of an off-white cream containing 0.00554% *Mentha Piperita* (Peppermint) Extract.
61. Institut d'Expertise Clinique. Sensitization and cutaneous compatibility study (face cream containing 20% *Mentha Piperita* (Peppermint) Leaf Water). Unpublished data submitted by the Personal Care Products Council on 3/1/2016. 2010. pp.1-11.

62. Kanerva, L., Rantanen, T, Aalto-Korte, K, Estlander, T, Hannuksela, M, Harvima, RJ, Hasan, T, Horsmanheimo, M, Jolanki, R, Kalimo, K, Lahti, A, Lammintausta, K, Lauerma, A, Niinimki - Section of Dermatology, FloOHHF, Turjanmaa, K, and Vuorela, AM. A multicenter study of patch test reactions with dental screening series. *Am J Contact Dermat.* 2001;12(2):83-87.
63. Consumer Product Testing Co. 2017. The MatTek Corporation sub-Draize mildness testing (MTT ET-50) using the EpiOcular™ tissue model in vitro toxicity testing system (trade name mixture containing 2.5% Mentha Piperita (Peppermint Extract)).
64. Warshaw, E. M. Zug K. A. Belsito D. V. Fowler J. F. Jr. DeKoven J. G. Sasseville D. Maibach H. I. Mathias C. G. T. DeLeo V. A. Taylor J. S. Fransway A. F. Marks J. G. Jr. Pratt M. D. Zirwas M. J. Geier J. and Uter W. Positive patch-test reactions to essential oils in consecutive patients: Results from North America and Central Europe. *Dermatitis.* 2017;28(4):246-252.
65. Lewis, F. M., Shah, M, and Gawkrödger, DJ. Contact sensitivity to food additives can cause oral and perioral symptoms. *Contact Dermatitis.* 1995;33(6):429-430.
66. Fleming, C. J. and Forsyth A. Short communications. D5 patch test reactions to menthol and peppermint oil. *Contact Dermatitis.* 1998;38:337
67. Foti, C., Conserva, A, Antelmi, A, Lospalluti, L, and Angelini, G. Contact dermatitis from peppermint and menthol in a local action transcutaneous patch. *Contact Dermatitis.* 2003;49(6):312-313.
68. Vermaat, H., van, MT, Rustemeyer, T, Bruynzeel, DP, and Kirtschig, G. Vulval allergic contact dermatitis due to peppermint oil in herbal tea. *Contact Dermatitis.* 2008;58(6):364-365.
69. Tran, A., Pratt, M, and DeKoven, J. Acute allergic contact dermatitis of the lips from peppermint oil in a lip balm. *Dermatitis.* 2010;21(2):111-115.
70. Guin, J. D. Rosemary cheilitis: one to remember. *Contact Dermatitis.* 2001;45:63
71. Kalavala, M., Hughes, TM, Goodwin, RG, Anstey, AV, and Stone, NM. Allergic contact dermatitis to peppermint foot spray. *Contact Dermatitis.* 2007;57(1):57-58.
72. Bayat, R. and Borici-Mazi R. A case of anaphylaxis to peppermint. *Allergy, Asthma and Clinical Immunology.* 2014;10(6):1-3.
73. Szema, A. M. and Barnett T. Allergic reaction to mint leads to asthma. *Allergy Rhinol.* 2011;2:43-45.
74. Amjadia, M. A. Mojab F. and Kamranpoura S. B. Th effect of peppermint oil on symptomatic treatment of pruritus in pregnant women. *Iranian Journal of Pharmaceutical Research.* 2012;11(4):1073-1077.
75. Kearns, G. L., Chumpitazi, BP, Abdel-Rahman, SM, Garg, U, and Shulman, RJ. Systemic exposure to menthol following administration of peppermint oil to paediatric patients. *BMJ.* 2015;5(8):1-6.
76. Tisserand, R. and Young R. Essential Oil Safety. A Guide for Health Care Professionals. 2nd ed. New York: Elsevier, 2014.
77. Wallace, R. B. Gryzlak B. M. Zimmerman M. B. and Nisly N. L. Application of FDA adverse event report data to the surveillance of dietary botanical supplements. *The Annals of Pharmacotherapy.* 2008;42:653-660.
78. International Agency for Research on Cancer (IARC). IARC Monograph on Pulegone. <https://monographs.iarc.fr/ENG/Monographs/vol108/mono108-05.pdf>. Last Updated 2017.
79. Da Rocha, M. S. Dodmane P. R. Arnold L. L. Pennington K. L. Anwar M. M. Adams B. R. Taylor S. V. Wermes C. Adams T. B. and Cohen S. M. Mode of action of pulegone on the urinary bladder of F344 rats. *Toxicol.Sci.* 2012;128(1):1-8.
80. Ale, S. O. Hostynek J. J. and Maibach H. L. Menthol: A review of its sensitization potential. *Exog.Dermatol.* 2002;1:74-80.

2017 FDA VCRP Data**Mentha Piperita (Peppermint) Oil**

01B - Baby Lotions, Oils, Powders, and Creams	1
02A - Bath Oils, Tablets, and Salts	24
02B - Bubble Baths	2
02D - Other Bath Preparations	8
03G - Other Eye Makeup Preparations	4
04B - Perfumes	1
04E - Other Fragrance Preparation	17
05A - Hair Conditioner	43
05B - Hair Spray (aerosol fixatives)	1
05F - Shampoos (non-coloring)	57
05G - Tonics, Dressings, and Other Hair Grooming Aids	31
05H - Wave Sets	1
05I - Other Hair Preparations	15
07E - Lipstick	88
07I - Other Makeup Preparations	34
08B - Cuticle Softeners	2
08E - Nail Polish and Enamel	2
08G - Other Manicuring Preparations	3
09A - Dentifrices	34
09B - Mouthwashes and Breath Fresheners	19
09C - Other Oral Hygiene Products	73
10A - Bath Soaps and Detergents	43
10B - Deodorants (underarm)	2
10E - Other Personal Cleanliness Products	26
11A - Aftershave Lotion	3
11B - Beard Softeners	2
11D - Preshave Lotions (all types)	2
11E - Shaving Cream	10
11G - Other Shaving Preparation Products	2
12A - Cleansing	33
12C - Face and Neck (exc shave)	30
12D - Body and Hand (exc shave)	45
12E - Foot Powders and Sprays	9
12F - Moisturizing	56
12G - Night	1
12H - Paste Masks (mud packs)	17
12I - Skin Fresheners	13
12J - Other Skin Care Preps	73
Total	827

Mentha Piperita (Peppermint) Leaf (No Posting)**Mentha Piperita (Peppermint) Leaf Extract**

02D - Other Bath Preparations	1
03D - Eye Lotion	4

04C - Powders (dusting and talcum, excluding aftershave talc)	1
04E - Other Fragrance Preparation	4
05A - Hair Conditioner	23
05B - Hair Spray (aerosol fixatives)	1
05F - Shampoos (non-coloring)	18
05G - Tonics, Dressings, and Other Hair Grooming Aids	3
05I - Other Hair Preparations	5
07B - Face Powders	2
07C - Foundations	2
07D - Leg and Body Paints	1
07E - Lipstick	5
07I - Other Makeup Preparations	2
08F - Nail Polish and Enamel Removers	1
08G - Other Manicuring Preparations	1
09A - Dentifrices	1
09B - Mouthwashes and Breath Fresheners	1
10A - Bath Soaps and Detergents	6
10E - Other Personal Cleanliness Products	6
11A - Aftershave Lotion	3
11G - Other Shaving Preparation Products	1
12A - Cleansing	14
12C - Face and Neck (exc shave)	24
12D - Body and Hand (exc shave)	5
12E - Foot Powders and Sprays	1
12F - Moisturizing	17
12G - Night	2
12H - Paste Masks (mud packs)	8
12I - Skin Fresheners	10
12J - Other Skin Care Preps	24
13A - Suntan Gels, Creams, and Liquids	1
Total	198

Mentha Piperita (Peppermint) Leaf Water

03G - Other Eye Makeup Preparations	2
10A - Bath Soaps and Detergents	1
10B - Deodorants (underarm)	1
11G - Other Shaving Preparation Products	1
12A - Cleansing	3
12C - Face and Neck (exc shave)	1
12F - Moisturizing	3
12H - Paste Masks (mud packs)	1
12I - Skin Fresheners	1
12J - Other Skin Care Preps	1
Total	15

Mentha Piperita (Peppermint) Extract

02A - Bath Oils, Tablets, and Salts	1
05A - Hair Conditioner	1
05E - Rinses (non-coloring)	1
05F - Shampoos (non-coloring)	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	1
07E - Lipstick	2
07I - Other Makeup Preparations	2
09B - Mouthwashes and Breath Fresheners	2
10A - Bath Soaps and Detergents	2
10B - Deodorants (underarm)	1
10E - Other Personal Cleanliness Products	3
11A - Aftershave Lotion	1
11E - Shaving Cream	3
12A - Cleansing	4
12C - Face and Neck (exc shave)	10
12D - Body and Hand (exc shave)	2
12E - Foot Powders and Sprays	3
12F - Moisturizing	17
12H - Paste Masks (mud packs)	10
12I - Skin Fresheners	1
12J - Other Skin Care Preps	3
Total	71



Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: November 27, 2017

SUBJECT: Draft Final Amended Report: Safety Assessment of *Mentha piperita* (Peppermint)-derived Ingredients as Used in Cosmetics (draft prepared for the December 4-5, 2017 CIR Expert Panel Meeting)

Key Issue

The Introduction states “the Panel noted that much of the data on this ingredient [peppermint oil] are considered relevant to the entire group”. If this is true, more of the ingredients in the report should have the same conclusion as peppermint oil.

Additional Considerations

Composition/Impurities - It is not clear why there are two subsections in this section on *Mentha Piperita* (Peppermint) Leaf Extract, Leaf and Leaf Water. It should be made clear that the newly added chromatographic profile of a *Mentha Piperita* (Peppermint) Leaf Extract is for the CO₂ extract.

Cosmetic Use - As there is more than one ingredient in this report, “this ingredient” needs to be revised to “these ingredients”.

Exposure Assessment, Risk Assessment - It is not clear why these two subsections are presented under the Chronic section.

Risk Assessment - Tisserand and Young (2014) recommend that peppermint oil should be avoided in cases of cardiac fibrillation and in individuals with glucose-6-phosphate dehydrogenase deficiency because of its menthol content (see menthol summary in the *Essential Oil Safety* book).

Cytotoxicity, *Mentha Piperita* (Peppermint) Oil - Although reference 45 may have stated that they “extracted” the essential oil, the material should be called an essential oil rather than an extract.

Cytotoxicity *Mentha piperita* - What type of preparation was studied in reference 46? The title says “polysaccharides from *Mentha piperita*”. This should be made clear in the description of this study.

Multicenter Studies - Please provide a reference for the ICDRG study in which 74 patients were patch tested with *Mentha Piperita* (Peppermint) Oil.

Summary - The details of the NACDG study need to be corrected to reflect the changes made in the description of this study earlier in the report (only 71 patients were tested with peppermint oil with a reaction rate of 0.53%).

Table 1, Carcinogenicity - It is not clear why the NTP bioassay on menthol is presented under the heading "Menthone".

Table 1, Skin Sensitization - What was the conclusion of the review article on the sensitization potential of menthol?



Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Jay Ansell, Ph.D. */JA*
Industry Liaison to the CIR Expert Panel

DATE: January 8, 2017

SUBJECT: Revised Tentative Amended Report: Safety Assessment of *Mentha piperita* (Peppermint)-derived Ingredients as Used in Cosmetics

The Council respectfully submits the following comments on the revised tentative amended report, Safety Assessment of *Mentha piperita* (Peppermint)-derived Ingredients as Used in Cosmetics.

Although there may be variations in spelling of 1,8-cineole (I,8-cineol) it should be spelled the same way throughout the report, e.g., Composition and impurities section it is spelled both as 1,8-cineol and I,8-cineole.

Method of Manufacture, Mentha Piperita (Peppermint) Leaf Extract - In the presentation of the method of manufacture for the Leaf Extract sold as a powder, the following sentence should be deleted: "The preparation of this material may be similar to the preparation of some of the ingredients in this report." The method of manufacture came from a company that sells cosmetic ingredients. They provided a description of a method of manufacture of a cosmetic ingredient (not a method "similar to the preparation" of cosmetic ingredients).

Penetration Enhancement, Penetration Inhibition - The identity of the receptor fluid should be stated in the descriptions of the *in vitro* dermal penetration studies (references 37 and 38).

ADME, Human, Oral, Mentha Piperita (Peppermint) Oil, old report summary - Please revise "180 mg of an enteric-coated Mentha Piperita (Peppermint) Oil-coated capsule" to "180 mg of Mentha Piperita (Peppermint) Oil in an enteric-coated capsule".

Sensitization, Human - The multicenter study (reference 62) is presented in the Clinical Study section, and in Table 6 and does not also need to be presented in the sensitization section. If it is left in the Sensitization section, the other multicenter study (reference 64) should also be mentioned in the Sensitization section.

Other Clinical Reports, Oral, Summary - The triple-blind study in pregnant women with itchy skin is not an oral study and it should be presented in a dermal exposure subsection.

Although the English used in the study (reference 74) is not precise, the study does state: "They should use the drug as topical twice a day for the area which was affected by skin itching." The subjects were given 60 ml of sesame oil with or without 0.5% peppermint oil to use on the skin twice a day over a 2 week period. The CIR report incorrectly states that the "subjects were instructed to consume 60 ml of peppermint oil (0.5% in sesame oil) and identical placebos, respectively, twice per day for 2 weeks." The described intake would result in a dose of peppermint oil of about 0.6 g (or 600 mg) each day. In addition, topical peppermint oil for the treatment of itchy skin has been the subject of at least one other study¹ that is not cited in the CIR report.

Summary - Please state the concentrations of peppermint oil that were cytotoxic to human cancer cell lines, as at high enough concentrations all materials can be cytotoxic.

Discussion - The ISO standard for peppermint oil with a level of 0.5-3% pulegone should be mentioned in the Discussion.

In addition to mentioning the positive NTP bioassay on pulegone, (0.5-3% of peppermint oil), the Discussion should also mention the negative NTP bioassay on menthol (32-49% of peppermint oil).

Table 1 - The title of the table should be revised as not all of the studies presented in the table were in the original report. The title should be "Toxicity Data on Components of *Mentha Piperita* (Peppermint)-Derived Ingredients (studies cited to reference 1 were described in the original CIR report)"

Table 1 - The conclusion of the NTP bioassay on menthol should be clearly stated (no evidence of carcinogenicity in rats or mice of either sex).

In the protocol description of the NTP bioassay on pulegone, "5" needs to be deleted after the doses for male rats. Please state the basis for the IARC conclusion for pulegone.

Table 4 - Reference 19, titled "Allergen compounds according to Cosmetic EU Regulation 1223/2009: Peppermint Leaf CO₂ Extract" is about the extract not the oil. In the title of Table 4, it needs to be moved from the oil to the extract (or moved to the subheading Allergen Compounds).

¹Elsaie LT, El Mohsen AM, Ibrahim IM, et al. 2016. Effectiveness of topical peppermint oil on symptomatic treatment of chronic pruritus. *Clin Cosmet Invest Dermatol*, 9: 333-338.

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8.¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 29 flavouring substances in the Flavouring Group Evaluation 18, as laid down in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have a genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. This revision deals with new toxicity data on the supporting substance myrcene [FL-no: 01.008], providing an appropriate NOAEL for the evaluation of candidate substance [FL-no: 02.146]. The Panel concluded that all 29 substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.146, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. However, based on mTAMDI calculations, for all candidate substances except [FL-no: 02.146] more reliable intake data are required for a re-evaluation. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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KEY WORDS

flavourings, safety, aliphatic, alicyclic, saturated, unsaturated, tertiary alcohols, FGE.18

¹ On request from the European Commission, Question No EFSA-Q-2013-00852, adopted on 07 May 2015.

² Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Konrad Grob, Rainer Gürtler, Trine Husøy, Wim Mennes, Maria Rosaria Milana, André Penninks, Vittorio Silano, Andrew Smith, Maria de Fátima Tavares Poças, Christina Tlustos, Fidel Toldrá, Detlef Wölfe and Holger Zorn. Correspondence: fip@efsa.europa.eu

³ Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby and EFSA staff: Annamaria Rossi, Maria Carfi and Maria Anastassiadou for the support provided to this scientific opinion.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8. EFSA Journal 2015;13(5):4118, 115 pp. doi:10.2903/j.efsa.2015.4118

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 29 flavouring substances in the Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 29 flavouring substances belong to chemical groups 6 and 8, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision 3 of FGE.18 (FGE.18Rev3) is due to the availability of new toxicity data for myrcene, [FL-no: 01.008] which was used for the safety evaluation of the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

FGE.18Rev3 deals with 29 saturated and unsaturated aliphatic acyclic and alicyclic tertiary alcohols, aromatic tertiary alcohols and their esters. Based on their structures, the candidate substances can be subdivided into 8 subgroups.

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four substances [FL-nos: 02.129, 02.147, 02.168 and 02.197] the stereoisomeric composition of the mixture has not been specified.

Seventeen of the 29 candidate substances are classified into structural class I, eleven into structural class II and one into structural class III according to the decision tree approach.

Twenty-one out of the 29 candidate substances have been reported to occur in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, 28 of the 29 flavouring substances in this group have intakes in Europe that vary from 0.0012 to 27 $\mu\text{g}/\text{capita}$ per day, which are below the thresholds of concern for structural classes I, II and III substance of 1800, 540 and 90 μg per person per day, respectively. The remaining substance, terpineol [FL-no: 02.230] belonging to structural class I, has an MSDI of 1200 $\mu\text{g}/\text{capita}$ per day, which is also below the threshold for this structural class.

2-Methylpropan-2-ol [FL-no: 02.052] provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence and the lack of structural alerts for genotoxicity for this substance and its metabolites do not raise concern for *in vivo* genotoxicity. For the other substances in this group the available data considered valid do not give rise to any safety concerns with respect to genotoxicity.

Twenty-eight of the candidate substances are anticipated to be metabolised to innocuous products. For the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no metabolism data are available and therefore it cannot be predicted to be metabolised to innocuous products.

In Revision 2 of FGE.18, for substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no appropriate NOAEL was available and additional data were requested. In the present revision of FGE.18 (Revision 3), new toxicity data on the supporting substance myrcene [FL-no: 01.008] became available, providing an appropriate NOAEL of 44 mg/kg bw per day which was used for the evaluation of the candidate substance [FL-no: 02.146]. Based on the MSDI intake of 12 µg/capita per day, the margin of safety calculated for [FL-no: 02.146] is 2.2×10^5 .

Therefore, none of the 29 candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] would give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances, on the basis of the default MSDI approach.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for 25 candidate substances. However, information on purity criteria and/or stereoisomeric composition has not been specified sufficiently for four substances [FL-no: 02.129, 02.147, 02.168 and 02.197]. Thus, the final evaluation of the materials of commerce cannot be performed for these substances, pending further information.

Thus, for 25 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach: [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.146, 02.149, 02.150, 02.171, 02.181, 02.184, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808].

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI approach, are 3900 µg per person per day, and for the two remaining substances 7000 and 14000 µg per person per day respectively. These seventeen mTAMDI are all above the threshold of concern of 1800 µg per person per day for a structural class I substance. For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg per person per day and for the remaining two substances, 110 and 1600 µg per person per day, respectively. For ten substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg per person per day. For the one substance assigned to structural class III the mTAMDI is 5700 µg per person per day, which is above the threshold of concern for structural class III substances of 90 µg per person per day.

In conclusion, for all candidate substances except [FL-no: 02.146] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008⁴ on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012⁵. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000.⁶

FGE.78Rev1

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2.⁷

The substances [FL-nos: 01.008, 01.018, 01.040 and 01.061] were among the 14 substances for which the Panel had “reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing information on stereoisomerism/composition of mixture)” and also among those for which “additional toxicity data was requested”.

FGE.25Rev2

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31.⁸

The substances with [FL-nos: 01.035, 01.064, 01.070 and 01.035] were among the 27 candidate substances for which “additional toxicity data” were required by EFSA. For [FL-no: 01.035] also “additional information on composition” was requested.

FGE.18Rev2

On 30 September 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8.

For the flavouring substance [FL-no: 02.146], the Panel considered that “additional data” are needed including “information on specifications/stereoisomerism/composition of mixture”.

⁴ Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p.34-50.

⁵ Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1-161.

⁶ Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8-16.

⁷ EFSA Journal 2011;9(6):2178

⁸ EFSA Journal 2011;9(6):2177

On 21 November 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted a statement on the re-evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] based on additional data on a supporting substance.⁹

The Panel concluded that “linalool [FL-no: 02.013] is not sufficiently structurally related to 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for a re-evaluation of [FL-no: 02.146]. Accordingly, a 90-day study on 3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] or on a sufficiently structurally related substance has to be provided in order to establish on appropriate NOAEL”.

New data and relationship with other substances

On 5 and 11 July 2013, the applicant submitted additional data on the following acyclic terpene hydrocarbons [FL-nos: 01.008, 01.018, 01.040, 01.061, 01.035, 01.064, 01.070 and 02.146] represented by myrcene [FL-no: 01.008].

As regards the related substances also evaluated in these opinions, namely [FL-nos: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026, 01.029 and 01.059], data was submitted and are currently being evaluated (EFSA-Q-2013-00185 to – 00193).

As regards substance with [FL-no: 01.014], data should be submitted by 31 December 2013.¹⁰

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment on this group of flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

SUPPORTING DOCUMENTS

Submission by the European Flavour Association

INTERPRETATION OF THE TERMS OF REFERENCE

The above background and terms of reference include also a previous mandate received from the European Commission on 6 February 2013.¹¹ The present scientific opinion FGE.18Rev3 covers the safety assessment of the following flavouring substance: 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

⁹ EFSA Journal 2012;10(12):2995

¹⁰ This substance is in the process of being deleted from the Union List (DG SANTE, 2015)

¹¹ SANCO.E3/SH/km D (2013) Ares(2013)15188

ASSESSMENT

1. History of the Evaluation of the Substances in the Present FGE

The first version of the Flavouring Group Evaluation 18, FGE.18, dealt with 24 aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters.

The first Revision of FGE.18, FGE.18Rev1, included the assessment of six additional candidate substances [FL-nos: 02.120, 02.206, 02.226, 02.230, 02.253 and 09171]. Information on toxicity and/or metabolism on three of the substances were included [FL-nos: 02.120, 02.206 and 02.230]. Since the publication of FGE.18 additional information on specifications on 12 substances became available [FL-nos: 02.052, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.191, 02.197, 09.614, 09.617 and 09.671].

The second Revision of FGE.18, FGE.18Rev2, included the assessment of two additional candidate substances [FL-nos: 02.129 and 02.146]. Information on toxicity and/or metabolism on the substance [FL-no: 02.129] was included. No information on toxicity and/or metabolism was available for [FL-no: 02.146]. A search in open literature did not provide any further data on toxicity or metabolism for these substances. Since the publication of FGE.18Rev1 additional information on toxicity on [FL-nos: 02.120, 02.140, 02.144 and 02.197] was included. Furthermore, additional information on specifications on six substances was included [FL-nos: 02.147, 02.168, 02.197, 02.226, 02.230 and 02.253] (EFFA, 2010). Based on the additional information made available by Industry, it was also considered appropriate to merge subgroups 7 and 8 into one (subgroup 7).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.18	3 March 2006	http://www.efsa.europa.eu/en/scdocs/scdoc/331.htm	24
FGE.18Rev1	29 January 2008	http://www.efsa.europa.eu/en/scdocs/scdoc/978.htm	30
FGE.18Rev2	30 September 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1847.htm	32
FGE.18Rev3	7 May 2015	http://www.efsa.europa.eu/en/efsajournal/pub/4118.htm	29

The present Revision of FGE.18, FGE.18Rev3, includes the safety assessment of (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for which a 90-day toxicity study on a supporting substance has been provided. No data on toxicity and/or metabolism are available for the substance itself and a search in the open literature did not provide any pertinent information either. In the EFSA statement of 2012 (EFSA, 2012) the Panel has proposed a suitable supporting substance for [FL-no: 02.146], namely myrcene [FL-no: 01.008]. The safety evaluation of myrcene has been finalised in FGE.78Rev2 based on a new 90-day toxicity study (Bauter, 2013) for this substance. These new data will accordingly be used in the safety evaluation of (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146]. Substances in subgroup 3 with [FL-nos: 02.185, 02.191 and 09.669] are no longer used as flavouring substances in the EU according to the Industry (DG SANCO, 2012) and therefore they will not be considered in the present FGE.

1.1. Description

The present Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000) (The Procedure – shown in schematic form in Appendix B of this FGE), deals with 29 saturated and unsaturated aliphatic acyclic and alicyclic tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000). The 29 flavouring substances under consideration (candidate substances), with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

The present FGE consists of seven aliphatic saturated tertiary alcohols and one ester of such [FL-nos: 02.041, 02.052, 02.147, 02.181, 02.184, 02.219, 02.253 and 09.356]; five are aliphatic unsaturated tertiary alcohols which possess isolated terminal double bonds and two are esters thereof [FL-nos: 02.123, 02.144, 02.150, 02.168, 02.226, 09.614 and 09.671]; one aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond [FL-no: 02.146]; one aliphatic unsaturated tertiary alcohol which does not possess terminal double bonds [FL-no: 02.140]; two monocyclic saturated tertiary alcohols and one ester thereof [FL-nos: 02.054, 02.171 and 09.617]; two monocyclic unsaturated tertiary alcohols [FL-nos: 02.129 and 02.230]; two mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond [FL-nos: 02.149 and 02.206]; one bicyclic unsaturated ester [FL-no: 09.808], one tricyclic saturated ester [FL-no: 09.171] and two bi- and tricyclic tertiary alcohols [FL-nos: 02.197 and 02.120] and one tertiary alcohol with an aromatic substituent [FL-no: 02.203].

A summary of the safety evaluation is summarised in Appendix A, Table 7.

The hydrolysis products of the candidate esters are listed in Appendix A, Table 8.

The 29 candidate substances are closely related structurally to 26 flavouring substances (supporting substances). Twenty-three of these were evaluated in the group of “Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances” and one, menthol, was evaluated in the group “Substances structurally related to menthol” at the 51st JECFA meeting (JECFA, 2000a). Two supporting substances were evaluated at the 63rd JECFA meeting (JECFA, 2005b), one in the group “Aliphatic and alicyclic hydrocarbons” and the other in the group “Aromatic hydrocarbons”. The names and structures for the 26 supporting substances are listed in Appendix A, Table 9, together with their evaluation status.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the FGE.18Rev3

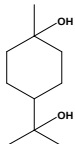
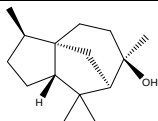
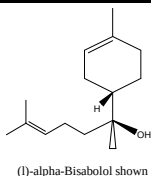
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.041	2-Methylbutan-2-ol		515 75-85-4	Liquid C ₅ H ₁₂ O 88.15	Slightly soluble Freely soluble	102 MS 96 %	1.402-1.408 0.805-0.813	
02.052	2-Methylpropan-2-ol		698 75-65-0	Liquid C ₄ H ₁₀ O 74.12	Slightly soluble Freely soluble	82 MS 95 %	1.384-1.390 0.780-0.790	
02.054	p-Menthane-1,8-diol		701 80-53-5	Solid C ₁₀ H ₂₀ O ₂ 172.27	Slightly soluble Freely soluble	260 116 MS 95 %	n.a. n.a.	
02.120 2030	(+)-Cedrol		4503 10190 77-53-2	Solid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Freely soluble	286 86 MS 95 %	n.a. n.a.	
02.123	2-Methylbut-3-en-2-ol		11794 115-18-4	Liquid C ₅ H ₁₀ O 86.13	Practically insoluble or insoluble Freely soluble	97 MS 98 %	1.413-1.419 0.818-0.827	
02.129 2031	(l)-alpha-Bisabolol	 (l)-alpha-Bisabolol shown	4666 10178 23089-26-1	Liquid C ₁₅ H ₂₆ O 222.37	Insoluble Soluble	^(g) MS >94 %	1.493-1.499 0.927-0.935	Secondary components (no amount given): (d)-alpha- Bisabolol (CAS No. 23178-88-3), (l)-epi-alpha-

Table 1: Specification Summary of the Substances in the FGE.18Rev3

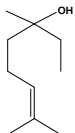
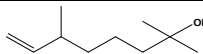
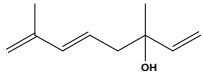
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
								Bisabolol (CAS No.78148-59-1), (d)-epi-alpha-Bisabolol (CAS No. 76738-75-5). Stereoisomeric composition to be specified.
02.140	1,2-Dihydrolinalool		2270-57-7	Liquid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	90 (16 hPa) MS 95 %	1.452-1.458 0.857-0.863	Racemate.
02.144	2,6-Dimethyloct-7-en-2-ol		18479-58-8	Liquid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	86 (15 hPa) MS 95 %	1.434-1.440 0.824-0.830	Racemate.
02.146	(E)-3,7-Dimethylocta-1,5,7-trien-3-ol ^(f)		10202 53834-70-1	Liquid C ₁₀ H ₁₆ O 152.24	Insoluble	62 (6 hPa) MS 93 %	1.489-1.495 0.878-0.886	Racemate; Secondary components 2-3 % linalool, 1-2 % linalool oxide and up to 1 % nerol oxide (Flavour Industry, 2012).

Table 1: Specification Summary of the Substances in the FGE.18Rev3

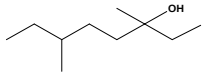
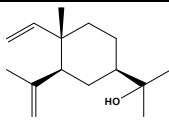
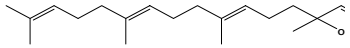
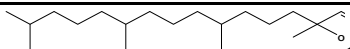
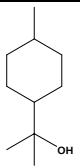
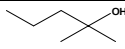
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.147	3,6-Dimethyloctan-3-ol ^(f)		151-19-9	Liquid C ₁₀ H ₂₂ O 158.28	Practically insoluble or insoluble Freely soluble	195 MS 95 %	1.434-1.440 0.831-0.837	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.149	(-)-alpha-Elemol		10205 639-99-6	Solid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Freely soluble	123 (5 hPa) 50 MS 95 %	n.a. n.a.	Register name (1R,3S,4S)- isomer.
02.150	(E,E)-Geranyl linalool		1113-21-9	Solid C ₂₀ H ₃₄ O 290.49	Practically insoluble or insoluble Freely soluble	153 (5 hPa) 51 MS 95 %	1.484-1.490 0.878-0.881	Racemate of (6E,10E)-isomer.
02.168	Isophytol ^(f)		10233 505-32-8	Liquid C ₂₀ H ₄₀ O 296.54	Practically insoluble or insoluble Freely soluble	138 (0.1 hPa) MS 95 %	1.457-1.462 0.847-0.853	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.171	p-Menthan-8-ol		498-81-7	Solid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	208 35 MS 95 %	1.460-1.466 0.909-0.915	
02.181	2-Methylpentan-2-ol		10274 590-36-3	Liquid C ₆ H ₁₄ O 102.18	Slightly soluble Freely soluble	122 MS 95 %	1.409-1.415 0.823-0.829	

Table 1: Specification Summary of the Substances in the FGE.18Rev3

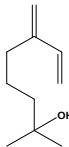
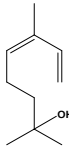
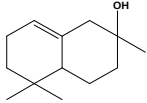
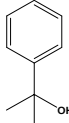
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.184	3-Methylpentan-3-ol		10277 77-74-7	Liquid C ₆ H ₁₄ O 102.18	Practically insoluble or insoluble Freely soluble	121 MS 95 %	1.415-1.421 0.824-0.830	
02.185	Myrcenol		543-39-5	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	91 (13 hPa) MS 95 %	1.470-1.476 0.873-0.879	Not longer supported by industry (DG SANCO, 2012)
02.191	Ocimenol		5986-38-9	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	94 (16 hPa) MS 95 %	1.480-1.486 0.871-0.877	Mixture of (E)- and (Z)-isomers (EFFA, 2010). Not longer supported by industry (DG SANCO, 2012)
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol ^(f)		10173 41199-19-3	Solid C ₁₃ H ₂₂ O 194.32	Practically insoluble or insoluble Freely soluble	82 (1 hPa) 68 MS 95 %	n.a. n.a.	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.203	2-Phenylpropan-2-ol		11704 617-94-7	Liquid C ₉ H ₁₂ O 136.19	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.529-1.535 0.971-0.977	

Table 1: Specification Summary of the Substances in the FGE.18Rev3

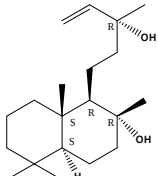
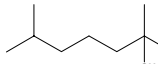
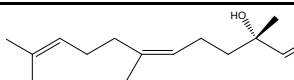
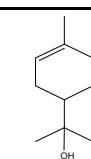
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.206 2029	(-)-Sclareol		4502 10311 515-03-7	Solid C ₂₀ H ₃₆ O ₂ 308.5	Practically insoluble or insoluble Freely soluble	182 (1.33 hPa) 106 MS 98 %	n.a. n.a.	
02.219	2,6-Dimethyl-2-heptanol		13254-34-7	Liquid C ₉ H ₂₀ O 144.26	Practically insoluble or insoluble Freely soluble	171 MS 98 %	1.421-1.427 0.816-0.822	
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol		67 142-50-7	Liquid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Soluble	291.92 20.98 MS 95 %	1.478-1.483 0.870-0.876	
02.230	Terpineol	 alpha-Terpineol shown	8000-41-7	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	210-24 to 235-6 MS 91-99 %	1.480-1.488 0.928-0.937	The specification covers alpha-, beta-, gamma- and delta-terpineol. Composition of mixture: 55-75 % alpha, 16-23 % gamma, 1-10 % cis-beta, 1-13 % trans-beta, 0-1 % delta (EFFA, 2007b).

Table 1: Specification Summary of the Substances in the FGE.18Rev3

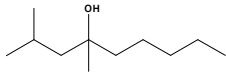
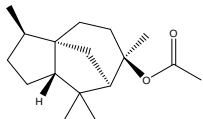
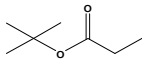
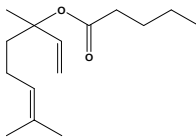
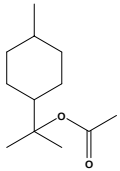
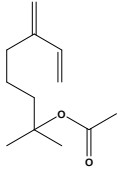
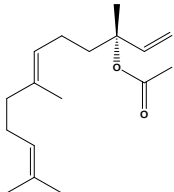
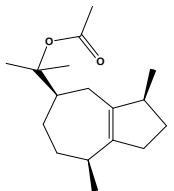
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.253 1850	2,4-Dimethyl-4- Nonanol		4407 74356-31-3	Liquid C ₁₁ H ₂₄ O 172.31	Practically insoluble or insoluble Freely soluble	212 MS 95 %	1.434-1.440 0.825-0.833	Racemate (EFFA, 2010).
09.171	Cedryl acetate		527 77-54-3	Solid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	158 (10.7 hPa) 156 MS 95 %	n.a. n.a.	
09.356	1,1-Dimethylethyl propionate		20487-40-5	Liquid C ₇ H ₁₄ O ₂ 130.19	Practically insoluble or insoluble Freely soluble	118 MS 95 %	1.390-1.396 0.862-0.868	
09.614	Linalyl valerate		10738 10471-96-2	Liquid C ₁₅ H ₂₆ O ₂ 238.37	Practically insoluble or insoluble Freely soluble	238 MS 95 %	1.450-1.456 0.897-0.903	Racemate
09.617	p-Menthan-8-yl acetate		58985-18-5	Liquid C ₁₂ H ₂₂ O ₂ 198.29	Practically insoluble or insoluble Freely soluble	115 (13 hPa) MS 95 %	1.449-1.455 0.930-0.940	
09.669	Myrcenyl acetate		10857 1118-39-4	Liquid C ₁₂ H ₂₀ O ₂ 196.29	Practically insoluble or insoluble Freely soluble	112 (13 hPa) MS 95 %	1.456-1.462 0.915-0.921	Not longer supported by industry (DG SANCO, 2012)

Table 1: Specification Summary of the Substances in the FGE.18Rev3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
09.671	(3S,6Z)-Nerolidyl acetate		10862 56001-43-5	Liquid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	107 (0.4 hPa) MS 95 %	1.467-1.473 0.901-0.907	
09.808	Guaiyl acetate		10659 134-28-1	Solid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	269 96 MS 95 %	n.a. n.a.	

(a): Solubility in water, if not otherwise stated.

(b): Solubility in 95 % ethanol, if not otherwise stated.

(c): At 1013.25 hPa, if not otherwise stated.

(d): At 20°C, if not otherwise stated.

(e): At 25°C, if not otherwise stated.

(f): Stereoisomeric composition not specified.

(g): Boiling point missing.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavours may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.)

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four of these flavouring substances [FL-nos: 02.129, 02.147, 02.168 and 02.197] the stereoisomeric composition has not been sufficiently specified (see Table 1).

1.3. Natural Occurrence in Food

Twenty-one out of 29 candidate substances have been reported to occur widely in fruits, liquorice, milk powder, cabbage, mushroom, various herbs, coffee, tea, chicken, wine and rum. Quantitative data on the natural occurrence in food have been reported for nine of these 29 substances:

Table 2: Candidate Substances Reported to Occur in Food (TNO, 2000)

FL-no:	Name:	Quantitative data reported
02.041	2-Methylbutan-2-ol	Up to 0.1 mg/kg in cherimoya, up to 0.1 mg/kg in loquat, up to 0.01 mg/kg in passion fruit, 0.00002 mg/kg in sapodilla fruit, 0.0007 mg/kg in chicken, 0.2 mg/kg in rum
02.052	2-Methylpropan-2-ol	0.25 mg/kg in grape, up to 0.1 mg/kg in mango, 0.0021 mg/kg in guava fruit
02.054	p-Menthane-1,8-diol	Up to 0.37 mg/kg in cranberry
02.120	Cedrol	1900 mg/kg in calamus (European), up to 100 mg/kg in cinnamon
02.123	2-Methylbut-3-en-2-ol	3.3 mg/kg in mango, up to 2.5 mg/kg in coffee, 1 mg/kg in tea, 0.6 mg/kg in black currants, 0.1 mg/kg in cranberry, up to 0.1 mg/kg in passion fruit, 0.05 mg/kg in bilberry, up to 0.05 mg/kg in cherimoya, 0.002 mg/kg in papaya, trace amounts in cabbage, trace amounts in cardamom, 0.003 mg/kg in milk powder
02.149	Elemol	0.37 mg/kg in grapefruit juice
02.171	p-Menthan-8-ol	0.01 mg/kg in grapefruit juice
02.181	2-Methylpentan-2-ol	0.01 mg/kg in plumcot
02.184	3-Methylpentan-3-ol	0.034 mg/kg in plumcot

According to TNO eight of the substances have not been reported to occur naturally in any food items:

Table 3: Candidate Substances Not Reported to Occur in Food (TNO, 2000)

FL-no:	Name:
02.140	1,2-Dihydrolinalool
02.147	3,6-Dimethyloctan-3-ol
02.150	Geranyl linalool
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol
02.219	2,6-Dimethyl-2-heptanol
02.253	2,4-Dimethyl-4-nonanol
09.671	Nerolidyl acetate
09.808	Guaiyl acetate

2. Specifications

Purity criteria for the 29 substances have been provided by the Flavouring Industry (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007b; EFFA, 2010; Flavour Industry, 2009a; Flavour Industry, 2009b) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for [FL-no: 02.129] are insufficient. Furthermore, the stereoisomeric composition / composition of mixture need to be specified for [FL-nos: 02.129,02.147, 02.168 and 02.197]. The specifications are adequate for 25 candidate substances (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population¹² (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation the total annual volume of production of the 29 candidate substances from use as flavouring substances in Europe has been reported to be approximately 10700 kg (EFFA, 2004b; EFFA, 2005b; EFFA, 2006a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2009b). For the supporting substances the total annual volume of production is approximately 58000 kg (JECFA, 2000a).

On the basis of the annual volume of production reported for the 29 candidate substances, MSDI values for each of these flavourings have been estimated (Appendix A, Table 7).

Ninety-four percent of the total annual volume of production for the candidate substances is accounted for by terpineol [FL-no: 02.230] with 10200 kg. The estimated MSDI of terpineol from use as a flavouring substance is 1200 µg/*capita* per day. The daily *per capita* intakes for each of the remaining substances are less than 30 µg (Appendix A, Table 7).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the 29 candidate substances, information on food categories and normal and maximum use levels^{13,14,15} were submitted for the 29 candidate substances by the Flavour Industry (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012). The 29 candidate substances are used in flavoured food products divided into

¹² EU figure 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

¹³ "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

¹⁴ The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

¹⁵ The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), as shown in Table 4. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 4: Use of 29 Candidate Substances in Various Food Categories

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except [FL-no: 02.197]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-nos: 02.146, 02.197, 02.226]
03.0	Edible ices, including sherbet and sorbet	All except [FL-nos: 02.197, 09.671]
04.1	Processed fruits	All except [FL-nos: 02.146, 02.197, 02.226, 02.230]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	[FL-no: 09.171]
05.0	Confectionery	All except [FL-no: 02.197]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-nos: 02.146, 02.197, 02.230]
07.0	Bakery wares	All except [FL-nos: 02.146, 02.197]
08.0	Meat and meat products, including poultry and game	All except [FL-nos: 02.129, 02.146, 02.197, 02.226]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-nos: 02.129, 02.146, 02.197, 02.226, 02.230, 02.253]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-nos: 02.146, 02.197]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-nos: 02.146, 02.129, 02.197, 02.226, 02.230]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 02.197]
15.0	Ready-to-eat savouries	All except [FL-nos: 02.146, 02.129, 02.197]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-nos: 02.146, 02.197, 02.226, 02.230]

According to the Flavour Industry the normal use levels for the 29 candidate substances are in the range of 0.2 - 200 mg/kg food, and the maximum use levels are in the range of 2 - 500 mg/kg (EFFA, 2002; EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012) (see Appendix C).

The mTAMDI values for the seventeen candidate substances from structural class I (See Section 6) range from 3900 to 14000 µg/person per day. For ten candidate substances from structural class II the

mTAMDI range from 1600 to 3900 µg/person per day whereas for [FL-no: 02.146] from structural class II is 110 µg/person per day. For the one substance from structural class III the mTAMDI value is 5700 µg/person per day (see Section 6).

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Appendix C.

4. Absorption, Distribution, Metabolism and Elimination

Six of the candidate substances in this group are esters [FL-nos: 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808]. Hydrolysis data are not available for any of these esters. However, *in vitro* hydrolysis data for the supporting substance linalyl acetate, indicate that these seven esters can be anticipated to be hydrolysed. The carboxylic acids resulting from the hydrolysis of these seven candidate substances are acetic acid, propanoic acid and valeric acid, which will all be incorporated in normal metabolic processes such as beta-oxidation and the citric acid cycle. The alcohols resulting from the hydrolysis of these esters are tertiary alcohols and their metabolisms are considered further below, together with the candidate tertiary alcohols in this Flavouring Group Evaluation.

A consideration of the chemical structures of the candidate substances, their anticipated pathways of metabolism and the extent to which data on one substance may support the metabolism of another substance has indicated that it is appropriate to divide the candidate substances in FGE.18Rev2 into eight subgroups of more closely related structures. This subdivision is shown in Table 5.

Table 5: Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
1	02.041	2-Methylbutan-2-ol	Aliphatic saturated tertiary alcohols and one ester thereof
	02.052	2-Methylpropan-2-ol	
	02.147	3,6-Dimethyloctan-3-ol	
	02.181	2-Methylpentan-2-ol	
	02.184	3-Methylpentan-3-ol	
	02.219	2,6-Dimethyl-2-heptanol	
	02.253	2,4-Dimethyl-4-nonanol	
	09.356	1,1-Dimethylethyl propionate	
2	02.123	2-Methylbut-3-en-2-ol	Aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two esters thereof
	02.144	2,6-Dimethyloct-7-en-2-ol	
	02.150	Geranyl linalool	
	02.168	Isophytol	
	02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	
	09.614	Linalyl valerate	
	09.671	Nerolidyl acetate	
3	02.146	3,7- Dimethylocta-1,5,7-trien-3-ol	Aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond
4	02.140	1,2-Dihydrolinalool	Aliphatic unsaturated tertiary alcohol (without terminal double bond)
5	02.054	p-Menthane-1,8-diol	Monocyclic saturated and unsaturated tertiary alcohols and one ester thereof
	02.129	Bisabola-1,12-dien-8-ol	
	02.171	p-Menthan-8-ol	

Table 5: Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
6	02.230	Terpineol	Monocyclic and bicyclic unsaturated tertiary alcohols with isolated terminal double bonds
	09.617	p-Menthan-8-yl acetate	
	02.149	Elemol	
	02.206	Sclareol	
7	02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	Bi- and tricyclic tertiary alcohols and esters
	02.120	Cedrol	
	09.171	Cedryl acetate	
	09.808	Guaiyl acetate	
8	02.203	2-Phenylpropan-2-ol	Tertiary alcohol with an aromatic substituent

Subgroup 1: Metabolism studies of the three candidate substances 2-methylbutan-2-ol [FL-no: 02.041], 2-methylpropan-2-ol [FL-no: 02.052] and 2-methylpentan-2-ol [FL-no: 02.181] show that these are conjugated with glucuronic acid before excretion in the urine. When rats were given 2-methylpropan-2-ol by gavage, acetone was excreted in small amounts, and when given 2-methylbutan-2-ol by gavage, diols were excreted. This indicates that an additional metabolic pathway of the three candidate substances is oxidation of methyl groups. From these metabolism studies it is anticipated that the candidate substances 2-methylbutan-2-ol [FL-no: 02.041], 2-methylpropan-2-ol [FL-no: 02.052], 3,6-dimethyloctan-3-ol [FL-no: 02.147], 2-methylpentan-2-ol [FL-no: 02.181], 3-methylpentan-3-ol [FL-no: 02.184], 2,6-dimethyl-2-heptanol [FL-no: 02.219], 2,4-dimethyl-4-nonanol [FL-no: 02.253] and the hydrolysis product 2-methylpropan-2-ol from the candidate substance 1,1-dimethylethyl propionate [FL-no: 09.356] are conjugated with glucuronic acid and excreted in the urine, or that they can undergo oxidation to yield the corresponding diols, which are also expected to be excreted as their respective glucuronic acid conjugates.

Subgroup 2: Linalool is a supporting substance to the candidate substances 2-methylbut-3-en-2-ol, linalyl valerate, nerolidyl acetate, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol and geranyl linalool [FL-nos: 02.123, 09.614, 09.671, 02.168, 02.226 and 02.150], which all have an isolated terminal double bond in close proximity to the tertiary alcohol group. As these substances or their respective alcohol moieties have a free hydroxyl group, they may be directly conjugated. Seventy-two hours after intragastrical application of 500 mg/kg bw ¹⁴C-labelled linalool to 12 weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetrahydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut (Rahman, 1974). In addition, the metabolism of linalool indicates that these candidate substances may also be metabolised by omega-oxidation of methyl groups and excreted in the urine as the oxidation product as such or after conjugation with glucuronic acid. No oxidation of the terminal double bond in linalool was observed, indicating no formation of epoxide intermediates.

For 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] the structure differs from the supporting substance linalool and the other candidate substances in this group in that the isolated terminal double bond is located distant from the tertiary alcohol group. However, any risk from epoxide formation of this compound is considered to be low since at low dose such epoxides formed are anticipated to be efficiently metabolised by conjugation with glutathione or by epoxide-hydrolase mediated hydrolysis,

and in line with the discussion in FGE.07Rev2, the candidate substance 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] can be evaluated via the A-side of the Procedure scheme. Moreover, the tertiary alcohol group can be directly conjugated with glucuronic acid.

Subgroup 3: The Panel stated that for the evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146], the monoterpene myrcene [FL-no: 01.008] could be used as a supporting substance (EFSA, 2012). This substance also has alcohol moiety that can be directly conjugated. In addition, further oxidation of methyl groups may occur. As shown for myrcene, oxidation of conjugated terminal double bonds in the candidate substance may occur, resulting in epoxide intermediates. However the available genotoxicity data for myrcene do not indicate a genotoxic potential for this substance even in the presence of metabolic activation. Nevertheless, it cannot be anticipated that these candidate substances will be metabolised to innocuous products.

Subgroup 4: 1,2-Dihydrolinalool [FL-no: 02.140] has been shown to be directly conjugated with glucuronic acid like the supporting substance linalool, and excreted. After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep dihydrolinalool and tetrahydrolinalool are formed as metabolites (Rahman, 1974). *In vivo* metabolism studies in rats on ¹⁴C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucuronides (Rahman, 1974). Additionally, it might be oxidised at the methyl groups, introducing new hydroxyl groups that also can be conjugated and excreted.

Subgroup 5: From the metabolism studies of alpha-terpineol and menthol it is anticipated that the candidate substances terpineol [FL-no: 02.230], p-menthane-1,8-diol [FL-no: 02.054], bisabola-1,12-dien-8-ol [FL-no: 02.129] and p-menthan-8-ol [FL-no: 02.171] (also an hydrolysis product of candidate substance: p-menthan-8-yl acetate [FL-no: 09.617]) may undergo allylic oxidation of the exocyclic methyl group. This could be further oxidised to a carboxylic acid group. Alternative or subsequent metabolism may occur by conjugation with glucuronic acid, followed by excretion in the urine.

Subgroup 6: A metabolism study on elemol [FL-no: 02.149] indicates that the substance is absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of elemol [FL-no: 02.149] and sclareol [FL-no: 02.206], which have the same structural features as elemol, would not be anticipated.

Subgroup 7: Two metabolism studies on cedrol indicate that the candidate substances cedrol [FL-no: 02.120], cedryl acetate [FL-no: 09.171] guaiyl acetate [FL-no: 09.808] and 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197] will be further hydroxylated and excreted in urine as such or as conjugates.

Subgroup 8: In metabolism studies, the supporting substance 1-isopropyl-4-methylbenzene [FL-no: 01.002] (synonym: p-cymene) was oxidised at the isopropyl side chain yielding 2-(p-tolyl)-2-propanol, which is not further oxidised, but excreted unchanged or as a glucuronic acid conjugate. It is anticipated that the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203] will follow the same pathway and be excreted unchanged or in conjugation with glucuronic acid.

In summary, 28 of the candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] are anticipated to be metabolised to innocuous products.

For the candidate substance [FL-no: 02.146], which contains a conjugated terminal double bond, data from a supporting substance, myrcene [FL-no: 01.008] indicate that [FL-no: 02.146] may be oxidised,

giving rise to epoxide intermediates. Thus, it cannot be anticipated that this candidate substance will be metabolised to innocuous products. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies and the Procedure can be applied for the safety evaluation of this candidate substance.

A more detailed discussion of the metabolism of the candidate substances in this evaluation is provided in Appendix D.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the 29 candidate substances the Procedure as outlined in Appendix B was applied, based on the MSDI approach. The stepwise evaluations of the 29 substances are summarised in Appendix A, Table 7.

Step 1

Seventeen of the 29 candidate substances are classified into structural class I, eleven candidate substances are classified into structural class II and one candidate substance is classified into structural class III according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

Twenty-eight of the candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] are anticipated to be metabolised to innocuous products and proceed via the A-side of the Procedure scheme.

One candidate substance [FL-no: 02.146] contains a conjugated terminal double bond and data from the supporting substance myrcene [FL-no: 01.008] indicate that this terminal double bond may be oxidised, giving rise to epoxide intermediates. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies and therefore the Panel decided to evaluate [FL-no: 02.146] according to the Procedure. Since it cannot be anticipated that [FL-no: 02.146] is metabolised to innocuous products, the evaluation of this candidate substance will proceed via the B-side of the Procedure scheme.

Step A3

Seventeen of the candidate substances [FL-nos: 02.054, 02.120, 02.140, 02.144, 02.149, 02.168, 02.171, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.614, 09.617, 09.671 and 09.808], which are anticipated to be metabolised to innocuous products have been assigned to structural class I. These substances have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1200 µg. These intakes are below the threshold of concern of 1800 µg/person per day for structural class I.

Ten of the candidate substances [FL-nos: 02.041, 02.052, 02.123, 02.147, 02.150, 02.181, 02.184, 02.197, 02.203 and 09.356], which are predicted to be metabolised to innocuous products have been assigned to structural class II. These substances have European daily *per capita* intakes (MSDI) of 0.0012 to 12 µg. These intakes are below the threshold of concern of 540 µg/person per day for structural class II.

One of the candidate substances [FL-no: 02.129] which is predicted to be metabolised to innocuous products has been assigned to structural class III. This substance has European daily *per capita* intake (MSDI) of 27 µg. This intake is below the threshold of concern of 90 µg/person per day for structural class III.

Based on results of the safety evaluation sequence these 28 candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] proceeding via the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances at estimated levels of intake.

Step B3

The candidate substance [FL-no: 02.146] which could not be predicted to be metabolised to innocuous products,] was assigned to structural class II according to the Cramer classification. The estimated European daily *per capita* intake (MSDI) is of 12 µg for [FL-no: 02.146] is below the threshold of concern of 540 µg per person per day and accordingly, [FL-no: 02.146] proceeds to step B4 of the Procedure.

Step B4

In its statement of 2012 (EFSA CEF Panel, 2012) the Panel identified myrcene [FL-no: 01.008] as supporting substance for [FL-no: 02.146] in the present FGE. The safety evaluation of myrcene has been finalised in FGE.78Rev2 based on a new 90-day toxicity study (Bauter, 2013) which provided a NOAEL of 44 mg/kg bw per day. When the MSDI of [FL-no: 02.146] of 12 µg/*capita* per day is compared to this NOAEL a margin of safety of 2.2×10^5 for [FL-no: 02.146] can be calculated.

6. Comparison of the Intake Estimations Based on the MSDI and the mTAMDI Approach

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI, are 3900 µg/person per day, and for the two remaining substances 7000 and 14000 µg/person per day. These seventeen mTAMDI are all above the threshold of concern of 1800 µg/person per day for a structural class I substance.

For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg/person per day and for the remaining two substances, 110 and 1600 µg/person per day, respectively. For 10 substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg/person per day. For the candidate substance [FL-no: 02.146] the estimated intake is below the threshold of concern for structural class II substances of 540 µg/person per day for a structural class II substance.

For substance [FL-no: 02.129] assigned to structural class III the mTAMDI is 5700 µg/person per day, which is above the threshold of concern for structural class III substances of 90 µg/person per day.

Thus, for 28 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, see Table 6.

Table 6: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI µg/capita per day	mTAMDI µg/person per day	Structural class	Threshold of concern µg/person per day
02.054	p-Menthane-1,8-diol	11	3900	Class I	1800
02.120	(+)-Cedrol	13	3900	Class I	1800
02.140	1,2-Dihydrolinalool	0.044	3900	Class I	1800
02.144	2,6-Dimethyloct-7-en-2-ol	0.0012	3900	Class I	1800
02.149	(-)-alpha-Elemol	1.6	3900	Class I	1800
02.168	Isophytol	0.037	3900	Class I	1800
02.171	p-Menthan-8-ol	0.012	3900	Class I	1800
02.206	(-)-Sclareol	0.67	3900	Class I	1800
02.219	2,6-Dimethyl-2-heptanol	0.012	3900	Class I	1800
02.226	[S-(cis)]-3,7,11-Trimethyl- 1,6,10-dodecatrien-3-ol	0.049	7000	Class I	1800
02.230	Terpineol	1200	14000	Class I	1800
02.253	2,4-Dimethyl-4-Nonanol	0.24	3900	Class I	1800
09.171	Cedryl acetate	0.99	3900	Class I	1800
09.614	Linalyl valerate	0.43	3900	Class I	1800
09.617	p-Menthan-8-yl acetate	0.012	3900	Class I	1800
09.671	(3S,6Z)-Nerolidyl acetate	0.061	3900	Class I	1800
09.808	Guaiyl acetate	0.0012	3900	Class I	1800
02.041	2-Methylbutan-2-ol	2.7	3900	Class II	540
02.052	2-Methylpropan-2-ol	0.012	3900	Class II	540
02.123	2-Methylbut-3-en-2-ol	0.0012	3900	Class II	540
02.147	3,6-Dimethyloctan-3-ol	0.0012	3900	Class II	540
02.150	(E,E)-Geranyl linalool	0.026	3900	Class II	540
02.181	2-Methylpentan-2-ol	0.12	3900	Class II	540
02.184	3-Methylpentan-3-ol	0.0012	3900	Class II	540
02.197	1,2,3,4,4a,5,6,7- Octahydro-2,5,5- trimethylnaphthalen-2-ol	0.026	1600	Class II	540
02.203	2-Phenylpropan-2-ol	0.0012	3900	Class II	540
09.356	1,1-Dimethylethyl propionate	0.0012	3900	Class II	540
02.146	(E)-3,7-Dimethylocta- 1,5,7-trien-3-ol	12	110	Class II	540
02.129	(l)-alpha-Bisabolol	27	5700	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same

pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2004b, 2005b, 2006a, 2007b; Flavour Industry, 2009a,b) the estimated combined daily *per capita* intake as flavourings of the 17 candidate substances assigned to structural class I is approximately 1250 µg, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 µg per person per day.

For the 11 candidate substances assigned to structural class II the combined intake is approximately 15 µg, which does not exceed the threshold of concern for a compound belonging to structural class II of 540 µg/person per day.

The 29 candidate substances are structurally related to 26 supporting substances of which 23 were evaluated by JECFA at its 51st meeting (JECFA, 2000a) and three were evaluated by the JECFA at its 63rd meeting (JECFA, 2005b). The total combined intake (in Europe) of the candidate and the supporting substances all assigned to structural class I is approximately 25500 µg/*capita* per day, which exceeds the threshold of concern for the corresponding structural class of 1800/person per day.

For one of the supporting substances from structural class I, menthol [FL-no: 02.015], The JECFA established an acceptable daily intake (ADI) of 4 mg/kg bw per day at their 51st meeting (JECFA, 2000a). The combined intake of the candidate and supporting substances from structural class I of 25500 µg/*capita* per day ~ 0.425 mg/kg bw per day is approximately 10 times below this ADI.

In Europe, the total estimated combined intake of the candidate and the supporting substances assigned to structural class II is 16 µg/*capita* per day, which does not exceed the threshold of concern for the corresponding structural class (540 µg/person per day).

The intake of the one candidate substance assigned to structural class III is 27 µg/*capita* per day. No supporting substances are assigned to structural class III and thus calculation of a combined intake for this substance is not applicable.

8. Toxicity

8.1. Acute Toxicity

Data are available for 16 of the 32 candidate substances and for 19 of the 26 supporting substances. The oral LD₅₀ values in rats, mice or rabbits ranged from 230 to 50000 mg/kg body weight (bw). The magnitudes of the LD₅₀ values indicate that the oral acute toxicity is low for the candidate and supporting substances.

The acute toxicity data are summarised in Appendix E, Table 16.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data are available for six of the candidate substances, 2-methylpropan-2-ol [FL-no: 02.052], cedrol [FL-no: 02.120], 2-methylbut-3-en-2-ol [FL-no: 02.123], bisabol-1,12-dien-8-ol [FL-no: 02.129], 2,6-Dimethyloct-7-en-2-ol [FL-no: 02.144], and sclareol [FL-no: 02.206] (see Appendix E, Table 17).

A number of studies have been conducted on 2-methyl-propan-2-ol [FL-no: 02.052], as described below:

In a 10-week study in male rats, exposure to drinking water containing 0.5 % 2-methylpropan-2-ol [FL-no: 02.052] (equivalent to 500 mg/kg bw per day) resulted in histopathological changes in the liver and kidney. No other tissues were examined or analyses performed (Acharya et al., 1997).

Ninety-day studies were performed in Fisher F344/N rats and B6C3F₁ mice, in conjunction with 2 year chronic toxicity/carcinogenicity studies, which are described below. The main findings after 90 days were, in rats, hyperplasia and inflammation of the urinary bladder in males receiving 2 % and males and females receiving 4 % 2-methylpropan-2-ol in the drinking water (equivalent to 2000 and 4000 mg/kg bw per day, respectively), increased incidence of nephropathy in females receiving 0.5 %, 1 % and 4 % (equivalent to 500, 1000 and 4000 mg/kg bw per day) and increased severity of nephropathy in males at all treatment doses (intakes of 250 mg/kg bw per day and above). In mice, transitional epithelial hyperplasia and inflammation were observed in the urinary bladder in 2 % and 4 % group males (equivalent to 5000 and 10,000 mg/kg bw per day, respectively) and 4 % group females (equivalent to 10,000 mg/kg bw per day) (Lindamood et al., 1992; NTP, 1995). These observations were consistent with earlier 90-day studies using the same species and strains and the same dose levels, except that there was no reference in the short report to renal effects in rats (Brown and Wheeler, 1979).

In the 2-year study on 2-methylpropan-2-ol [FL-no: 02.052] in F344/N rats, the animals (60/sex/group) were dosed via drinking water containing, for males 0, 0.125, 0.25 or 0.5 % (equivalent to 0, 90, 200 or 420 mg/kg bw per day) and for females 0, 0.25, 0.5 or 1 % (equivalent to 0, 180, 330 or 650 mg/kg bw per day), 2-methylpropan-2-ol. Survival was significantly reduced in the 0.5 % male group and 1 % female group. Severity of nephropathy and incidence and severity of transitional cell nephropathy were reported to be increased in all treated groups. Foci of mineralisation were observed in the renal papillas in all treated groups, and the incidence of renal mineralisation was significantly increased in the 0.5 % male group. The incidences of focal renal tubular hyperplasia and adenoma were observed to be increased in a dose-related manner in all treated male groups but did not reach statistical significance. Combined incidences of renal tubular adenomas and carcinomas were significantly increased in the 0.25 % male group, although not in the 0.5 % dose group. Renal tubular hyperplasia was observed in one female in the 1 % dose group (NTP, 1995). A review subcommittee for the US National Toxicology Program (NTP) concluded that the study showed 'some evidence of carcinogenic activity' in the males and 'no evidence of carcinogenic activity' in the females (NTP, 1995).

Further examination of renal samples from the 90-day study in F344/N male rats revealed a significant increase in the quantity of hyaline droplets and number of intracytoplasmic deposits of abnormal shape (crystalline rhomboid structures) in treated groups compared to controls, indicating that the nephropathy observed in male rats was at least in part due to alpha-2u-globulin (Takahashi et al., 1993). The NTP review subcommittee noted that the increased severity of nephropathy also seen in females indicated that the mechanism for renal toxicity is not limited to increased accumulation of alpha-2 μ -globulin (NTP, 1995); however, tumours were observed in males only and appear to be related to a dose-related increase in renal tubule hyperplasia, which occurred in males only.

The B6C3F₁ mice (60/sex/group) received drinking water containing 0, 0.5, 1 or 2 % 2-methylpropan-2-ol [FL-no: 02.052] (equivalent to 0, 540, 1040, 2070 mg/kg bw per day in males and 0, 510, 1020 or 2100 mg/kg bw per day in females) for 2 years. Survival of the 2 % male group was significantly reduced compared to controls. Incidence of follicular cell hyperplasia of the thyroid were significantly increased in all treated male groups and in the 1 % and 2 % female groups. Incidence of follicular cell adenoma was significantly increased in the 2 % female group. The combined incidences of follicular cell adenomas and carcinomas was observed to be increased in the 1 % male group, although this did not reach statistical significance. However, the incidence of adenoma exceeded the highest incidence seen in historic NTP drinking water controls. Chronic inflammation of the urinary bladder was significantly increased in the 2 % male and female groups, and transitional cell hyperplasia in the urinary bladder was significantly increased in the 2 % female group. No NOAEL could be derived from this study for males; the NOAEL in females was 0.5 % in the diet, equivalent to 510 mg/kg bw

per day (NTP, 1995). An NTP review subcommittee concluded that the study demonstrated ‘equivocal evidence of carcinogenic activity’ in male mice and ‘some evidence of carcinogenic activity’ in females (NTP, 1995). The Panel concluded that these tumours appear to be secondary to follicular cell hyperplasia, which was observed in all treated male groups and mid- and high-dose female groups.

The Panel concluded, taking into account the clear lack of a genotoxic potential *in vivo*, that the carcinogenic effects observed in male rats and in mice appear likely to be due to threshold-based mechanisms. Therefore, the overall results do not preclude evaluating 2-methylpropan-2-ol and structurally related candidate substances through the Procedure.

In a 90-day study, 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] was administered by gavage to ten male and ten female Sprague-Dawley rats at dose levels of 10, 50, 500 and 1000 mg/kg bw per day. A control group of ten males and ten females was dosed with vehicle (corn oil) alone (Dunster et al. 2006). In female rats, treatment related effects such as decrease in body weight, platelet counts, thromboplastin time, haemoglobin, hematocrit, erythrocyte counts, increase in serum aspartate aminotransferase, alanine aminotransferase, urinary creatinine were observed doses of 500 and/or 1000 mg/kg bw per day. No such changes were observed in females at the lower doses tested and therefore, 50 mg/kg bw per day was considered to constitute a No Observed Adverse Effect Level (NOAEL) for females. In the kidney of male rats, a greater incidence and/or severity of groups of basophilic tubules and/or globular accumulations of eosinophilic material were observed at all doses tested. Dunster et al., 2007 considered these findings to be consistent with the presence of hydrocarbon nephropathy, which results from the excessive accumulation of alpha2-urinaryglobulin. The latter was indicated by positive staining with Mallory' s Heidenhain stain in renal proximal tubular epithelial cells, frequently associated with tubular degenerative changes. alpha2-Urinaryglobulin is found only in the proximal tubular epithelium of adult male rats. Dunster et al. 2007, concluded that this effect is not indicative of a hazard to human health and that 10 mg/kg per day should be regarded as the NOAEL for males (Dunster et al., 2007).

A NOAEL of 150 mg/kg bw per day in rats is available from a 28-day oral toxicity study conducted on 2-methylbut-3-en-2-ol [FL-no: 02.123] (BASF, 1994).

A NOAEL of 850 mg/kg bw per day in rats is available from a 28-day oral toxicity study conducted on bisabola-1,12-dien-8-ol [FL-no: 02.129] (Habersang et al., 1979).

A 32-day oral toxicity study on both sclareol [FL-no: 02.206] and cedrol [FL-no: 02.120] was conducted in Charles River Laboratories CD(SD) rats. Two groups of 10 rats received dose levels of approximately 8.8 mg/kg bw per day of sclareol and two groups of 10 rats received dose levels of approximately 8.4 mg/kg bw per day of cedrol. The animals were dosed seven days per week via gavage. No adverse effects were observed (IOFI, 2006).

Data are available for six supporting substances [FL-nos: 01.008, 02.013, 02.015, 09.013, 09.423 and 09.830] and two other related substances (linalyl cinnamate and geranyl acetate) administered as a mixture together with citronellyl acetate (See Annex E, Table 17).

Linalool [FL-no: 02.013] was reported to result in no significant adverse effects compared to control when administered to rats at 50 mg/kg bw per day as a 50 % mixture with citronellol via the diet for 84 days. No further details are available. Similarly linalyl acetate [FL-no: 09.013], linalyl isobutyrate [FL-no: 09.423] and geranyl acetate [FL-no: 09.011] administered as a mixture at doses of dosed 24, 27 and 48 mg/kg bw per day respectively resulted in no adverse effects (Oser, 1967).

No adverse effects were reported in rats (10/sex/group) given diets containing 0, 1000, 2500 or 10,000 ppm linalyl isobutyrate [FL-no: 09.423] (equivalent to 0, 50, 125 or 500 mg/kg bw per day) (Hagan et al., 1967) for 18 weeks. Few study details are available. Similarly no adverse effects were reported in a similar study on terpinyl acetate [FL-no: 09.830] given diet containing 0, 1000, 2500 or 10,000 ppm (equivalent to 0, 50, 125 or 500 mg/kg bw per day) for 20 weeks (Hagan et al., 1967).

Since the publication of FGE.18Rev2 (EFSA CEF Panel, 2011) new data on myrcene have become available (Bauter, 2013). The results are described in more detail in FGE.78Rev2 (EFSA, 2015). In this FGE a NOAEL of 44 mg/kg bw per day was derived which can be used for the evaluation of [FL-no: 02.146] in the current FGE.

The repeated dose toxicity data are summarised in Appendix E, Table 17.

8.3. Developmental / Reproductive Toxicity Studies

Data are available for two candidate substances: 2-methylpropan-2-ol [FL-no: 02.052] and 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144].

In a study on 2-methylpropan-2-ol [FL-no: 02.052], doses of 0.5, 0.75 or 1 % liquid diet (equivalent to approximately 3400, 4900 and 6400 mg/kg bw per day) administered to mice on days 6-20 of gestation were associated with reduced performance of offspring in four neurobehavioural tests: righting reflex, cliff avoidance and open field (conducted on days 2, 4, 6, 8 and 10 post-parturition) and roto-rod (conducted on days 14, 16, 18, 20 and 22). The authors indicated that there was some evidence of recovery in the first three tests but not the latter during the periods of study (Daniel and Evans, 1982). In a second study, administration of 1557 mg/kg bw per day to pregnant CBA/J and C57BL/6J mice by oral gavage during days 6-18 of gestation resulted in a significant increase in the incidence of foetal resorptions and a significant decrease in the number of live births per litter. No foetal malformations were observed (Faulkner et al., 1989). The Panel noted that these experimental studies were conducted using very high doses and were not of concern when compared with the low exposure arising from use as flavouring substances.

In a study on 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], doses of 0, 250, 500 or 1000 mg/kg bw per day were administered to Charles River Laboratory CD(SD) rats via corn oil gavage on gestational days 7-17.

Observations for viability, adverse clinical signs, abortion, and premature delivery were conducted before and approximately one hour following treatment and once thereafter. Body weight gains in the high-dose group were reduced by 5 % when compared to controls; weight losses were observed after the first two doses. Although these observations were not significant, they were considered to be evidence of a threshold level for maternal toxicity. Both maternal absolute and relative feed consumption values were significantly reduced in the 1000 mg/kg bw per day group compared to vehicle control. Reduced feed consumption was most prominent on gestational days 7-10, which correlated with the weight losses and reduced weight gains that occurred during the initial days of the dosing period.

Body weights for combined male and female foetuses were reduced approximately 3% in the 1000 mg/kg bw per day group compared to vehicle controls (the reduction was statistically significant for females). No other litter parameters were affected by dosages of 2,6-dimethyloct-7-en-2-ol as high as 1000 mg/kg bw per day. Upon inspection, there were no foetal gross external alterations or foetal soft tissue or skeletal malformations observed in the experiment. There were no observable soft tissue variations, and skeletal variations were limited to two reversible minor changes. First, there was evidence of a threshold (but statistically significant) increase in supernumerary ribs, along with associated significant increases and decreases in the respective numbers of thoracic and lumbar vertebrae. Second, there was evidence of a small but statistically significant retardation in ossification of the metatarsal bones in the hind paws, evident as a reduction in the mean number of ossified metatarsal bones.

The results indicate that 1000 mg 2,6-dimethyloct-7-en-2-ol/kg bw per day produced threshold levels of maternal and developmental toxicity. As such, the maternal and developmental no-observable-adverse-effect levels (NOAELs) for 2,6-dimethyloct-7-en-2-ol are considered to be 500 mg/kg per day (Politano et al., 2008).

Data are available for three supporting substances [FL-nos: 02.013, 02.015 and 01.008] (See Annex E, Table 18).

A NOAEL of 365 mg/kg bw per day was reported for linalool [FL-no: 02.013]. A higher dose of 729 mg/kg bw per day resulted in decreased live litter size and increased pup mortality; maternal toxicity was observed at all doses tested (Hoberman and Christian, 1989). NOAELs of 185 to 425 mg/kg bw per day (highest doses tested) were reported for menthol [FL-no: 02.015] in teratological studies in rats, mice, hamsters and rabbits (Food and Drug Research Laboratories, Inc., 1973). Three studies have been performed in rats on myrcene [FL-no: 01.008]; two developmental toxicity studies (one in which myrcene was administered to dams on days 6-15 of gestation and one in which dosing was from day 15 of gestation until weaning) and a single generation study. NOAELs were 500 mg/kg bw per day, 250 mg/kg bw per day and 300 mg/kg bw per day, respectively (Delgado et al., 1993a; Delgado et al., 1993b; Paumgartten et al., 1998).

The developmental/reproductive toxicity data are summarised in Appendix E, Table 18.

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for nine candidate [FL-nos: 02.052, 02.041, 02.120, 02.123, 02.129, 02.140, 02.144, 02.168 and 02.197] and for eight supporting substances [FL-nos: 01.002, 01.008, 02.013, 02.014, 02.015, 02.097, 09.013 and 09.830]. Data from *in vivo* tests are available for two candidate [FL-nos: 02.052 and 02.123] and for three supporting substances [FL-nos: 01.008, 02.013 and 02.015].

2-Methylpropan-2-ol [FL-no: 02.052] was negative in reversion tests in *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100, without and with metabolic activation by rat and hamster liver S9 (Zeiger et al., 1987). A borderline (less than two-fold) increase in revertants in strain TA1535 was observed in two other studies (Haworth et al., 1981a; Haworth et al., 1981b), which were not available for evaluation. A marginal increase in sister chromatid exchange (SCE) was reported from two studies with Chinese hamster ovary (CHO) cells, which could not be evaluated because the papers were submitted incompletely (Putman, 1985; Thilagar et al., 1981). A borderline increase in mutant frequency was observed in mouse-lymphoma TK +/- cells in a single test in the absence of metabolic activation, whereas negative results were obtained in repeated experiments with S9 (McGregor et al., 1988). Again, similar results are quoted in the summary of an unpublished study not available for evaluation (Kirby et al., 1981). Finally, a slight increase of petite (mitochondrial) mutations was reported in yeast after treatment with 2-methylpropan-2-ol (Jiménez et al., 1988), but this effect is not considered relevant for genotoxicity assessment.

1,2-Dihydrolinalool [FL-no: 02.140] was negative in *S. typhimurium* TA97, TA100, TA102, TA1535, without and with metabolic activation (Gocke, 1999). In one test, 1,2-dihydrolinalool was negative without S9 but increased the number of revertants in TA98 with metabolic activation. This positive finding could not be reproduced in subsequent tests with TA98 (Gocke, 1999).

The remaining candidate substances, for which genotoxicity data have become available in this Revision 2 of FGE.18; Bisabola-1,12-dien-8-ol [FL-no: 02.129], cedrol [FL-no: 02.120], 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197] were all negative in reversion tests in the following test objects, without and with metabolic activation by rat liver S9: [FL-no: 02.129]: *S. typhimurium* TA97a, TA98, TA100, TA102, TA1535, TA1537 up to 5000 µg/plate; [FL-no: 02.120]: *S. typhimurium* TA97a, TA98, TA102, TA1535 up to 5000 µg/plate; [FL-no: 02.144]: *S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 up to 5000 µg/plate; and [FL-no: 02.197]: *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA up to 250 µg/plate without metabolic activation and up to 500 µg/plate with metabolic activation. The studies were considered valid.

A negative result was obtained in a sister chromatid exchange (SCE) study on bisabola-1,12-dien-8-ol [FL-no: 02.129] with Chinese hamster ovary (CHO) cells. The data were presented in a review, but considered valid.

In vivo, 2-methylpropan-2-ol gave clearly negative results in a rat bone marrow micronucleus test, after intraperitoneal (i.p.) administration of a range of doses (six doses from 39 to 1250 mg/kg bw), which reached complete lethality at the highest dose (NTP, 1997). Negative results were also obtained in the mouse peripheral blood micronucleus assay, after 13 weeks of oral exposure to 3000 to 40000 ppm in drinking water. There was no deviation in the PCE/NCE ratio in treated animals, but signs of general toxicity were observed at the two highest doses, indicating significant systemic exposure (NTP, 1995). In another study, 2-methylpropan-2-ol was negative in the mouse bone marrow micronucleus assay when given by ip injections at doses up to 1250 mg/kg bw (three daily administrations) (NTP, 1996). The alleged positive result obtained with 2-methylpropan-2-ol in a rat bone marrow chromosomal aberration test after oral administration of 1/5 of the LD₅₀ (Barilyak and Kozachuk, 1988) is considered inconclusive, because the result is not adequately supported by experimental data.

Terpineol [FL-no: 02.230] (the mixture of alpha-terpineol, beta-terpineol, delta-terpineol and gamma-terpineol) was tested negative in a rec assay in *Bacillus subtilis* (Oda et al., 1978).

Alpha-terpineol [FL-no: 02.014] was reported to give weakly positive results (as a dose-dependent increase in mutation frequency both with and without S9 activation with a maximum increase of 2.2-fold compared with the control) in an Ames-type mutagenicity assay in one (TA102) of four *S. typhimurium* strains tested (TA97a, TA98, TA100, TA102). alpha-Terpineol was incorporated into agar plates up to 2500 µg/plate, either with or without S9 metabolic activation (Gomes-Carneiro et al., 1998).

In other studies, alpha-terpineol gave consistently negative results in Ames assays in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, either with or without S9 metabolic activation (Florin et al., 1980; Lorillard, 1983; Heck et al., 1989).

In an *in vivo/in vitro* study designed to investigate the mutagenicity of the metabolites of beta-terpineol [FL-no: 02.097], Sprague-Dawley rats were administered a single dose of 0.5 ml (452 mg) of beta-terpineol by gavage and the urine was collected for 24-hours. The urine (500 microl) was hydrolysed with beta-glucuronidase. Hydrolysed and un-hydrolysed urine samples, ether extracts of the urine, and aqueous fractions of the urine-ether extracts were then separately incubated with *S. typhimurium* strains TA98 and TA100 without S9 activation. Neither beta-terpineol, nor any of the urinary solutions isolated from the urine of rats given 452 mg doses of beta-terpineol, showed any evidence of mutagenicity in either TA98 or TA100 without metabolic activation (Rockwell and Raw, 1979).

In gene mutation tests in mouse lymphoma cells, alpha-terpinol was non-mutagenic when applied of doses up to 250 nl/ml (with S9) and 300 nl/ml (without S9) (Lorillard, 1982); negative results were also obtained in another study in which alpha-terpineol was tested up to 0.5 µl/ml (without S9) and 0.75 µl/ml (with S9) (Kirby et al., 1984). Based on the negative results obtained in gene mutation tests in mammalian cells, and in view of the sensibility of the TK+/- system to mutagens specifically active toward the *S. typhimurium* strain TA102, the Panel concluded that alpha-terpineol does not raise concern for genotoxicity.

Overall, 2-methylpropan-2-ol provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence does not raise concern for *in vivo* genotoxicity.

2-Methylbut-3-en-2-ol [FL-no: 02.123] was reported to be negative in two bacterial gene mutation tests and in an *in vivo* micronucleus test; however, the unpublished study reports are not available for re-evaluation.

The available data considered valid do not give rise to safety concerns with respect to genotoxicity.

The genotoxicity data are summaries in Appendix E, Tables 19 and 20.

CONCLUSIONS

The present revision 3 of the Flavouring Group Evaluation 18 (FGE.18Rev3) is due to the availability of new toxicity data for myrcene, [FL-no: 01.008] which was used for the safety evaluation of the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

In this revision seven of the candidate substances are aliphatic saturated tertiary alcohols and one is an ester of such, five are aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two are esters of such, one is an aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond, one is an aliphatic unsaturated tertiary alcohol without terminal double bond, two are monocyclic saturated tertiary alcohols and one is an ester of such, two are monocyclic unsaturated tertiary alcohols, two are mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond, two are bicyclic unsaturated esters, two are bi- and tricyclic tertiary alcohols and one is a tertiary alcohol with an aromatic substituent.

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four of these substances [FL-nos: 02.146, 02.147, 02.168 and 02.197] the stereoisomeric composition has not been specified sufficiently.

Seventeen of the 29 candidate substances are classified into structural class I, eleven candidate substances are classified into structural class II and one is classified into structural class III according to the decision tree approach.

Twenty-one out of the 29 candidate substances have been reported to occur in a wide range of food items.

According to the default MSDI approach, 28 of the 29 flavouring substances in this group have intakes in Europe that vary from 0.0012 to 27 $\mu\text{g/capita}$ per day, which are below the thresholds of concern for structural classes I, II and III substance of 1800, 540 and 90 $\mu\text{g/person}$ per day, respectively. The remaining substance, terpineol [FL-no: 02.230] belonging to structural class I, has an MSDI of 1200 $\mu\text{g/capita}$ per day, which is also below the threshold for this structural class.

On the basis of the reported annual production volumes in Europe, the combined intakes of seventeen candidate substances belonging to class I and of eleven candidate substances belonging to class II would result in combined intakes of approximately 1250 and 15 $\mu\text{g/capita}$ per day, respectively. These values are lower than the thresholds of concern for structural class I and class II substances of 1800 and 540 $\mu\text{g/person}$ per day, respectively.

For flavouring substances belonging to structural class I, the total combined intake of the candidate and supporting substances is approximately 25500 $\mu\text{g/capita}$ per day. This total combined intake exceeds the threshold for structural class I of 1800 $\mu\text{g/person}$ per day. For one of the supporting substances from structural class I, menthol [FL-no: 02.015], the JECFA established an acceptable daily intake (ADI) of 4 mg/kg bw per day at their 51st meeting. The combined intake of the candidate and supporting substances from structural class I of 25500 $\mu\text{g/capita}$ per day $\sim$ 0.425 mg/kg bw per day is approximately 10 times below this ADI. For flavouring substances belonging to structural class II, the total combined intake of the candidate and supporting substances is approximately 16 $\mu\text{g/capita}$ per day, which is below the threshold of concern for structural class II (540 $\mu\text{g/person}$ per day).

2-Methylpropan-2-ol [FL-no: 02.052] provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence and the lack of structural alerts for genotoxicity for this substance and its metabolites do not raise concern for *in vivo* genotoxicity. For

the other substances in this group the available data do not give rise to safety concerns with respect to genotoxicity.

Twenty-eight of the candidate substances are anticipated to be metabolised to innocuous products. For the candidate substance (E)-3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no metabolism data are available and therefore it cannot be predicted to be metabolised to innocuous products.

In Revision 2 of FGE.18, for substance (E)-3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no appropriate NOAEL was available and additional data were requested. In the present revision of FGE.18 (Revision 3), new toxicity data on the supporting substance myrcene [FL-no: 01.008] became available, providing an appropriate NOAEL of 44 mg/kg bw per day which was used for the evaluation of the candidate substance [FL-no: 02.146]. Based on the MSDI intake of 12 µg/capita per day, the margin of safety calculated for [FL-no: 02.146] is 2.2×10^5 .

Therefore, none of the 29 candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] would give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances, on the basis of the default MSDI approach.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for 25 candidate substances. However, information on purity criteria and/or stereoisomeric composition has not been specified sufficiently for four substances [FL-no: 02.129, 02.147, 02.168 and 02.197]. Thus, the final evaluation of the materials of commerce cannot be performed for these substances, pending further information.

Thus, for 25 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach: [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.146, 02.149, 02.150, 02.171, 02.181, 02.184, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808].

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI approach, are 3900 µg/person per day, and for the two remaining substances 7000 and 14000 µg/person per day respectively. These seventeen mTAMDI are all above the threshold of concern of 1800 µg/person per day for a structural class I substance. For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg/person per day and for the remaining two substances, 110 and 1600 µg/person per day, respectively. For ten substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg/person per day. For the one substance assigned to structural class III the mTAMDI is 5700 µg/person per day, which is above the threshold of concern for structural class III substances of 90 µg/person per day.

In conclusion, for all candidate substances except [FL-no: 02.146] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

DOCUMENTATION PROVIDED TO EFSA

1. Bauter MR, 2013. Myrcene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33546. May 20, 2013. Unpublished report submitted by EFFA to DG SANCO.
2. BASF (Baden Aniline and Soda Factory), 1979. Bericht über die gewerbetoxikologische vorprüfung. 2,6-dimethylheptanol-2. BASF Aktiengesellschaft. Dr. Gelbke. Date 18.02.77. Unpublished data submitted by EFFA to FLAVIS Secretariat.
3. Bertens AMC and van de Waart EJ, 2000. Evaluation of the ability of linalylacetate to induce chromosome aberrations in cultured peripheral human lymphocytes. Hoffmann-LaRoche. Report no. 38576, study no. 289968. June 22, 2000. Unpublished report submitted by EFFA to FLAVIS Secretariat. Bertens AMC and van de Waart EJ, 2000. Evaluation of the ability of linalylacetate to induce chromosome aberrations in cultured peripheral human lymphocytes. Hoffmann-LaRoche. Report no. 38576, study no. 289968. June 22, 2000. Unpublished report submitted by EFFA to FLAVIS Secretariat.
4. Brown RJ and Wheeler RJ, 1979. Subchronic test of t-butanol (C55367) in B6C3F1 mice and Fischer 344 rats in drinking water with cover letter dated 3/23/94. EPA Doc 86940000200, microfiche no. OTS0556795. Date 10/26/79. Unpublished data submitted by EFFA to FLAVIS Secretariat.
5. Buck NR and Renwick AG, 1998. Report on the metabolism of linalyl acetate in rat tissue and intestinal homogenates. University of Southampton. Clinical Pharmacology. School of Medicine. Report 1.2. Date 28/11/98. Unpublished report submitted by EFFA to FLAVIS Secretariat.
6. DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/02 2012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.
7. DG SANTE (Directorate General for Health and Food safety), 2015. Information from DG SANTE concerning the status of substance [FL-no: 01.014] in FGE.78 (http://ec.europa.eu/food/committees/regulatory/scfcah/toxic/docs/sum_20150211_en.pdf).
8. Dunster JS, Watson P and Brooks PN, 2007. Dymrycetol: Ninety day repeated dose oral (Gavage) toxicity study in the rat. SafePharm Laboratories. Project No. 2153-003. Unpublished report submitted by EFFA to FLAVIS Secretariat.
9. Eastman Kodak Company, 1994. Toxicity report (summary) of t-butyl alcohol with cover letter dated 02/15/94. EPA Doc 86940000084, microfiche no. OTS0556680. Date 10/05/63. Unpublished data submitted by EFFA to FLAVIS Secretariat.
10. EFFA (European Flavour and Fragrance Association), 2002. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
11. EFFA (European Flavour and Fragrance Association), 2004a. Submission 2003-1. Flavouring group evaluation of 25 flavouring substances (candidate chemicals) of the chemical group 6 (annex I of 1565/2000/EC) structurally related to aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances [JECFA/WHO FAS 42/51] used as flavouring substances. 1 December 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.29.

12. EFFA (European Flavour and Fragrance Association), 2004b. Submission 2003-1. Flavouring group evaluation of 25 flavouring substances (candidate chemicals) of the chemical group 6 (annex I of 1565/2000/EC) structurally related to aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances [JECFA/WHO FAS 42/51] used as flavouring substances. 1 December 2003. FLAVIS/8.29. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat.
13. EFFA (European Flavour and Fragrance Association), 2004c. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.
14. EFFA (European Flavour and Fragrance Association), 2005a. Submission 2004-3. Flavouring group evaluation of 32 flavouring substances (candidate chemicals) of chemical group 31 (annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons [FEMA 2004-2] used as flavouring substances. 24 June 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.38.
15. EFFA (European Flavour and Fragrance Association), 2005b. Supplement of 2 flavouring substances (candidate chemicals) to the flavouring group evaluation of the chemical group 05 (Annex I of 1565/2000/EC) structurally related to saturated aliphatic, alicyclic secondary alcohols, ketones and related saturated and unsaturated esters [FAO/WHO JECFA 42/51], or aliphatic secondary alcohols, ketones and related esters used as flavouring substances. 4 July 2005. Addendum to FGE.18 (EFFA submission 2003-1). Unpublished data submitted to FLAVIS Secretariat. Flavis/8.91.
16. EFFA (European Flavour and Fragrance Association), 2006a. Addendum of 7 flavouring substances (candidate chemicals) to the flavouring group evaluation of the chemical group 06 (Annex I of 1565/2000/EC) structurally related to aliphatic, alicyclic and alicyclic terpenoid tertiary alcohols and structurally related substances used as flavouring substances. November 2006. Addendum to FGE.18 (EFFA submission 2003-1). Unpublished data submitted by EFFA to FLAVIS Secretariat. Flavis/8.64.
17. EFFA (European Flavour and Fragrance Association), 2006b. Addendum of 1 flavouring substance (candidate chemical) to the flavouring group evaluation of the chemical group 31 (Annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons [JECFA/WHO FAS 54/63] used as flavouring substances. Addendum to FGE.25 (EFFA submission 2004-3). 21 December 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.106.
18. EFFA (European Flavour and Fragrance Association), 2007a. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages. FLAVIS/8.70.
19. EFFA (European Flavour and Fragrance Association), 2007b. Addendum of 4 flavouring substances (candidate chemicals) to the flavouring group evaluation of the chemical group 6 (Annex I of 1565/2000/EC) structurally related to aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters [FAO/WHO JECFA 42/51] used as flavouring substances. 21 December 2007. Addendum to FGE.18 (EFFA submission 2003-1). Unpublished data submitted by EFFA to FLAVIS Secretariat. Flavis/8.90.
20. EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

21. Flavour Industry, 2009a. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-18Rev2.
22. Flavour Industry, 2009b. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-18Rev2.
23. Flavour Industry, 2012. Unpublished information submitted by Flavour Industry to the EU Commission, EFSA and the FLAVIS Secretariat. A-18Rev3 [FL-no: 02.146].
24. Food and Drug Research Laboratories, Inc., 1973. Teratologic evaluation of FDA 71-57 (menthol natural, Brazilian). Food and Drug Research Laboratories, Inc. Morgareidge K Lab. no. 1573k, study no. FDABF-GRAS-134, June 1, 1973. Unpublished report submitted by EFFA to FLAVIS Secretariat.
25. Food and Drug Research Laboratories, Inc., 1975. Mutagenic evaluation of compound FDA 71-57, menthol. Litton Bionetics, Inc. Weir, R.J. January 14, 1975. Unpublished report submitted by EFFA to FLAVIS Secretariat.
26. Gocke E, 1999. Mutagenicity evaluation of Dihydro Linalool (Ro 02-8243/000) in the Ames Test. Roche Ltd. Project No. 486M99. October 20, 1999. Unpublished report submitted by EFFA to FLAVIS Secretariat.
27. Haworth SR, Lawlor TE, Williams NA, Burke PJ, Hans LJ, Reichard GL, Wagner VO and Olewine SM, 1981a. Salmonella/mammalianmicrosome preincubation mutagenicity assay using Arconol with cover letter dated 03/24/94. EPA Doc 86940000260, microfiche no. OTS0572363. Date 05/08/81. Unpublished data submitted by EFFA to FLAVIS Secretariat.
28. Haworth SR, Lawlor TE, Smith JK, Williams NA, Burke PJ, Reichard GL, Hans LJ, Olewine SM and Wagner VO, 1981b. Salmonella/mammalianmicrosome preincubation mutagenicity assay with t-butyl alcohol with cover letter dated 03/24/94. EPA Doc 86940000253, microfiche no. OTS0572356. Date 05/08/81. Unpublished data submitted by EFFA to FLAVIS Secretariat.
29. Herken H, 1961. Pharmakologisches gutachten über die vertraglichkeit von natürlichem (l-) und synthetischem (d,l-) menthol. Unpublished report from the director, Pharmakologischen Institute der Freien Universität, Berlin-Duhlem, submitted to the World Health Organization by Schering AG. Cited in JECFA, 1976. Toxicological evaluation of certain food additives. Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 21-29 April, 1976. Food Additive Series 10, pp. 64-69.
30. Hoberman AM and Christian MS, 1989. Reproductive and developmental toxicity screening test of coriander oil administered orally via gavage to Cr1: Cd TM (SD) BR female rats. Argus Research Laboratories Inc. Report no. 36124, study no. 412-005. 12 April, 1989. Unpublished report submitted by EFFA to FLAVIS Secretariat.
31. Hoffman-LaRoche Inc., 1967. Acute toxicity, eye and skin irritation tests on aromatic compounds. Report no. 30645. August 20, 1968. Unpublished report submitted by EFFA to FLAVIS Secretariat.
32. Hoffman-LaRoche Inc., 1967b. Acute toxicity, eye and skin irritation tests on aromatic compounds. Roche Chemical Division. Report no. 30642. September 20, 1967. Unpublished report submitted by EFFA to FLAVIS Secretariat.
33. IOFI (International Organization of the Flavor Industry), 1995. European inquiry on volume of use.

34. IOFI (International Organization of the Flavor Industry), 2006. Sclareol and cedrol: subchronic toxicity study (28 day repeated dose oral gavage study in rats). Unpublished report to IOFI submitted by EFFA to FLAVIS Secretariat.
35. Johnson DE, 1981a. Acute oral toxicity (LD50) study in rats with t-butyl alcohol with cover letter dated 03/24/94. Arco Chemical Co. EPA Doc 86940000248, microfiche no. OTS0572351. Date 07/24/81. Unpublished data submitted by EFFA to FLAVIS Secretariat.
36. Johnson DE, 1981b. Acute oral toxicity (LD50) study in rats with Arconol with cover letter dated 03/24/94. Arco Chemicals Co. EPA Doc 86940000255, microfiche no. OTS0572358. Date 08/03/81. Unpublished data submitted by EFFA to FLAVIS Secretariat.
37. King M-T and Harnasch D, 2002. Mutagenicity study of Bisabolol alpha nat. H&R in the Salmonella typhimurium/mammalian microsome reverse mutation assay (Ames test). Freiburger Labor für Mutagenitätsprüfung. Project no. AM01202N. August 5, 2002. Unpublished report submitted by EFFA to FLAVIS Secretariat.
38. King M-T, 2000. Mutagenicity study of dihydromyrcenol in the salmonella typhimurium/mammalian microsome reverse mutation assay (Ames-Test). Freiburger Labor für Mutagenitätsprüfung. Project No. AM04000N. August 31, 2000. Unpublished report submitted by EFFA to FLAVIS Secretariat.
39. Kirby PE, Pizzarello RF, Williams PE, Wattam RE, Clarke JJ, Condon MB, Johnson JL, Madden G, Hoynak GJ, Stroud RM and Reichard GL, 1981. Evaluation of test article t-butyl alcohol 99.9% (MRI #635) & arconol (MRI #636) for mutagenic potential employing the L5178Y TK+/- mutagenesis assay w/cover letter dated 03/24/94. Arco Chemical Co. EPA Doc 6940000262, microfiche no. OTS0572365. Date 08/06/81. Unpublished data submitted by EFFA to FLAVIS Secretariat.
40. Kirby PE, Douglas-Tabor Y, Simmons RT, Voglezon RA, Rogers-Back AM, Brauninger RM, O'Keefe TR and Fernandez-Madrid AM, 1984. Mouse lymphoma mutagenesis assay with #70437 (alpha-terpineol). Short-term test program sponsored by The Division of Cancer Etiology, National Cancer Institute. Study no. ML-NCI#109. Unpublished data submitted by EFFA to FLAVIS Secretariat.
41. Levenstein I, 1975. Acute oral toxicity (rat - 5 gms./kg. body weight dose). Dermal toxicity (rabbit - 5 gms./kg. body weight dose). Linalyl butyrate. Leberco Laboratories, Inc. Assay no. 51746. January 30, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.
42. Lewis EM, 2006. Oral (gavage) development toxicity study of linalool in rats. Charles River Laboratories. Project No. TIF 00009. 7 July 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat.
43. Lorillard (Lorillard Tobacco Company), 1982. Mutagenicity evaluation of alpha-terpineol in the mouse lymphoma mutation assay. LBI Project no. 20989. Unpublished report submitted by EFFA to FLAVIS Secretariat.
44. Lorillard (Lorillard Tobacco Company), 1983. Mutagenicity evaluation of alpha-terpineol in the Ames Salmonella/microsome plate test. Lorillard Research Center. LBI Project no. 20988. Unpublished report submitted by EFFA to FLAVIS Secretariat.
45. Meerts IATM and van de Waart EJ, 2001. Micronucleus test in bone marrow cells of the mouse with linalool. Hoffman-LaRoche. Report no. 38577, project no. 328826. Date 18 October, 2001. Unpublished report submitted by EFFA to FLAVIS Secretariat.

46. Moreno OM, 1971. Acute oral LD 50 in rats of dimethyl benzyl carbinyl acetate, pseudo linalyl acetate, oil fir needles siberian, terpeneol, alpha-hexyl cinnamate aldehyde. Bio/Dynamics Inc. Project no. 10-71. March 24, 1971. Unpublished data submitted by EFFA to FLAVIS Secretariat.
47. Moreno OM, 1972a. Acute oral toxicity in rats. Myrcenol. Toxicological Resources. Project no. 846-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.
48. Moreno OM, 1972b. Acute oral toxicity in rats. Myrcenyl acetate. Toxicological Resources. Project no. 813-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.
49. Moreno OM, 1973a. Acute oral toxicity in rats. Dermal toxicity in rabbits. 3,6-Dimethyl-3-octanol. MB Research Laboratories, Inc. Project no. MB 73-191. June 19, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
50. Moreno OM, 1973b. Acute oral toxicity in rats. Dermal toxicity in rabbits. Dihydro myrcenol. MB Research Laboratories, Inc. Project no. MB 73-90. June 4, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
51. Moreno OM., 1973p. Acute oral toxicity in rats. Dermal toxicity in rabbits. Linalyl propionate. MB Research Laboratories, Inc. Project no. MB 73-99. May 14, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
52. Moreno OM, 1973d. Acute oral toxicity in rats. Dermal toxicity in rabbits. Terpinyl propionate. MB Research Laboratories, Inc. Project no. MB 73-314. November 12, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
53. Moreno OM, 1973e. Acute oral toxicity in rats. Dermal toxicity in rabbits. Guaiacwood acetate. MB Research Laboratories, Inc. Project no. 72-17. Date 2/1/73. Unpublished data submitted by EFFA to FLAVIS Secretariat.
54. Moreno OM, 1973f. Acute oral toxicity in rats. Dermal toxicity in rabbits. Dihydro terpeneol. MB Research Laboratories, Inc. Project no. MB 73-91. July 5, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
55. Moreno OM, 1975. Acute oral toxicity in rats. Dermal toxicity in rabbits. Linalyl isovalerate. MB Research Laboratories, Inc. Project no. MB 75-762. April 10, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.
56. Moreno OM, 1976b. Acute oral toxicity in rats. Dermal toxicity in rabbits. Tetrahydro linalool. MB Research Laboratories, Inc. Project no. MB 76-1034. March 13, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.
57. Moreno OM, 1976c. Acute oral toxicity in rats. Dermal toxicity in rabbits. Nerolidyl acetate. MB Research Laboratories, Inc. Project no. MB 76-1356. October 7, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.
58. Moreno OM, 1976d. Acute oral toxicity in rats. Dermal toxicity in rabbits. Terpinyl formate. MB Research Laboratories, Inc. Project no. MB 75-934. January 5, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.
59. Moreno OM, 1977. Acute oral toxicity in rats. Dermal toxicity in rabbits. 4-Terpeneol. MB Research Laboratories, Inc. Project no. MB 77-1548. April 8, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

60. Moreno OM, 1982a. Oral toxicity in rats. Dermal toxicity in rabbits. Geranyl linalool. MB Research Laboratories, Inc. Project no. MB 82-6239. Date 9/29/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.
61. Moreno OM, 1982b. Oral toxicity in rats. Dermal toxicity in rabbits. Terpinyl isobutyrate. MB Research Laboratories, Inc. Project no. MB 82-6246. Date 9/29/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.
62. National Cancer Institute, 1983. Mutagenicity of G70437. alpha-Terpineol. Unpublished data submitted by EFFA to FLAVIS Secretariat.
63. Piccirillo VJ and Hartman WC, 1980. Acute oral toxicity study in rats (LD50) with 79-054-01 (methyl 1-acetoxycyclohexyl ketone). Borriston Research Laboratories, Inc. Project no. 204-Q. March 21, 1980. Unpublished report submitted by EFFA to FLAVIS Secretariat.
64. Putman DL, 1985. An in vitro evaluation of t-butyl alcohol 99.9% to produce sister chromatid exchanges in Chinese hamster ovary cells with cover letter dated 03/24/94. Arco Chemical Co. EPA Doc 86940000254, microfiche no. OTS0572357. Date 06/05/81. Unpublished data submitted by EFFA to FLAVIS Secretariat.
65. Rhône-Poulenc Inc., 1992a. Acute toxicity and local tolerance. Linalool and dehydrolinalool. Document no. 23272. 11 September, 1992. Unpublished data submitted by EFFA to FLAVIS Secretariat.
66. Russell T, 1973. Acute oral toxicity (rat - 5 gm/kg body weight dose). Acute dermal toxicity (rabbit - 5 gm/kg body weight dose). 3,7-Dimethyl-1,6-octadien-3-yl formate. Toxicological Resources. Project nos. 1277a-73, 1277b-73. January 25, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
67. Scheerbaum D, 2001. Reverse Mutation Assay (Ames test) with *Salmonella typhimurium*. Cedrol Cryst. Angewandte Biologie. Project No. 000915HR. 5 March 2001. Unpublished report submitted by EFFA to FLAVIS Secretariat.
68. Thilagar A, Kumaroo PV, McCoy S and Reichard G, 1981. An in vitro evaluation of t-butyl alcohol - Arconol Batch # A209411 to produce sister chromatid exchanges in Chinese hamster ovary cells with cover letter dated 03/24/94. Arco Chemical Co. EPA Doc 86940000261, microfiche no. OTS0572364. Date 06/05/81. Unpublished data submitted by EFFA to FLAVIS Secretariat.
69. Watanabe S, 2002. Ambrinol-20: reverse Mutation Test "Ames Test" with *S. typhimurium* and *E.coli*. Takasago International Corporation. Project No. M-200. 9 May 2002. Unpublished report submitted by EFFA to FLAVIS Secretariat.
70. Wohl AJ, 1974. Acute oral toxicity (rat - 5 gms./kg. body weight dose). Dermal toxicity (rabbit - 5 gms./kg. body weight dose). Ocimenol. August 19, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

REFERENCES

- Acharya S, Mehta K, Rodriguez, S Pereira J, Krishnan S and Rao CV, 1997. A histopathological study of liver and kidney in male Wistar rats treated with subtoxic doses of t-butyl alcohol and trichloroacetic acid. *Experimental and Toxicologic Pathology* 49(5), 369-373.
- Amberg A, Bernauer U, Scheutzw D and Dekant W, 1999. Biotransformation of [(12)C]- and [(13)C]-tert-amyl methyl ether and tert-amyl alcohol. *Chemical Research in Toxicology* 12(10), 958-964.
- Andersen PH and Jensen NJ, 1984. Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. *Mutation Research* 138, 17-20.
- Asakawa Y, Ishida T, Toyota M and Takemoto T, 1986. Terpenoid biotransformation in mammals IV. Biotransformation of (+)-longifolene, (-)-caryophyllene, (-)-caryophyllene oxide, (-)-cyclocolorenone, (+)-nootkatone, (-)-elemol, (-)-abietic acid and (+)-dehydroabietic acid in rabbits. *Xenobiotica* 16(8), 753-767.
- Atzl G, Bertel M, Daxenbichler G and Gleispach H, 1972. Determination of etheral oils from the urine by gas-liquid chromatography. *Chromatographia* 5(4), 250-255.
- Bakke OM and Scheline RR, 1970. Hydroxylation of aromatic hydrocarbons in the rat. *Toxicology and Applied Pharmacology* 16(3), 691-700.
- Bang L and Ourisson G, 1975. Hydroxylation of cedrol by rabbits. *Tetrahedron Letters*, 24, 1881-1884
- Barilyak IR and Kozachuk SY, 1988. Investigation of the cytogenetic effect of a number of monohydric alcohols on rat bone marrow cells. *Cytology and genetics* 22(2), 51-54.
- BASF (Baden Aniline and Soda Factory), 1972. BASF Aktiengesellschaft, Abteilung Toxikologie; unveroeffentlichte Untersuchung (XXII/49), 13.10.1972. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 115-18-4, EINECS Name 2-methylbut-3-en-2-ol. Section 1-5.
- BASF (Baden Aniline and Soda Factory), 1989. BASF Aktiengesellschaft, Abteilung Toxikologie; unveroeffentlichte Untersuchung (89/43), 20.03.1989. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 115-18-4, EINECS Name 2-methylbut-3-en-2-ol. Section 1-5.
- BASF (Baden Aniline and Soda Factory), 1991. BASF Aktiengesellschaft, Abteilung Toxikologie; unveroeffentlichte Untersuchung (89/43), 22.07.1991. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 115-18-4, EINECS Name 2-methylbut-3-en-2-ol. Section 1-5.
- BASF (Baden Aniline and Soda Factory), 1992. BASF Aktiengesellschaft, Abteilung Toxikologie; unveroeffentlichte Untersuchung (89/809), 10.06.1992. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 115-18-4, EINECS Name 2-methylbut-3-en-2-ol. Section 1-5.
- BASF (Baden Aniline and Soda Factory), 1994. BASF AG, Abteilung Toxikologie; unveroeffentlichte Untersuchung (21C0809/89100), 07.03.1994. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 115-18-4, EINECS Name 2-methylbut-3-en-2-ol. Section 1-5.
- Beaugé F, Clément M, Nordmann J and Nordmann R, 1981. Liver lipid disposal following t-butanol administration to rats. *Chemico-Biological Interactions* 38, 45-51.

- Bernauer U, Amberg A, Scheutzw D and Dekant W, 1998. Biotransformation of 12C- and 2-13C-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and tert-butyl alcohol in rats: Identification of metabolites in urine by 13C nuclear magnetic resonance and gas chromatography/mass spectrometry. *Chemical Research in Toxicology* 11, 651-658.
- Boyle R, McLean S, Foley WJ and Davies NW, 1999. Comparative metabolism of dietary terpene, p-cymene, in generalist and specialist folivorous marsupials. *Journal of Chemical Ecology* 25, 2109-2126.
- Brown B, Schaffarzik RW and Dreisbach RH, 1955. Anticonvulsant properties of certain secondary and tertiary alcohols. *Journal of Pharmacology and Experimental Therapeutics* 115, 230-239.
- Chadha A and Madyastha KM, 1984. Metabolism of geraniol and linalool in the rat and effects on liver and lung microsomal enzymes. *Xenobiotica* 14(5), 365-374.
- CIR, (Cosmetic Ingredient Review), 1999. Final report on the safety assessment of bisabolol. *International Journal of Toxicology* 18(Suppl. 3), 33-40.
- Cirvello JD, Radovsky A, Heath JE, Farnell DR and Lindamood C, 1995. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. *Toxicology and Industrial Health* 11(2), 151-165.
- CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.
- Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. *Food and Cosmetics Toxicology* 16(3), 255-276.
- Daniel MA and Evans MA, 1982. Quantitative comparison of maternal tertiary butanol on postnatal development. *Journal of Pharmacology and Experimental Therapeutics* 222(2), 294-300.
- Delgado IF, Carvalho RR, de Almeida Nogueira ACM, Mattos AP, Figueiredo LH, Oliveira SHP, Chahoud I and Paumgartten FJR, 1993a. Study on embryo-foetotoxicity of beta-myrcene in the rat. *Food and Chemical Toxicology* 31, 31-35.
- Delgado IF, de Almeida Nogueira ACM, Souza CAM, Costa AMN, Figueiredo LH, Mattos AP, Chahoud I and Paumgartten FJR, 1993b. Peri- and postnatal developmental toxicity of beta-myrcene in the rat. *Food and Chemical Toxicology* 31, 623-628.
- Dow Chemical Company, 1982. Unpublished data. 2-phenyl-2-propanol. In: Clayton GD and Clayton FE (Eds.). *Patty's Industrial Hygiene and Toxicology* 2C. 3rd Ed. John Wiley & Sons, New York, 4607-4701.
- Eder E, Neudecker T, Lutz D and Henschler D, 1980. Mutagenic potential of allyl and allylic compounds. Structure-activity relationship as determined by alkylating and direct in vitro mutagenic properties. *Biochemical Pharmacology* 29, 993-998.
- EFSA (European Food Safety Authority), 2004. Minutes of the 7th Plenary Meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: <http://www.efsa.europa.eu/en/events/event/afc040712-m.pdf/>.

- EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food (AFC) related to Flavouring Group Evaluation 78: Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal (2009) 931, 1-59.
- EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2011. Scientific Opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8. EFSA Journal 2011; 9(5):1847, 91 pp. doi:10.2903/j.efsa.2011.1847.
- EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Statement on evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] based on additional data on a supporting substance. EFSA Journal 2012;10(12):2995. [5 pp.] doi:10.2903/j.efsa.2012.2995
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev3. EFSA Journal 2015;13(4):4067. [72 pp.] doi:10.2903/j.efsa.2015.4067
- Eisenberg Jr F, Field JB and Stetten Jr DW, 1955. Studies on glucuronide conjugation in man. Archives of Biochemistry and Biophysics 59(1), 297-299.
- Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.
- Faulkner TP, Wiechart JD, Hartman DM and Hussain AS, 1989. The effects of prenatal tertiary butanol administration in CBA/J and C57BL/6J mice. Life Sciences 45, 1989-1995.
- Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 18, 219-232.
- Gaworski CL, Vollmuth TA, Dozier MM, Heck JD, Dunn LT, Ratajczak HV and Thomas PT, 1994. An immunotoxicity assessment of food flavouring ingredients. Food and Chemical Toxicology 32(5), 409-415.
- Gomes-Carneiro MR, Felzenszwalb I and Paumgartten FJ, 1998. Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay. Mutation Research 416, 129-136.
- Gomes-Carneiro MR, Dias DMM, De-Oliveira ACAX and Paumgartten FJR, 2005. Evaluation of mutagenic and antimutagenic activities of alpha-bisabolol in the salmonella/microsome assay. Mutation Research 585, 105-112.
- Habersang S, Leuschner F, Isaac O and Thiemer K, 1979. Pharmacological studies with compounds of chamomile. IV. Studies on toxicity of (-)-alpha-bisabolol. Journal of Medicinal Plants Research 37, 115-123.

- Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM, Long EL, Nelson AA and Brouwer JB, 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. *Food and Cosmetics Toxicology* 5(2), 141-157.
- Hall RL, 1979. Report on the hydrolysis of linalyl acetate and ethyl formate by artificial gastrointestinal juices. McCormick and Company, Inc. Private communication to FEMA.
- Heck JD, Vollmuth TA, Cifone MA, Jagannath DR, Myhr B and Curren RD, 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *Toxicologist* 9(1), 257-272.
- Heymann E, 1980. Carboxylesterases and amidases. In: Jakoby WB (Ed.). *Enzymatic basis of detoxication*. 2nd Ed. Academic Press, New York, pp. 291-323.
- Hill RM, Barer J, Leighton Hill L, Butler CM, Harvey DJ and Horning MG, 1975. An investigation of recurrent pine oil poisoning in an infant by the use of gas chromatographic-mass spectrometric methods. *The Journal of Pediatrics*. 87(1), 115-118.
- Horning MG, Butler CM, Stafford M, Stillwell RN, Hill RM, Zion TE, Harvey DJ and Stillwell WG, 1976. Metabolism of drugs by the epoxide-diol pathway. *Advances in Mass Spectroscopy in Biochemistry and Medicine*, Vol. I. Spectrum Publications, Inc., 91-108.
- Ishida T, Asakawa Y, Takemoto T and Aratani T, 1981. Terpenoids biotransformation in mammals [I]: biotransformation of alpha-pinene, beta-pinene, pinane, 3-carene, carene, myrcene and p-cymene in rabbits. *Journal of Pharmacological Sciences* 70(4), 406-415.
- Ishidate Jr M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food and Chemical Toxicology* 22(8), 623-636.
- Ivett JL, Brown BM, Rodgers C, Anderson BE, Resnick MA and Zeiger E, 1989. Chromosomal aberrations and sister chromatid exchange tests in chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. *Environmental and Molecular Mutagenesis* 14, 165-187.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1980. Evaluation of certain food additives. Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 648, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1998. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9-18 June 1998. FAO Food and Nutrition paper 52 Add. 6.

- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000a. Evaluation of certain food additives. Fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000b. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Committee of Food Additives. Fifty-fifth Meeting. Geneva, 6-15 June 2000. FAO Food and Nutrition paper 52 Add. 8.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2001. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Committee of Food Additives 57th session. Rome, 5-14 June 2001. FAO Food and Nutrition paper 52 Add. 9.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Committee of Food Additives 61st session. Rome, 10-19 June 2003. FAO Food and Nutrition paper 52 Add. 11.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005a. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005b. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Safety evaluation of certain food additives and contaminants. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.
- Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh OG, 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food and Cosmetics Toxicology 2, 327-343.
- Jiménez J, Longo E and Benítez T, 1988. Induction of petite yeast mutants by membrane-active agents. Applied and Environmental Microbiology 54(12), 3126-3132.
- Kaffenberger RM and Doyle MJ, 1990. Determination of menthol and menthol glucuronide in human urine by gas chromatography using an enzyme-sensitive internal standard and flame ionization detection. Journal of Chromatography 527, 59-66.
- Kamil IA, Smith JN and Williams RT, 1953. Studies in detoxication. 46. The metabolism of aliphatic alcohols. The glucuronic acid conjugation of acyclic aliphatic alcohols. Biochemical Journal 53(1), 129-136.
- Kauderer B, Zamith H, Paumgarten JR and Speit G, 1991. Evaluation of the mutagenicity of beta-myrcene in mammalian cells in vitro. Environmental and Molecular Mutagenesis 18, 28-34.
- Kouzi SA, McChesney JD and Walker LA, 1993. Identification of four biliary metabolites of the diterpene sclareol in the laboratory rat. Xenobiotica. 23(6), 621-632.
- Lindamood C, Farnell DR, Herschell DG, Prejean JD, Collins JJ, Takahashi K and Maronpot RR, 1992. Subchronic toxicity studies of t-butyl alcohol in rats and mice. Fundamental and Applied Toxicology 19, 91-100.

- Madyastha KM and Srivatsan V, 1987. Metabolism of beta-myrcene in vivo and in vitro: its effects on rat-liver microsomal enzymes. *Xenobiotica* 17, 539-549.
- Madyastha KM and Srivatsan V, 1988. Biotransformation of alpha-Terpeniol in the rat: Its effects on the liver microsomal cytochrome P-450 system. *Bulletin of Environmental Contamination and Toxicology* 41, 17-25.
- McGregor DB, Brown A, Cattanaach P, Edwards I, McBride D and Caspary WJ, 1988. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay II: 18 coded chemicals. *Environmental and Molecular Mutagenesis* 11, 91-118.
- Melnick RL, 2002. Carcinogenicity and mechanistic insights on the behavior of epoxides and epoxide-forming chemicals. *Annals of the New York Academy of Sciences* 982, 177-189.
- Moreno OM, 1976a. Report to RIFM, 8 October. Cited in Ford, R.A., Api, A.M., Letizia, C., 1992. 2,6-dimethyl-2-heptanol. *Food and Chemical Toxicology* 23S.
- Munch JC, 1972. Aliphatic alcohols and alkyl esters: Narcotic and lethal potencies to tadpoles and to rabbits. *Industrial Medicine* 41(4), 31-33.
- Murthy PBK, Ahmed MM and Regu K, 1991. Lack of genotoxicity of menthol in chromosome aberration and sister chromatid exchange assays using human lymphocytes in vitro. *Toxicology In Vitro* 5(4), 337-340.
- Myhr BC and Caspary WJ, 1991. Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program. *Environmental and Molecular Mutagenesis* 18, 51-83.
- National Cancer Institute, 1979. Bioassay of dl-menthol for possible carcinogenicity. U.S. Department of Health, Education, and Welfare. April 1978. NCI Technical Report Series, no. 98.
- Nohmi T, Miyata R, Yoshikawa K and Ishidate M, 1985. [Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests]. *Eisei Shikenjo hokoku. Bulletin of National Institute of Hygienic Sciences* 103(60), 60-64. (In Japanese)
- NTP (National Toxicology Program), 1984. Chromosome Aberrations (2-Methylpropan-2-ol). Study ID: A263726. [Online].: <http://ntp.niehs.nih.gov/>
- NTP (National Toxicology Program), 1987. NTP technical report on the carcinogenesis studies of food grade geranyl acetate (71 % geranyl acetate, 29 % citronellyl acetate) (CAS no. 105-87-3) in F344/N rats and B6C3F1 mice (gavage study). October 1987. NTP-TR 252. NIH Publication no. 88-2508.
- NTP (National Toxicology Program), 1994. Annual plan for fiscal year 1994. Genetic toxicology. Public Health Service. Department of Health and Human Services, pp. 61-69.
- NTP (National Toxicology Program), 1995. NTP technical report on the toxicology and carcinogenesis studies of t-butyl alcohol (CAS no. 75-65-0) in F344/N rats and B6C3F1 mice (drinking water studies). May 1995. NTP TR-436, NIH Publication no. 95-3167.
- NTP (National Toxicology Program), 1996. Bone Marrow Micronucleus study (2-Methylpropan-2-ol). Study no. A26596. [Online].: <http://ntp.niehs.nih.gov/>
- NTP (National Toxicology Program), 1997. NTP technical report on toxicity studies of t-butyl alcohol (CAS No. 75-65-0) administered by inhalation to F344/N rats and B6C3F1 mice. July 1997. NTP TR-53. NIH Publication no. 97-3942.

- NTP (National Toxicology Program), 1999. Salmonella Study Summary (beta-myrcene). Study no. A96914.
- NTP (National Toxicology Program), 2000. Salmonella Study (isophytol). Study no. A23522. [Online].: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.studysummary&study_no=A23522&cas_no=505%2D32%2D8&endpointlist=SA
- NTP (National Toxicology Program), 2001. Peripheral Blood Micronucleus (beta-myrcene). Study no. A06528. [Online].: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?cas_no=123%2D35%2D3&endpointlist=MN
- NTP (National Toxicology Program), 2004. Draft study results of a 13-week gavage study in rats (beta-myrcene). Study no. C099023. [Online].: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. Accessed 01/03/2005.
- Oda Y, Hamono Y, Inoue K, Yamamoto H, Niihara T and Kunita N, 1978. [Mutagenicity of food flavors in bacteria]. Osaka-Furitsu Koshu Eisei Kenkyu Hokoku Shokuhin Eisei Hen 9, 177-181. (In Japanese)
- Oda Y, Hamono Y, Inoue K, Yamamoto H, Niihara T and Kunita N, 1979. [Mutagenicity of food flavors in bacteria]. Osaka Furitsu Koshu Eisei Kenkyusho kenkyu hokoku. Shokuhin eisei hen 9, 177-181. (In Japanese)
- Oser BL, 1967. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. Citronellol. Geneva, 21-23 August 1967. FAO Nutrition Meetings Report Series no. 44a, pp. 1-5, 21-22.
- Parke DV, Rahman KHM and Walker R, 1974. The absorption, distribution and excretion of linalool in the rat. Biochemical Society Transactions 2, 612-615.
- Patty FA, 1982. Patty's Industrial Hygiene and Toxicology, 3rd Ed. John Wiley & Sons, New York.
- Paumgartten FJ, De-Carvalho RR, Souza CC, Madi K and Chahoud I, 1998. Study of the effects of beta-myrcene on rat fertility and general reproductive performance. Brazilian Journal of Medical and Biological Research 31(7), 955-965.
- Poet TS, Valentine JL and Borghoff SJ, 1997. Pharmacokinetics of tertiary butyl alcohol in male and female Fischer 344 rats. Toxicology Letters 92(3), 179-186.
- Politano VT, Lewis EM, Hoberman AM, Christian MS, Diener RM and Api AM, 2008. Developmental toxicity of dimyrcetol in rats. The Toxicologist 102(1), 313.
- Quick AJ, 1928. Quantitative studies of beta-oxidation. IV. The metabolism of conjugated glycuronic acids. Journal of Biological Chemistry 80, 535-541.
- Rahman KQMQ, 1974. Studies on the metabolism of linalool, a naturally occurring food flavour. Being a thesis presented for the award of a degree of doctor of philosophy in the university of Surrey. Department of biochemistry, University of Surrey, Guildford, Surrey, England. December 1974.
- Rockwell P and Raw I, 1979. A mutagenic screening of various herbs, spices and food additives. Nutrition and Cancer 1(4), 10-15.
- Roscheisen C, Zamith H, Paumgartten F and Speit G, 1991. Influence of beta-myrcene on sister chromatid exchanges induced by mutagens in V79 and HTC cells. Mutation Research 264, 43-49.

- Sasaki YF, Imanishi H, Ohta T and Yasuhiko S, 1989. Modifying effects of components of plant essence on the induction of sister-chromatid exchanges in cultured Chinese hamster ovary cells. *Mutation Research* 226, 103-110.
- SCF (Scientific Committee for Food), 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.
- SCF (Scientific Committee for Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.
- Schaffarzick RW and Brown BJ, 1952. The anticonvulsant activity and toxicity of methylparfynol (Dormison) and some other alcohols. *Science* 116, 663-665.
- Shelby MD, Erexson GL, Hook GJ and Tice RR, 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environmental and Molecular Mutagenesis* 21(2), 160-179.
- Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK and Gori GB, 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain a mice. *Cancer Research* 33(12), 3069-3085.
- Storer RD, McKelvey TW, Kraynak AR, Elia MC, Barnum JE, Harmon LS, Nichols WW and DeLuca JG, 1996. Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. *Mutation Research* 368(2), 59-101.
- Takahashi K, Lindamood C and Maronpot RR, 1993. Retrospective study of possible alpha-2μ-globulin nephropathy and associated cell proliferation in male Fischer 344 rats dosed with t-butyl alcohol. *Environmental Health Perspectives* 101(Suppl. 5), 281-286.
- Thorup I, Wurtzen G, Carstensen J and Olsen P, 1983. Short-term toxicity study in rats dosen with pulegone and menthol. *Toxicology Letters* 19(3), 207-210.
- TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2000. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database. Zeist, The Netherlands. TNO Triskelion, 1963-2000.
- Trifilieff E, Luu B and Ourisson G, 1975. Hydroxylation du Cedrol par le chien. *Tetrahedron Letters* 48, 4307-4310. [In French]
- Walde A, Ve B, Scheline RR and Monge P, 1983. p-Cymene metabolism in rats and guinea pigs. *Xenobiotica* 13, 503-512.
- Wokes F, 1932. The antiseptic value and toxicity of menthol isomers. *Quarterly Journal of Pharmacy and Pharmacology* 5, 233-244.
- Wright SE, 1945. Detoxication mechanisms in the sheep. *University of Quensland Papers* 1 (25-26), 1-12.
- Yamaguchi T, Caldwell J and Farmer PB, 1994. Metabolic fate of [3H]-l-menthol in the rat. *Drug Metabolism and Disposition* 22(4), 616-624.

- Yamahara J, Kimura H, Kobayashi M, Okamoto T, Sawada T, Fujimura H and Chisaka T, 1985. Cholagogic action and characteristics of (+/-) alpha-terpineol-beta-D-O-glucopyranoside, a new monoterpenoid glucoside. *Chemical and Pharmaceutical Bulletin* 33(4), 1669-1675.
- Yoo YS, 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. *Osaka City Medical Journal* 34(3-4), 267-288.
- Zamith HP, Vidal MNP, Speit G and Paumgartten FJR, 1993. Absence of genotoxic activity of beta-myrcene in the in vivo cytogenetic bone marrow assay. *Brazilian Journal of Medical and Biological Research* 26, 93-98.
- Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K and Speck W, 1987. Salmonella mutagenicity tests. 3. Results from the testing of 255 chemicals. *Environmental and Molecular Mutagenesis* 9(Suppl. 9), 1-110.
- Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1988. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environmental and Molecular Mutagenesis* 11(Suppl. 12), 1-158.
- Zeller H, 1969. [Acute toxicity, irritation, and sensitization studies on linalyl acetate]. BASF. Report no. 4418. 29 July, 1969. (In German)

Appendix A. Summary of Safety Evaluation

Table 7: Summary of Safety Evaluation Applying the Procedure

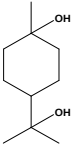
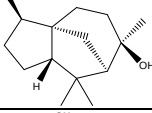
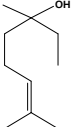
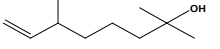
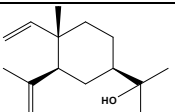
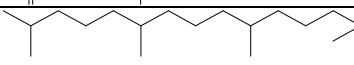
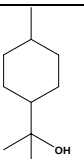
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02.054	p-Menthane-1,8-diol		11	Class I A3: Intake below threshold	d	f	
02.120 2030	(+)-Cedrol		13	Class I A3: Intake below threshold	d	f	
02.140	1,2-Dihydrolinalool		0.044	Class I A3: Intake below threshold	d	f	
02.144	2,6-Dimethyloct-7-en-2-ol		0.0012	Class I A3: Intake below threshold	d	f	
02.149	(-)-alpha-Elemol		1.6	Class I A3: Intake below threshold	d	f	
02.168	Isophytol		0.037	Class I A3: Intake below threshold	d	g	
02.171	p-Menthan-8-ol		0.012	Class I A3: Intake below threshold	d	f	

Table 7: Summary of Safety Evaluation Applying the Procedure

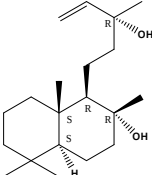
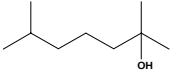
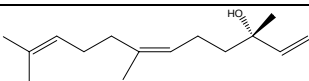
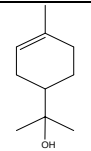
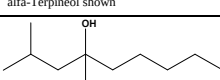
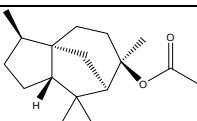
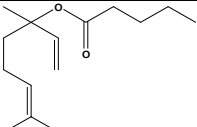
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.206 2029	(-)-Sclareol		0.67	Class I A3: Intake below threshold	d	f	
02.219	2,6-Dimethyl-2-heptanol		0.012	Class I A3: Intake below threshold	d	f	
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol		0.049	Class I A3: Intake below threshold	d	f	
02.230	Terpineol	 alfa-Terpineol shown	1200	Class I A3: Intake below threshold	d	f	
02.253 1850	2,4-Dimethyl-4-Nonanol		0.24	Class I A3: Intake below threshold	d	f	
09.171	Cedryl acetate		0.99	Class I A3: Intake below threshold	d	f	
09.614	Linalyl valerate		0.43	Class I A3: Intake below threshold	d	f	

Table 7: Summary of Safety Evaluation Applying the Procedure

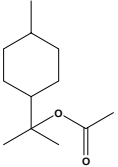
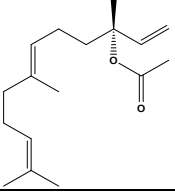
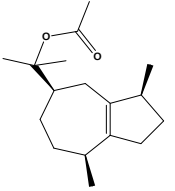
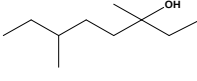
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
09.617	p-Menthan-8-yl acetate		0.012	Class I A3: Intake below threshold	d	f	
09.671	(3S,6Z)-Nerolidyl acetate		0.061	Class I A3: Intake below threshold	d	f	
09.808	Guaiyl acetate		0.0012	Class I A3: Intake below threshold	d	f	
02.041	2-Methylbutan-2-ol		2.7	Class II A3: Intake below threshold	d	f	
02.052	2-Methylpropan-2-ol		0.012	Class II A3: Intake below threshold	d	f	
02.123	2-Methylbut-3-en-2- ol		0.0012	Class II A3: Intake below threshold	d	f	
02.147	3,6-Dimethyloctan-3- ol		0.0012	Class II A3: Intake below threshold	d	g	

Table 7: Summary of Safety Evaluation Applying the Procedure

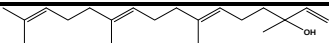
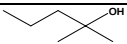
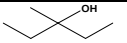
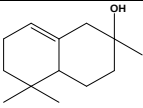
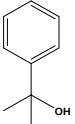
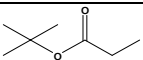
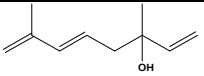
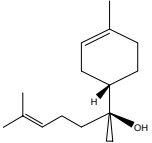
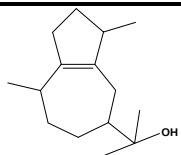
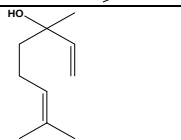
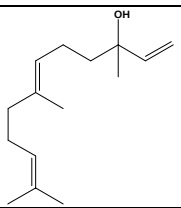
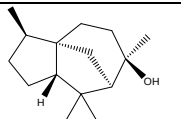
FL-no	EU Register name	Structural formula	MSDI ^a ($\mu\text{g/capita}$ per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.150	(E,E)-Geranyl linalool		0.026	Class II A3: Intake below threshold	d	f	
02.181	2-Methylpentan-2-ol		0.12	Class II A3: Intake below threshold	d	f	
02.184	3-Methylpentan-3-ol		0.0012	Class II A3: Intake below threshold	d	f	
02.197	1,2,3,4,4a,5,6,7- Octahydro-2,5,5- trimethylnaphthalen- 2-ol		0.026	Class II A3: Intake below threshold	d	g	
02.203	2-Phenylpropan-2-ol		0.0012	Class II A3: Intake below threshold	d	f	
09.356	1,1-Dimethylethyl propionate		0.0012	Class II A3: Intake below threshold	d	f	
02.146	(E)-3,7- Dimethylocta-1,5,7- trien-3-ol		12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		No safety concern at the estimated level of intake based on the MSDI approach.

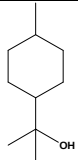
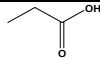
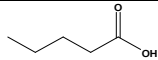
Table 7: Summary of Safety Evaluation Applying the Procedure

FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.129 2031	(l)-alpha-Bisabolol	 (l)-alpha-Bisabolol shown	27	Class III A3: Intake below threshold	d	g	
(a):	EU MSDI: Amount added to food as flavour in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.						
(b):	Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.						
(c):	Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.						
(d):	No safety concern based on intake calculated by the MSDI approach of the named compound.						
(e):	Data must be available on the substance or closely related substances to perform a safety evaluation.						
(f):	No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).						
(g):	Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.						
(h):	No conclusion can be drawn due to lack of information on the purity of the material of commerce.						

EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 8: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status ^a JECFA status ^b CoE status ^c EFSA status	Structural class ^d Procedure path (JECFA) ^e	Comments
	Guaiol				Not evaluated as flavour
02.013	Linalool 356		No safety concern (JECFA, 2000a) Category A (CoE, 1992)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
02.018	Nerolidol 1646		Category B (CoE, 1992)	Class I A3: Intake below threshold	
02.052	2-Methylpropan-2-ol		Category B (CoE, 1992) FGE.18	Class II A3: Intake below threshold	FGE.18: No safety concern based on the intake calculation by the MSDI approach
02.120	(+)-Cedrol 2030		FGE.18	Class I A3: Intake below threshold	

02.171	p-Menthan-8-ol		FGE.18	Class I A3: Intake below threshold	FGE.18: No safety concern based on the intake calculation by the MSDI approach
08.002	Acetic acid 81		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.003	Propionic acid 84		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake below threshold	

- (a): Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4: Not acceptable due to evidence of toxicity.
- (b): No safety concern at estimated levels of intake.
- (c): Category A: Flavouring substance which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.
- (d): Threshold of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.
- (e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

SUPPORTING SUBSTANCES SUMMARY

Table 9: Supporting Substances Summary

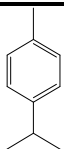
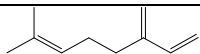
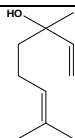
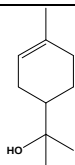
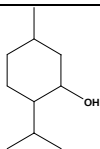
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01.002	1-Isopropyl-4-methylbenzene		2356 620 99-87-6	1325 JECFA specification (JECFA, 2005a).	926	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	
01.008	Myrcene		2762 2197 123-35-3	1327 JECFA specification (JECFA, 2005a)	290	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	EFSA conclusion: B4: Adequate NOAEL exists (EFSA, 2015).
02.013	Linalool		2635 61 78-70-6	356 JECFA specification (JECFA, 1998)	2200	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)
02.014	alpha-Terpineol		3045 62 98-55-5	366 JECFA specification (JECFA, 1998)	2600	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998)	16000	No safety concern (JECFA, 2000a) Category A	ADI: 0-4 (JECFA, 2000a).

Table 9: Supporting Substances Summary

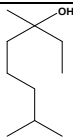
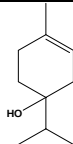
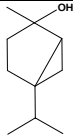
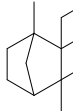
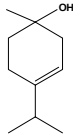
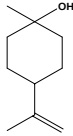
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
						(CoE, 1992)	
02.028	3,7-Dimethyloctan-3-ol		3060 77 78-69-3	357 JECFA specification (JECFA, 1998)	47	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.072	4-Terpinenol		2248 2229 562-74-3	439 JECFA specification (JECFA, 2000b)	150	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.085	Sabinene hydrate		3239 10309 546-79-2	441 JECFA specification (JECFA, 2000b)	0.91	No safety concern (JECFA, 2000a)	
02.095	2-Ethylfenchol		3491 10208 18368-91-7	440 JECFA specification (JECFA, 2001)	0.74	No safety concern (JECFA, 2000a)	
02.096	1-Terpinenol		3563 10252 586-82-3	373 JECFA specification (JECFA, 2000b)	35	No safety concern (JECFA, 2000a)	
02.097	beta-Terpineol		3564 10254 138-87-4	374 JECFA specification (JECFA, 2001)	1.3	No safety concern (JECFA, 2000a)	

Table 9: Supporting Substances Summary

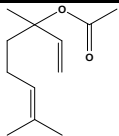
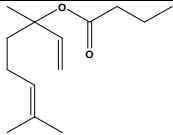
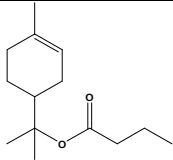
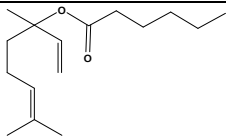
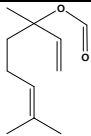
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.013	Linalyl acetate		2636 203 115-95-7	359 JECFA specification (JECFA, 1998)	1700	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)
09.050	Linalyl butyrate		2639 276 78-36-4	361 JECFA specification (JECFA, 1998)	8.4	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.052	Terpinyl butyrate		3049 278 2153-28-8	370 JECFA specification (JECFA, 2001)	5.1	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.068	Linalyl hexanoate		2643 318 7779-23-9	364 JECFA specification (JECFA, 1998)	0.85	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.080	Linalyl formate		2642 347 115-99-1	358 JECFA specification (JECFA, 2003)	6.9	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)

Table 9: Supporting Substances Summary

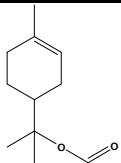
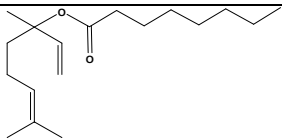
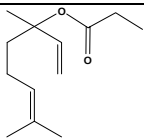
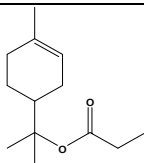
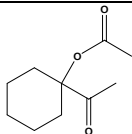
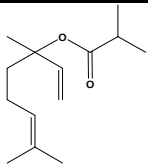
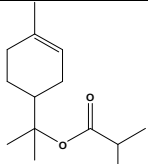
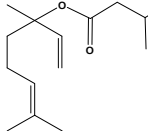
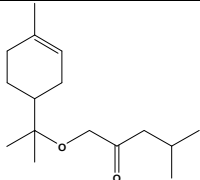
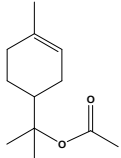
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.081	alpha-Terpinyl formate		3052 348 2153-26-6	367 JECFA specification (JECFA, 2001)	0.12	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.116	Linalyl octanoate		2644 397 10024-64-3	365 JECFA specification (JECFA, 2000b)	0.12	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.130	Linalyl propionate		2645 411 144-39-8	360 JECFA specification (JECFA, 2003)	13	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.142	Terpinyl propionate		3053 423 80-27-3	369 JECFA specification (JECFA, 1998)	0.024	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.293	1-Acetoxy-1-acetylcyclohexane		3701 52789-73-8	442 JECFA specification (JECFA, 2001)	ND	Additional data required (JECFA, 2000a)	

Table 9: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.423	Linalyl isobutyrate		2640 298 78-35-3	362 JECFA specification (JECFA, 1998)	30	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.425	Terpinyl 2-methylpropionate		3050 300 7774-65-4	371 JECFA specification (JECFA, 2000b)	0.61	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.454	Linalyl isovalerate		2646 449 1118-27-0	363 JECFA specification (JECFA, 2000b)	4.6	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.461	Terpinyl isovalerate		3054 456 1142-85-4	372 JECFA specification (JECFA, 2001)	0.12	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.830	Terpineol acetate		3047 205 8007-35-0	368 JECFA specification (JECFA, 1998)	220	No safety concern (JECFA, 2000a)	

(a): EU MSDI: Amount added to food as flavouring substance in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.

(b): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

(c): No safety concern at estimated levels of intake.

(d): Category A: Flavouring substance which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

Appendix B. Procedure for the Safety Evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure A.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 µg/person per day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products¹⁶ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous¹⁷ (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

¹⁶ "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

¹⁷ "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

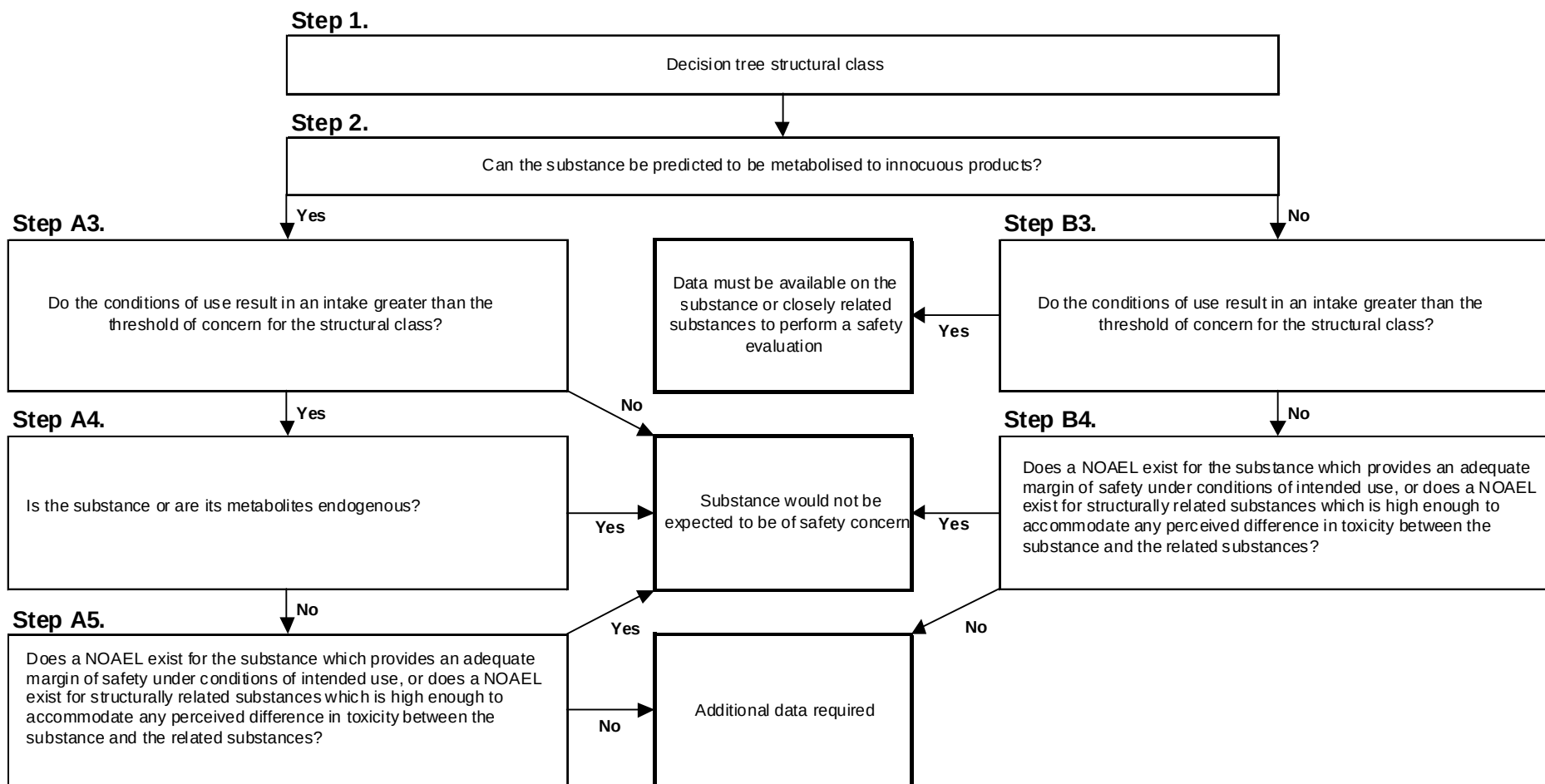


Figure A.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

Appendix C. Use Levels / mTAMDI

C.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table 10) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004c).

Table 10: Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic (“soft”) beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The “normal and maximum use levels” are provided by Industry for the 29 candidate substances in the present flavouring group (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012) (Table 11).

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFSA, 2005a; EFSA, 2006b; EFSA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.041	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.052	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.054	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.120	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.123	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.129	10	10	8	10	-	20	10	4	-	-	-	-	20	-	10	10	-	10
	50	50	50	30	-	100	30	20	-	-	-	-	100	-	30	30	-	500
02.140	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.144	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.146	0,2	-	0,2	-	-	0,5	-	-	-	-	-	-	-	-	0,2	0,2	-	-
	2	-	2	-	-	5	-	-	-	-	-	-	-	-	2	2	-	-
02.147	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.149	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.150	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.168	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFFA, 2005a; EFFA, 2006b; EFFA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.171	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.181	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.184	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.185	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.191	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-
02.203	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.206	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.219	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.226	2	-	2	-	-	200	2	2	-	-	-	-	1	-	2	30	2	-
	8	-	80	-	-	500	7	6	-	-	-	-	5	-	5	100	10	-
02.230	20	10	50	-	-	50	-	50	5	-	-	-	50	-	10	60	50	-
	150	50	300	-	-	300	-	300	10	-	-	-	300	-	300	300	300	-
02.253	7	5	10	7	-	10	5	10	2	-	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	-	-	-	25	50	25	50	100	25
09.171	7	5	10	7	7	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	35	50	25	50	10	10	-	-	25	50	25	50	100	2

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFFA, 2005a; EFFA, 2006b; EFFA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
09.356	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.614	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.617	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.669	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.671	7	5	-	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	-	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.808	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

C.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table 12. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table 12: Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g per day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table 13):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table 13: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (See Table 14) are presented for the 29 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012). The mTAMDI values are only given for highest reported normal use levels (See Table 14).

Table 14: Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per day)
02.054	p-Menthane-1,8-diol	3900	Class I	1800
02.120	(+)-Cedrol	3900	Class I	1800
02.140	1,2-Dihydrolinalool	3900	Class I	1800
02.144	2,6-Dimethyloct-7-en-2-ol	3900	Class I	1800
02.149	(-)-alpha-Elemol	3900	Class I	1800
02.168	Isophytol	3900	Class I	1800
02.171	p-Menthan-8-ol	3900	Class I	1800
02.206	(-)-Sclareol	3900	Class I	1800
02.219	2,6-Dimethyl-2-heptanol	3900	Class I	1800
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	7000	Class I	1800
02.230	Terpineol	14000	Class I	1800
02.253	2,4-Dimethyl-4-Nonanol	3900	Class I	1800
09.171	Cedryl acetate	3900	Class I	1800
09.614	Linalyl valerate	3900	Class I	1800
09.617	p-Menthan-8-yl acetate	3900	Class I	1800
09.671	(3S,6Z)-Nerolidyl acetate	3900	Class I	1800
09.808	Guaiyl acetate	3900	Class I	1800
02.041	2-Methylbutan-2-ol	3900	Class II	540
02.052	2-Methylpropan-2-ol	3900	Class II	540
02.123	2-Methylbut-3-en-2-ol	3900	Class II	540
02.147	3,6-Dimethyloctan-3-ol	3900	Class II	540
02.150	(E,E)-Geranyl linalool	3900	Class II	540
02.181	2-Methylpentan-2-ol	3900	Class II	540
02.184	3-Methylpentan-3-ol	3900	Class II	540
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	1600	Class II	540
02.203	2-Phenylpropan-2-ol	3900	Class II	540
09.356	1,1-Dimethylethyl propionate	3900	Class II	540
02.146	(E)-3,7-Dimethylocta-1,5,7-trien-3-ol	110	Class II	540
02.129	(l)-alpha-Bisabolol	5700	Class III	90

Appendix D. Metabolism

D.1 Introduction

A consideration of the chemical structures of the candidate substances in this Flavouring Group Evaluation, their anticipated pathways of metabolism and the extent to which data on one substance may support the metabolism of another substance has indicated that it is appropriate to divide the candidate substances in this evaluation into eight subgroups of more closely related structures (See Table 15).

Table 15: Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
1	02.041	2-Methylbutan-2-ol	Aliphatic saturated tertiary alcohols and one ester thereof
	02.052	2-Methylpropan-2-ol	
	02.147	3,6-Dimethyloctan-3-ol	
	02.181	2-Methylpentan-2-ol	
	02.184	3-Methylpentan-3-ol	
	02.219	2,6-Dimethyl-2-heptanol	
	02.253	2,4-Dimethyl-4-nonanol	
2	09.356	1,1-Dimethylethyl propionate	Aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two esters thereof
	02.123	2-Methylbut-3-en-2-ol	
	02.144	2,6-Dimethyloct-7-en-2-ol	
	02.150	Geranyl linalool	
	02.168	Isophytol	
	02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	
	09.614	Linalyl valerate	
3	09.671	Nerolidyl acetate	Aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond
	02.146	3,7- Dimethylocta-1,5,7-trien-3-ol	
4	02.140	1,2-Dihydrolinalool	Aliphatic unsaturated tertiary alcohol (without terminal double bond)
5	02.054	p-Menthane-1,8-diol	Monocyclic saturated and unsaturated tertiary alcohols and one ester thereof
	02.129	Bisabola-1,12-dien-8-ol	
	02.171	p-Menthan-8-ol	
	02.230	Terpineol	
	09.617	p-Menthan-8-yl acetate	
6	02.149	Elemol	Monocyclic and bicyclic unsaturated tertiary alcohols with isolated terminal double bonds
	02.206	Sclareol	
7	09.808	Guaiyl acetate	Bi- and tricyclic tertiary alcohols and esters
	02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	
	02.120	Cedrol	
	09.171	Cedryl acetate	
8	02.203	2-Phenylpropan-2-ol	Tertiary alcohol with an aromatic substituent

D.2 Absorption, Distribution and Elimination

Specific information on absorption, distribution and excretion is available for the candidate substances, 2-methylpropan-2-ol (tertiary butanol) [FL-no: 02.052], elemol [FL-no: 02.149], sclareol [FL-no: 02.206] and terpineol [FL-no: 02.230].

2-Methylpropan-2-ol [FL-no: 02.052] was administered by gastric intubation to female Wistar rats at a single dose of 1853 mg/kg bw and blood concentrations were monitored for 20 hours. 2-Methylpropan-2-ol was eliminated slowly from the blood of rats in this study, with concentrations in blood at 2, 5 and 20 hours measured as 13.2, 12.6 and 11.4 mM, respectively (Beaugé et al., 1981). The high bolus dose of 2-methylpropan-2-ol may have exceeded the capacity of the metabolic pathways for detoxication and contributed to the relatively slow clearance time from the blood.

The pharmacokinetic profile for 2-methylpropan-2-ol was characterised in male (four/dose) and female (three/dose) F-344 rats following intravenous administration of 37.5, 75, 150 and 300 mg 2-methylpropan-2-ol/kg bw. Blood samples were collected in heparinised syringes at 5, 10, 20, 30, 40 and 60 min, and 4, 8, 12, 16 and 24 hours from the cannula implanted in the right jugular vein of each animal. The data fit a two-compartment model with the distribution half-life ($T_{1/2\alpha}$) about 3 min and the elimination half-life ($T_{1/2\beta}$) about 3.8 hours in male and female rats for doses less than 300 mg/kg bw. Consistent with another study (Beaugé et al., 1981), the elimination of 2-methylpropan-2-ol appears to get saturated at higher doses, as evidenced by a disproportional increase in area under the concentration – time curve and decreased rate of clearance in this study. After administration of a 300 mg/kg bw dose of 2-methylpropan-2-ol, the means of $T_{1/2\beta}$ were increased to 5.0 and 4.3 hours in male and female rats, respectively. The steady-state volume of distribution for 2-methylpropan-2-ol was approximately 4.5-fold greater than total body water, suggesting significant tissue distribution (Poet et al., 1997).

Potential pharmacokinetic outcomes in mammalian plasma were analysed by intravenous administration of sclareol [FL-no: 02.206] at 100 mg/kg bw to two male Wistar rats. Plasma samples were collected at 5, 15, 30, 60, 180, 360, 720 and 1440 min after administration, and sclareol concentrations were quantified by gas-liquid chromatography using an internal standard. At 5 min post-injection, plasma levels of sclareol were 84.9 µg/ml. The sclareol plasma concentration dropped to 42.9 µg/ml after 180 min, and was not detectable at 720 min. This study indicated a rapid biphasic disappearance of sclareol from plasma following intravenous treatment. The authors suggest that sclareol may be distributed in fatty tissue due to its high lipophilicity. Sclareol was administered to two male Wistar rats by intravenous administration at 100 mg/kg bw, and to male Wistar rats by oral gavage at 1 g/kg bw in 3:1 propylene glycol-ethanol. Urine and faecal samples were collected from all rats at periodic intervals over 144 or 72 hours, respectively. Bile samples were collected only from rats given intravenous injections at periodic intervals over 30 hours. No sclareol was detected in urine or urine treated with β -glucuronidase at any time. No sclareol was detected in faecal samples from intravenoustreated rats, but 9 % of the initial sclareol dose was found in faecal samples from rats given oral administration. The bile samples from rats given intravenous administration showed very low levels (0.02 %) of sclareol over a 3 hour period. Very low levels (0.04 %) of oxidised metabolites were found in bile after 3 hours, including 3- α -hydroxysclareol (0.24 %), 3- β -hydroxysclareol (0.075 %), 18-hydroxysclareol (0.056 %), and 3-ketosclareol (0.03 %). Sclareol or its oxidised metabolites were not observed in bile samples collected at any other time during the 30 hours study. The authors hypothesized that the low sensitivity of the assay technique may have prevented detection of low levels of sclareol and its metabolites over the course of the experiment. While only a very small percentage of intravenously administered sclareol (<0.05 %) could be accounted for in these experiments, the authors suggested that other mammalian metabolites were formed that were not detected in the assays used (Kouzi et al., 1993).

Incubation of sclareol with 37 different microorganisms found that sclareol is oxidised to more polar metabolites, including 3-ketosclareol, 2 α -hydroxysclareol, 3 β -hydroxysclareol, 18-hydroxysclareol, 2 α ,18-dihydroxysclareol, and three glucoside conjugates (Kouzi et al., 1993).

Intravenous administration of sclareol to rats, however, found very low levels of the hydroxylated and glycosidic metabolites or the parent compound in the urine.

A metabolism study on the structurally related elemol [FL-no: 02.149] indicates that substances of this type are absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of this candidate substance would not be anticipated.

alpha-Terpineol, the major component of terpineol [FL-no: 02.230], undergoes allylic oxidation of the exocyclic methyl group, which can then be further oxidised to a carboxylic acid group (Madyastha and Srivatsan, 1988). In a minor pathway, the double bond of alpha-terpineol is epoxidized and then hydrolysed to yield the triol metabolite 1,2,8-trihydroxy-p-menthane, which also has been reported in humans following inadvertent oral ingestion of a pine oil disinfectant containing alpha-terpineol (Horning et al., 1976). It is expected that after single dose exposures, alpha-terpineol would undergo metabolism via glucuronic acid conjugation and excretion in the urine (Chadha and Madyastha, 1984; Hill et al., 1975; Wright, 1945).

(-)-Elemol (2 g) was orally given to rabbits (2-3 kg) and the urine was collected for the three following days (72 hours). In total 80 % of the administered dosage was recovered from the urine (Asakawa et al., 1986).

In addition, there is information on absorption and excretion for the four supporting substances linalool, myrcene, menthol and 1-isopropyl-4-methylbenzene (synonym: *p*-cymene).

[1,2-¹⁴C]-Linalool was orally administered to rats at a single dose of 500 mg/kg bw. The majority (55 %) of the radioactivity was excreted in the urine as the glucuronic acid conjugate, whereas 23 % was excreted as CO₂ in expired air, and 15 % was excreted in the faeces within 72 hours of administration. Only 3 % of the radioactivity was detected in tissues after 72 hours, with 0.5 % in the liver, 0.6 % in the gut, 0.8 % in the skin and 1.2 % in the skeletal muscle (Parke et al., 1974).

When given to male Japanese White rabbits by gavage at a dose of 670 mg/kg bw per day for two days, approximately 25 % of the total administered amount (19 g to six rabbits) of myrcene could be recovered from the urine excreted over a period of three days following administration (Ishida et al., 1981).

In humans 79 % of a 1000 mg oral dose (Quick, 1928) or 78 % of a 10 to 20 mg oral dose (Atzl et al., 1972) of menthol administered to volunteers was eliminated as the glucuronic acid conjugate. For eight days 750 mg l-menthol was administered orally to two human volunteers followed by oral or intravenous administration of 200 mg [6-¹³C]-glucuronolactone or [6-¹³C]-sodium glucuronate. For two days after administration of the isotopic compound, menthyl glucuronide was excreted in an average daily yield ranging from approximately 27 % to 84 % of the l-menthol administered (Eisenberg et al., 1955). Four males were given an oral dose of 180 mg peppermint oil. Between 37 and 116 mg menthol glucuronide were excreted in the urine after 14 hours (Kaffenberger and Doyle, 1990).

Non-cannulated and bile duct-cannulated male Fischer 344 rats were administered a single oral dose of 500 mg [3-³H]-1-menthol/kg bw. Urine and faeces were collected over the next 24 and 48 hours from non-cannulated rats. In the bile duct-cannulated rats, bile samples were collected at 2-hours intervals for the first 6 hours and then from 6 to 24 hours. The 0-24 hours urine was collected in the same rats. In the bile duct-cannulated rats, total recovery of the dose of the radiolabeled substance in the urine or bile was 74.2 % after 24 hours. The amount of radioactivity found in the urine after 24 hours from the non-cannulated and the bile duct-cannulated rats differed from 19 to 7.3 %, but indicates that most of the compound is excreted in the bile during the first 24 hours (Yamaguchi et al., 1994).

Of an oral dose of 100 mg/kg bw of p-cymene given to male Wistar rats or Dunkin Hartley guinea pigs, 80 % or 71 %, respectively, was excreted in the urine within the following 48 hours in the form of extractable metabolites. It was speculated that the rest of the dose was either excreted via the faeces or as unextractable metabolites in the urine (Walde et al., 1983).

In conclusion, the candidate substance 2-methylpropan-2-ol is anticipated to be absorbed and to undergo rapid excretion when administered at lower doses. However, when administered at higher doses the metabolic capacity may be exceeded. The candidate substances elemol and sclareol are absorbed but excreted rather slowly. The candidate substance terpineol is anticipated to be absorbed and excreted. The supporting substances, menthol and p-cymene, are readily absorbed and excreted rapidly with approximately 70 % recovery within 24 hours and 70-80 % recovery within 48 hours, respectively. Linalool is absorbed but rather slowly excreted. The slowest absorption and excretion was observed for myrcene where only 25 % of administered dose was recovered in urine after 72 h.

D.3 Biotransformation

Ester hydrolysis

Aliphatic esters are hydrolysed to the component alcohols and carboxylic acid by carboxyl-esterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980). In mammals these enzymes occur within the body in most tissues including the gut lumen and intestinal wall, but predominate in the hepatocytes (Heymann, 1980). The wide range of tissue distribution and the multiplicity of the esterases generally give rapid hydrolyse of esters *in vivo*.

No hydrolysis studies on the candidate esters [FL-no: 09.171, 09.356, 09.614, 09.669, 09.671, 09.808 and 09.617] are available. Two hydrolysis studies on a supporting substance, linalyl acetate [FL-no: 09.013], were found. In an *in vitro* hydrolysis study, linalyl acetate was easily hydrolysed in water and simulated gastric and pancreatic fluids. The mean half-lives for linalyl acetate hydrolysis were 5.5 and 52.5 min in gastric and pancreatic fluids, respectively (Hall, 1979). In neutral gastric juice, linalyl acetate is slowly ($t_{1/2} = 121$ min) hydrolysed to a mixture of linalool and the ring-closed isomer alpha-terpineol. In acidic artificial gastric juice, linalyl acetate is rapidly hydrolysed ($t_{1/2} < 5$ min) to yield linalool (Buck and Renwick, 1998). Linalyl acetate was slowly hydrolysed ($t_{1/2} = 153 - 198$ min) in intestinal fluid with or without pancreatin. Linalyl acetate also hydrolyses in homogenates of rat intestinal mucosa, blood and liver, but at rates much slower than in acidic gastric juice (rate constant for hydrolysis, $k = 0.01$ to 0.0055 min^{-1} vs. $> 5 \text{ min}^{-1}$ in gastric juice). Based on these observations it can be concluded that linalyl acetate hydrolyses in gastric juice to yield linalool and acetic acid (Buck and Renwick, 1998). Further, it has been demonstrated that linalyl acetate can be hydrolysed to linalool in *in vitro* studies after incubation with rat caecal flora (Rahman, 1974).

Subgroup 1 - Aliphatic saturated tertiary alcohols and ester

For three of the eight candidate substances in this subgroup, 2-methylpropan-2-ol [FL-no: 02.052], 2-methylbutan-2-ol [FL-no: 02.041] and 2-methylpentan-2-ol [FL-no: 02.181], there are one or more metabolism studies.

2-Methylpropan-2-ol (tert-Butanol [FL-no: 02.052])

[2-¹³C]-2-Methylpropan-2-ol was orally administered in a gel capsule at a dose of 5 mg/kg bw to one human volunteer (44 year old, 80 kg male). Urine was collected in 12-hours intervals for 48 hours and analysed by ¹³C-NMR. All of the urine samples of this human volunteer showed the presence of 2-methylpropan-2-ol, 2-methylpropan-2-ol glucuronide, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol. In contrast to the rat urine samples in which 2-methylpropan-2-ol sulphate was observed as a major metabolite, the sulphate was only present in trace amounts in the human volunteer urine samples. The low recovery of 2-methylpropan-2-ol sulphate in the urine of the human volunteer is likely based on a low affinity of human sulphotransferase for 2-methylpropan-2-ol as compared to rats. On the other hand, 2-hydroxyisobutyrate was the major metabolite excreted by the human volunteer. The likely pathway for the formation of the 2-hydroxyisobutyrate and 2-methyl-1,2-

propanediol metabolites involves methyl group oxidation of 2-methylpropan-2-ol by CYP-450 to yield 2-methyl-1,2-propanediol. Further oxidation of 2-methyl-1,2-propanediol results in the formation of 2-hydroxyisobutyrate, and acetone is likely formed by further oxidation of the intermediate diol and/or 2-hydroxyisobutyrate (Bernauer et al., 1998).

Three male rats per experiment were administered a single 250 mg/kg bw dose of [12C]- or [13C]- 2-methylpropan-2-ol dissolved in corn oil by oral gavage. All animals were maintained in individual metabolism cages for 72 hours, after which they were sacrificed by cervical dislocation. Urine was collected at 24 and 48 hours and analysed by 13C-NMR. In the 24-hour urine samples, it was determined that 2-methylpropan-2-ol sulphate, 2-methyl-1,2-propanediol, and 2-hydroxyisobutanoate (i.e. alpha-hydroxyisobutanoate) are the major metabolites of 2-methylpropan-2-ol in rats; minor metabolites were identified as free 2-methylpropan-2-ol, 2-methylpropan-2-ol glucuronide, and [13C]-acetone. Identical metabolites were present at lower concentrations in the urine collected between 24 and 48 hours after dosing. Extensive biotransformation of tert-butyl alcohol in rats by conjugation and oxidation was confirmed by the results of these experiments (Bernauer et al., 1998).

2-Methylpropan-2-ol was administered by gastric intubation to chinchilla rabbits at a 297 mg/kg bw. 2-Methylpropan-2-ol was conjugated to a large extent with glucuronic acid, and conjugates were readily isolated from urine. Of the administered dose, 24.4 % was excreted as glucuronic acid conjugate in the urine within 24 hours after administration. The investigators suggested that the volatile alcohol may also be eliminated to some extent via the lungs. No aldehydes or ketones were detected in the expired air of a rabbit administered 6 ml of 2-methylpropan-2-ol (Kamil et al., 1953).

2-Methylbutan-2-ol (tert-amyl-alcohol [FL-no: 02.041])

2-Methylbutan-2-ol was administered by gastric intubation to chinchilla rabbits at a single dose of 441 mg/kg bw. 2-Methylbutan-2-ol was conjugated to a large extent with glucuronic acid and 58 % of the dose was excreted as such conjugates via the urine within 24 hours post dosing (Kamil et al., 1953).

Three male rats were administered a single 250 mg/kg bw dose of [2-13C]-2-methylbutan-2-ol dissolved in corn oil by oral gavage. All animals were maintained in individual metabolism cages for 48 hours. Urine was collected at 24 and 48 hours and analysed by ¹³C-NMR. In the 24-hour urine samples, it was determined that 2-methylbutan-2-ol glucuronide, 2-methyl-2,3-butanediol and 2-methyl-2,3-butanediol glucuronide are the major metabolites of 2-methylbutan-2-ol; minor metabolites were identified as free 2-methylbutan-2-ol, 2-hydroxy-2-methylbutanoic acid and 3-hydroxy-3-methylbutanoic acid. The low concentration of 2-methylbutan-2-ol recovered in the urine suggests extensive metabolism of the alcohol. In the 48-hour urine samples, only 2-methyl-2,3-butanediol and 2-methyl-2,3-butanediol glucuronide were detected, suggesting the rapid excretion of 2-methylbutan-2-ol glucuronide following oral exposure. Glucuronidation appears to be the major pathway of metabolism, resulting in urinary excretion of the 2-methylbutan-2-ol glucuronide. In addition, it appears that 2-methylbutan-2-ol is oxidised to 2-methyl-2,3-butanediol, which is further conjugated to 2-methyl-2,3-butanediol glucuronide and excreted in the urine. A minor metabolic pathway seems to be oxidation of the carbon atom at the C4 position resulting in 2-methyl-2,4-butanediol as an intermediate, which is further oxidised to 3-hydroxy-3-methylbutanoic acid. Finally, oxidation of the methyl side chain is another minor transformation pathway that results in the formation of 2-methyl-1,2-butanediol as an intermediate, which is further oxidised to produce 2-hydroxy-2-methylbutanoic acid (Amberg et al., 1999) (See Figure D.1.)

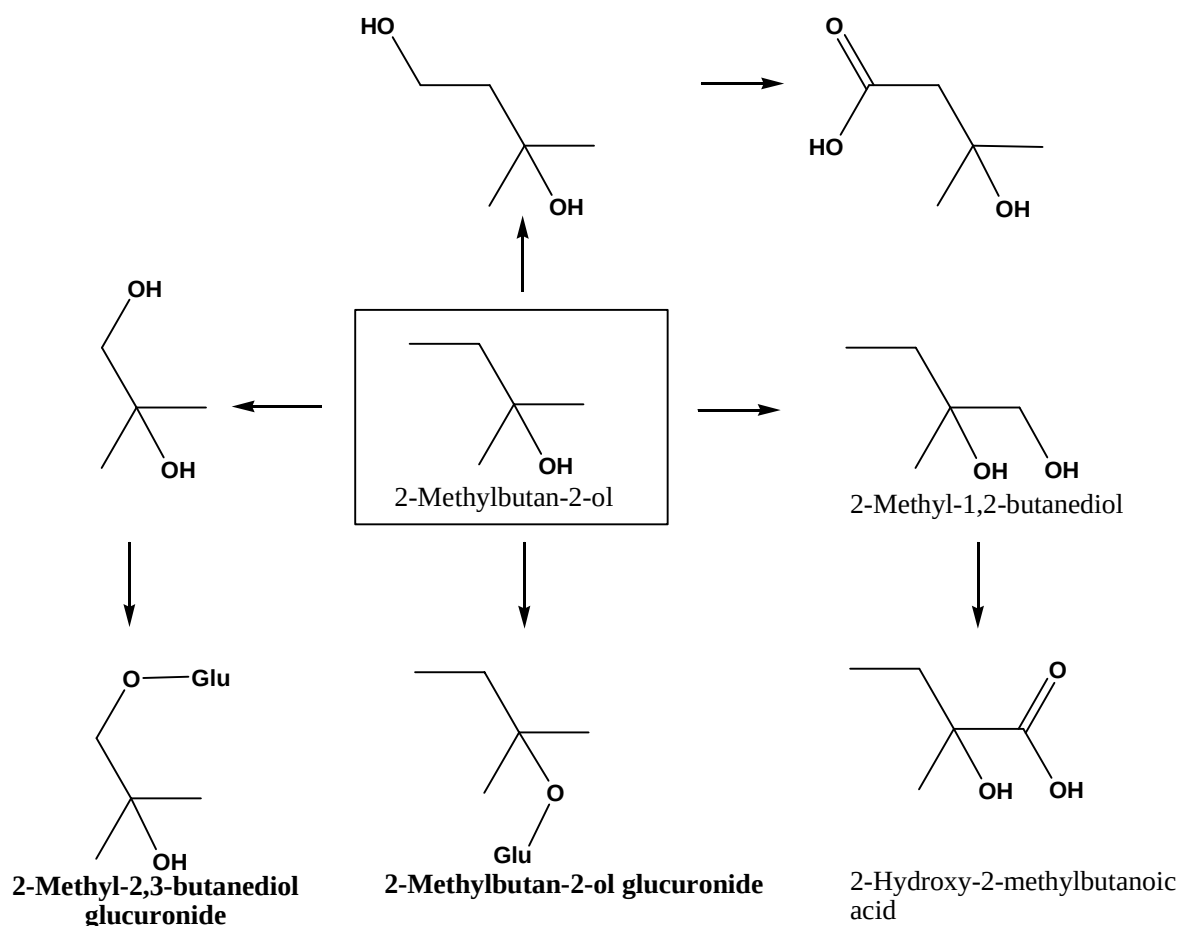


Figure D.1. Metabolism of 2-methylbutan-2-ol (tert-amyl-alcohol) in rats (Amberg et al., 1999). Main metabolism products are in bold.

2-Methylpentan-2-ol (tert-hexylalcohol [FL-no: 02.181])

2-Methylpentan-2-ol was administered by gastric intubation to chinchilla rabbits at a single dose of 851 mg/kg bw. 2-Methylpentan-2-ol was conjugated to a large extent with glucuronic acid, and 57 % of the dose was excreted within 24 hours post dosing as such conjugates via the urine (Kamil et al., 1953).

Subgroup 2 - Aliphatic unsaturated tertiary alcohols and esters with isolated terminal double bonds

There are no metabolism studies on the candidate substances 2-methylbut-3-en-2-ol, 2,6-dimethyloct-7-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate, [FL-no: 02.123, 02.144, 02.150, 02.168, 02.226, 09.614 and 09.671]. However, there is a metabolism study for linalool, supporting substance to 2-methylbut-3-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate [FL-no: 02.123, 02.150, 02.168, 02.226, 09.614 and 09.671,], which have the isolated double bond in close proximity to the tertiary alcohol group.

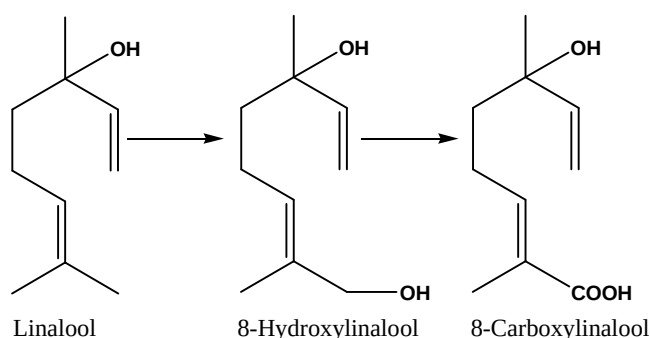


Figure D.2 Metabolism of linalool (Chadha & Madyastha 1984).

Seventy-two hours after intragastrical administration of 500 mg/kg bw ^{14}C -labelled linalool/kg bw to 12-weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetra hydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut (Rahman, 1974).

In another study, male rats were given a daily 800 mg/kg bw oral dose of linalool for 20 days. Urinary metabolites formed by CYP-450-mediated allylic oxidation of linalool included 8-hydroxylinalool and 8-carboxylinalool (Chadha and Madyastha, 1984) (Figure D.2). No oxidation of the terminal double bond was observed, indicating no formation of epoxide intermediates.

No metabolism studies on supporting substances to 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], which has the isolated double bond far from the tertiary alcohol group, are available.

Subgroup 3: Aliphatic unsaturated tertiary alcohols with a conjugated terminal double bond

No metabolism studies are found for the candidate substance, 3,7-dimethylocta-1,5,7-trien-3-ol, [FL-no: 02.146]. For the supporting substance, myrcene, two studies on metabolism are found.

In the urine of rabbits orally administered myrcene (single dosage of 670 mg/kg bw) via gavage, more than 80 % of the metabolites were neutral metabolites, the rest were acidic metabolites. The main metabolites identified in urine were myrcene-3,10-glycol, myrcene-1,2-glycol and uroterpenol (40.7, 20.8 and 11.8 %, respectively, of the neutral metabolites after 72 hours). Additionally, the glycols underwent further oxidation to yield 2-hydroxymyrcene-1-carboxylic acid and 3-hydroxymyrcene-10-carboxylic acid (no quantitative data were given for these acidic metabolites).

The authors suggested that uroterpenol (or limonene-8,9-diol) may have been formed from limonene, which is derived from cyclization of myrcene in the acidic conditions of the rabbit stomach (Ishida et al., 1981).

When rats were administered 800 mg/kg bw myrcene per day via gavage for 20 days, the principal metabolites isolated from the urine were 10-hydroxylinalool (or myrcene-3,10-glycol) and, to a lesser extent, 7-methyl-3-methylene-oct-6-ene-1,2-diol (or myrcene-1,2-glycol). Other minor metabolites included the hydroxy acids of both the 3,10- and 1,2-glycols (10-carboxylinalool (or 3-hydroxymyrcene-10-carboxylic acid) and 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid (or 2-hydroxymyrcene-1-carboxylic acid), respectively) and a cyclic diol, 1-hydroxymethyl-4-isopropenylcyclohexanol (or p-menth-8-ene-1,7-diol), formed by intramolecular cyclization of an open chain metabolite (Madyastha and Srivatsan, 1987).

It was demonstrated that the biotransformation of myrcene was cytochrome P450 (CYP)-mediated and that it could be enhanced by pretreatment of animals with phenobarbital (Madyastha & Srivatsan,

1987). These studies indicate the oxidation of the terminal double bonds of myrcene via intermediate epoxidation of the 1,2- and 3,10- double bonds (Figure D.3).

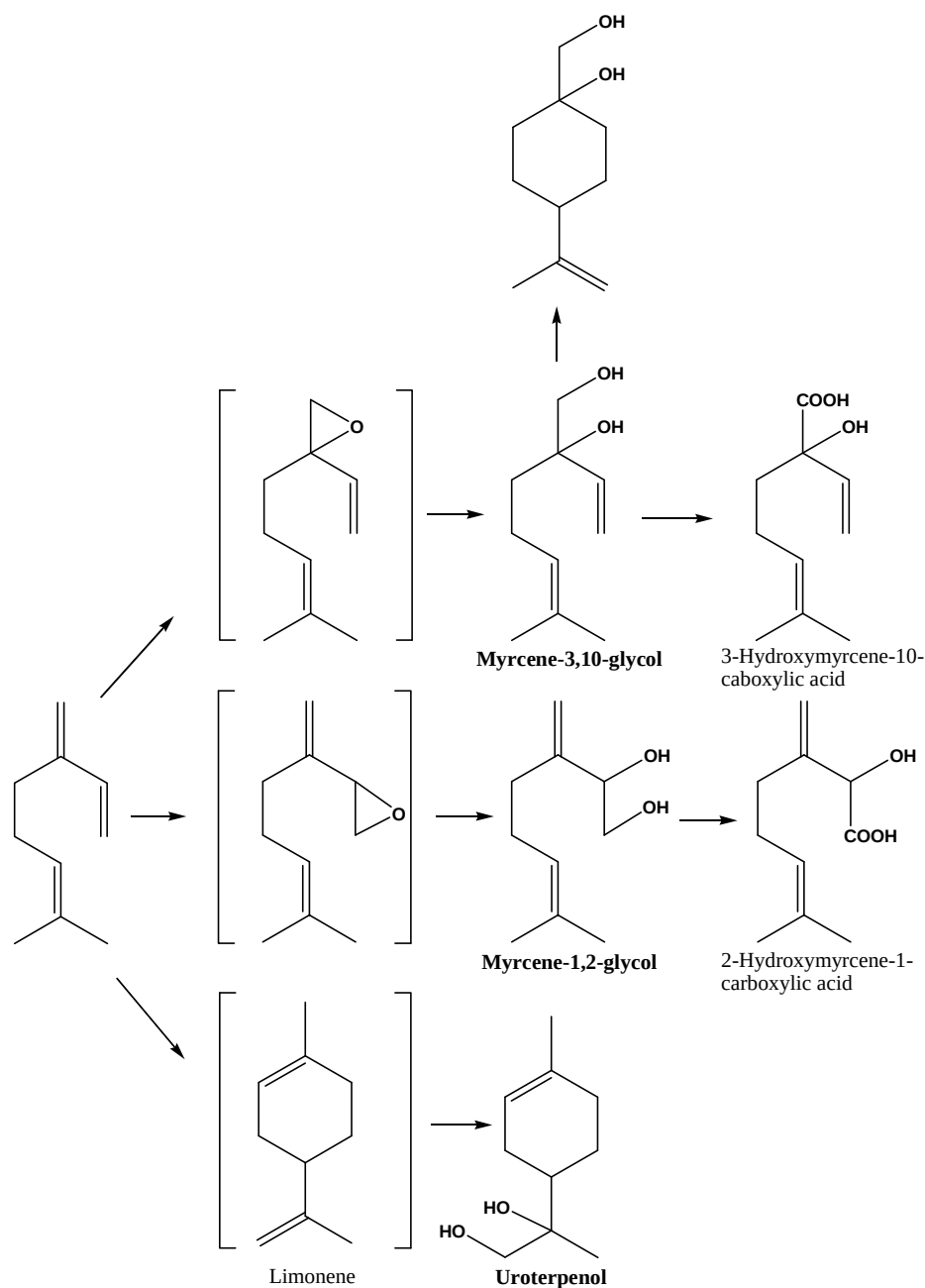


Figure D.3 Metabolism of myrcene (Ishida et al., 1981 and Madyastha & Srivatsan 1987). Intermediate products are in brackets and main metabolism products are in bold.

Subgroup 4: Aliphatic unsaturated tertiary alcohols

After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep, dihydrolinalool and tetrahydrolinalool are formed as metabolites (Rahman, 1974). *In vivo* metabolism studies in rats on ¹⁴C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucuronides (Rahman, 1974).

Subgroup 5: Monocyclic saturated tertiary alcohols and esters

There are no metabolism studies on the candidate alicyclic substances p-menthane-1,8-diol, bisabolol, 1,12-dien-8-ol, p-menthan-8-ol, terpineol and p-menthan-8-yl acetate [FL-no: 02.054, 02.129, 02.171, 02.230 and 09.617]. p-Menthan-8-yl acetate is anticipated to be hydrolysed to give p-menthan-8-ol. There are metabolism studies on two substances, alpha-terpinol and menthol, which are supporting substances to p-menthane-1,8-diol, p-menthan-8-ol, and p-menthan-8-yl acetate (Figure D.4).

In a repeated dose study, male albino rats were orally administered the alicyclic tertiary alcohol alpha-terpineol at a daily dose of 600 mg/kg bw for 20 days. Oxidation of the allylic methyl group was observed to yield the corresponding carboxylic acid, which was hydrogenated, to a small extent, to yield the corresponding saturated carboxylic acid (Madyastha and Srivatsan, 1988).

In rats, the vast majority of orally administered menthol is eliminated in either the urine or faeces as the glucuronic acid or various oxidation products (Madyastha and Srivatsan, 1988; Yamaguchi et al., 1994).

Non-cannulated and bile duct-cannulated male Fischer 344 rats were given a single oral dose of 500 mg/kg bw [3-³H]-1-menthol. Urine and faeces were collected over the next 24 and 48 hours from non-cannulated rats. In the bile duct-cannulated rats, bile samples were collected at 2-hours intervals for the first 6 hours and then from 6 to 24 hours. The 0-24 hours urine was collected in the same rats (Yamaguchi et al., 1994). The major metabolites found were menthol glucuronide in the bile and a variety of oxidation products in the urine. Menthol glucuronide formed in the liver passes into the bile with subsequent elimination or entry into enterohepatic circulation. Oxidation products of menthol are primarily p-menthane-3,8-diol, 3,8-dihydroxy-p-menthane-7-carboxylic acid and 3-hydroxy-p-menthane-7-carboxylic acid (Figure III.4). Minor metabolites are p-menthane-3,7-diol, p-menthane-3,7,8-triol, p-menthane-3,9-diol and 3-hydroxy-p-menthane-9-carboxylic acid. The monohydroxylated menthols are excreted in the urine in part as glucuronide (Yamaguchi et al., 1994).

The study by Madyastha & Srivatsan (Madyastha and Srivatsan, 1988) supports the finding of p-menthane-3,8-diol and 3,8-dihydroxy-p-menthane-7-carboxylic acid as the main metabolites of menthol in urine after giving rats an oral dosage of 800 mg/kg bw. They also found p-menthane-3,9-diol as a minor metabolite.

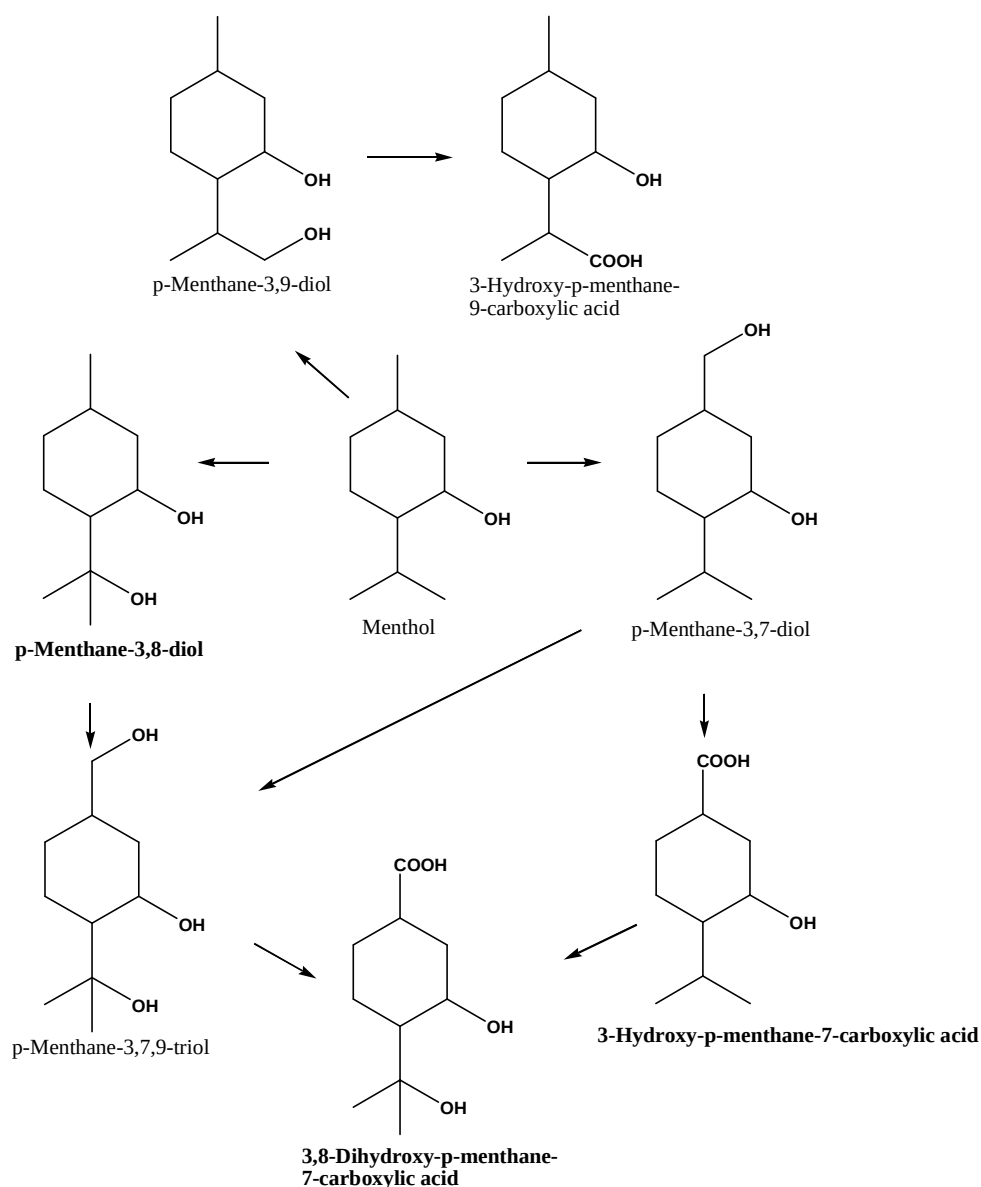


Figure D.4. Metabolism of menthol in rats (Yamaguchi et al., 1994). Main metabolism products are in bold.

Subgroup 6: Monocyclic unsaturated tertiary alcohols with isolated terminal double bonds

There is one metabolism study on one of the two candidate substances in this group, elemol [FL-no: 02.149]. (-)-15-Hydroxyelemol was the only metabolite (10 % of the natural metabolites) detected after 72 hours in the urine of rabbits (2-3 kg) given 2g (-)-elemol. Most of the (-)-elemol (70 %) was recovered as elemol conjugated with glucuronic acid or sulphate. No oxidation of the double bond of the vinyl group was observed, indicating that no epoxide intermediates was formed.

Subgroup 7: Bi- and tricyclic tertiary alcohols and esters

For the candidate substances guaiyl acetate [FL-no: 09.808], 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197] and cedryl acetate [FL-no: 09.171] there are no metabolism studies available. Two metabolism studies were found for the candidate substance cedrol [FL-no: 02.120]. In the study by Bang and Ourisson (1975) rabbits were given natural cedrol (1 g) suspended in a 1 % methyl-cellulose mucilage (20 ml) administered by gavage followed by 25 ml water. The urine was collected at 24, 48 and 96 hours after administration. The urine was acidified to pH 4.5 and hydrolysed at 37° C for 24 hours with 6 ml snail (D-glucuronide)-glucuronidase. Five percent of the

dose was excreted as conjugation product of unchanged cedrol. Thirtyfive percent of the dose was recovered as a mixture of stereochemically different 3-hydroxycedrol metabolites. Twelve percent of the dose was recovered as a C7-C8 dehydrated C3-hydroxylation product of cedrol (both 3-R and 3-S) (Bang and Ourisson, 1975).

Trifilieff et al. (1975) administered 2 grams of cedrol to a phenobarbital-pretreated fasted dog. The metabolites described below (see also Figure III.5) were found in the first 24 hours urine. Urine was acidified with hydrochloric acid and treated with glucuronidase-sulphatase to hydrolyse conjugates. Three metabolite fraction, isolated by chromatography on silica columns and acetylation, were obtained in the following amounts: 130 mg of diol (1), 100 mg of a mixed fraction of diols (2 plus 3), and 40 mg of an acid (4). Diol (1) was identified by spectral data and correlation with α -epi-isobiotol (= 3-hydroxycedrol) and the corresponding ketone. In contrast to the rabbit the dog produced only the 3S isomer. Diols (2 plus 3) were identified as 15-hydroxycedrol and 2-hydroxycedrol, respectively, by separation after dehydration and further derivation and identification of the reaction products by comparison of the spectral data with literature data. The acid (4) was identified as the C14 carboxylic acid derivation product of 2-hydroxycedrol. In total only 270 mg (13 % of the dose) was recovered, but elimination of cedrol (-conjugates) was not studied / reported (Trifilieff et al., 1975). For structures of cedrol and the four metabolites in the dog see scheme below (figure III.5):

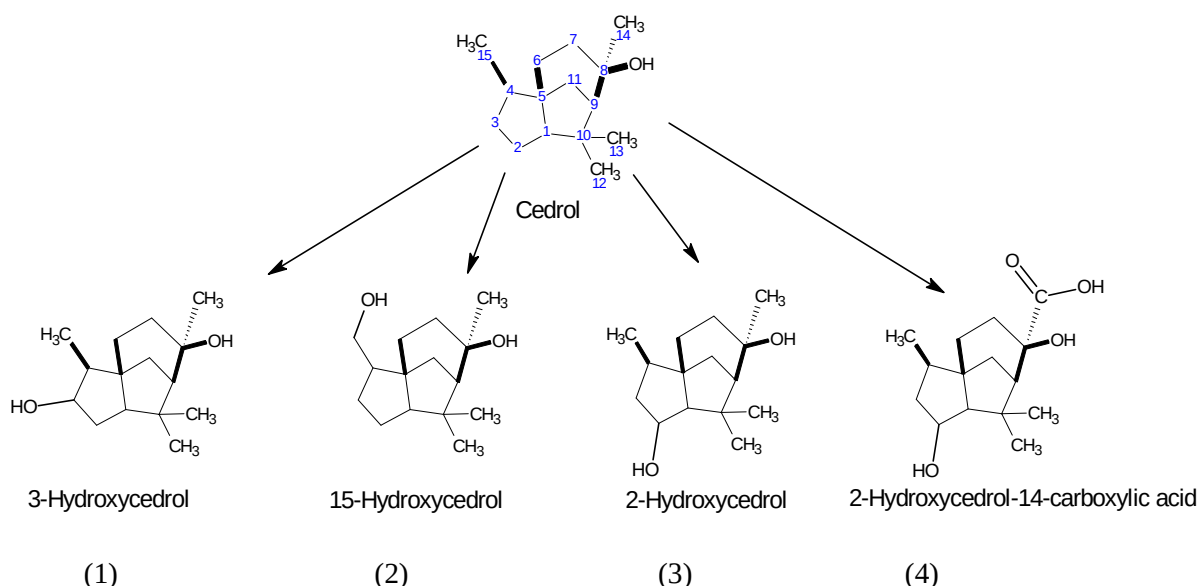


Figure D.5 Metabolism of cedrol in a dog treated with phenobarbital. The carboxylic acid is presumably formed from 2-hydroxycedrol, but this was not further studied (after Trifilieff et al, 1975).

Subgroup 8: Aromatic tertiary alcohols

No metabolism studies were found for the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203]. However, several metabolism studies were found for the supporting substance p-cymene.

The main metabolites in the urine of rabbits given a single oral dose of 670 mg p-cymene /kg bw were p-cymen-9-ol and p-cymen-8-ol (50 % and 28 %, respectively, of the neutral metabolites). Acidic metabolites identified were α -p-tolylpropionic acid, α -tolyl- α -hydroxypropionic acid, p-isopropylbenzoic acid and p-1-hydroxyisopropylbenzoic acid. Ring hydroxylation did not occur (Ishida et al., 1981).

Following an oral dose of 100 mg/kg bw of p-cymene to male rats, the principal urinary metabolites were p-isopropylbenzoic acid (19 % of the administered dose) and 2-p-carboxyphenylpropionic acid (16 %). Other less important urinary metabolites included 2-p-tolylpropan-1-ol (8 %), 2-p-tolylpropan-

2-ol (9 %), 2-p-carboxyphenylpropan-2-ol (9 %), 2-p-(hydroxymethyl)phenylpropionic acid (4 %), 2-p-carboxyphenylpropan-1-ol (11 %), p-isopropylbenzoylglycine (2 %), p-isopropylbenzyl alcohol (1 %), and 2-p-tolylpropionic acid (1 %) (Walde et al., 1983). When the same dose was given to male guinea pigs, similar urinary metabolites were identified, however in different quantities. The primary urinary metabolite in guinea pigs was p-isopropylbenzoylglycine (31 %), indicating that conjugation with glycine was more prevalent in guinea pigs than in rats. Another major metabolite in guinea pigs was 2-p-tolylpropan-2-ol (14 %). In addition, whereas ring hydroxylation of p-cymene was not reported in rats (Bakke and Scheline, 1970; Walde et al., 1983) and rabbits (Ishida et al., 1981), trace amounts of the ring hydroxylation metabolites hydroxyl-p-cymene and hydroxycarvacrol (dehydroxyl-p-cymene) were detected in guinea pig urine. Ring hydroxylation in guinea pigs only occurred in ortho position to the methyl group (Walde et al., 1983).

Boyle et al. (1999) studied the metabolite pattern of p-cymene in rats following oral doses equivalent to 50 and 200 mg/kg bw. The major metabolites in 0-48 hours urine after administration of the 50 mg/kg bw dose were 2-p-tolylpropan-2-ol (39 % of recovered dose) and 2-p-carboxyphenylpropan-2-ol (19 %). The former metabolite is the product of allylic hydroxylation of the isopropyl substituent, while the latter metabolite is the product of allylic hydroxylation of both the isopropyl substituent and the methyl substituent. Minor metabolites in rat urine were 2-p-carboxyphenylpropan-1-ol (10 %), 2-p-carboxyphenylpropionic acid (14 %), and p-isopropylbenzoic acid (17 %). A large percentage of the urinary metabolites at this dose was conjugated (66 % conjugated vs 34 % free) both to glucuronic acid and glycine. The same metabolites were observed after the high dose, but conjugation was considerably reduced (18 % conjugated vs 82 % free), suggesting saturation of the conjugation pathway (Boyle et al., 1999).

Oxidation of terminal double bonds

Ten candidate substances [FL-no: 02.123, 02.168, 02.144, 02.146, 02.150, 02.206, 02.226, 09.614, 09.671 and 02.149] possess terminal double bonds. These double bonds may be oxidised to the corresponding epoxide. Epoxides are highly reactive molecules, due to the large strain associated with the three membered ring structures, and they react easily with nucleophilic sites of cellular macromolecules. For this reason, several aliphatic alkene-derived epoxides have been demonstrated to be carcinogenic (Melnick, 2002).

Two of the main metabolites of one of the supporting substances, myrcene, were found to be produced through epoxidation of the double bonds followed by hydrolysis to diols by epoxide hydroxylase (see above). Theoretically these reactions may also occur in the candidate substances with conjugated terminal double bonds. However, linalool or elemol were not found to give oxidation of terminal double bonds, indicating that terminal double bonds as such will not give rise to intermediate epoxides.

D.4 Summary and Conclusion

Six candidate flavouring substances in this group are esters [FL-no: 09.171, 09.356, 09.614, 09.671, 09.808 and 09.617]. Hydrolysis data are not available for any of these esters. However, for the supporting substance, linalyl acetate, *in vitro* hydrolysis data indicate that these esters may be hydrolysed, which would also be expected based on general knowledge about ester hydrolysis. The carboxylic acids resulting from the hydrolysis of these six candidate flavouring substances are acetic acid, propanoic acid and valeric acid, which will be incorporated in normal physiological processes such as beta-oxidation and citric acid cycle. The alcohols resulting from the hydrolysis of these esters are tertiary alcohols as are all the remaining candidate substances in this flavouring group. The tertiary alcohols in this FGE are subdivided into eight subgroups according to their chemical structures.

Subgroup 1: Metabolism studies of the three candidate substances, 2-methylpropan-2-ol [FL-no: 02.052], 2-methylbutan-2-ol [FL-no: 02.041] and 2-methylpentan-2-ol [FL-no: 02.181] show that these are conjugated with glucuronic acid before excretion in the urine. When rats were treated with 2-methylpropan-2-ol, acetone was excreted in small amounts, and when administered 2-methylbutan-2-

ol, diols were excreted. This indicates that an additional metabolism pathway of the three candidate substances is oxidation of the methyl group. From these metabolism studies it is anticipated that the candidate substances 2-methylpropan-2-ol, 2-methylbutan-2-ol, 3-methylpenta-3-ol, 2-methylpentan-2-ol, 2,6-dimethyl-2-heptanol, 2,4-dimethyl-4-nonanol, 3,6-dimethyloctan-3-ol and 1,1-dimethylethyl propionate [FL-no: 02.052, 02.041, 02.184, 02.181, 02.219, 02.253, 02.147 and 09.356] are conjugated with glucuronic acid and excreted in the urine, or that they can undergo oxidation to yield the corresponding diols. They are also expected to be excreted as their respective glucuronic acid conjugates.

Subgroup 2: Linalool is a supporting substance to the candidate substances 2-methylbut-3-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate [FL-no: 02.123, 02.150, 02.168, 02.226, 09.614 and 09.671,], which have the isolated double bond in close proximity to the tertiary alcohol group. As these substances or their respective alcohol moieties have a free hydroxyl group, they may be directly conjugated. Seventy-two hours after intragastrical administration of 500 mg/kg bw ¹⁴C-labelled linalool to 12-weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetra hydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut. In addition, the metabolism of linalool indicates that these candidate substances may also be metabolised by omega-oxidation of methyl groups, excreted in the urine as such or in conjugation with glucuronic acid. No oxidation of the terminal double bond in linalool was observed, indicating no formation of epoxide intermediates.

For 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] the structure differs from the supporting substance linalool and the other candidate substances in this group in that the isolated terminal double bond is located distant from the tertiary group. However, any risk from epoxide formation of this compound is considered to be low since the substance can be directly conjugated with glucuronic acid or the alcohol group can be expected to be readily attacked by oxidation processes, ultimately yielding the corresponding carboxylic acid. Biochemical attack of this carboxylic acid via beta-oxidation or conjugation with glucuronic acid is expected to be more efficient and rapid than microsomal oxidation. Any epoxides formed may be metabolised by conjugation with glutathione or by epoxide-hydrolase mediated hydrolysis.

Subgroup 3: Myrcene is a supporting substance to the candidate substance 3,7-dimethylocta-1,5,7-trien-3-ol, [FL-no: 02.146]. This substance also has an alcohol moiety that can be directly conjugated. In addition, further oxidation of methyl groups may occur. As shown for myrcene, oxidation of conjugated terminal double bonds in the candidate substances may occur, giving rise to epoxide intermediates. It should be noted that the available genotoxicity data for myrcene do not indicate a genotoxic potential for this substance even in the presence of metabolic activation. However, it cannot be anticipated that these candidate substances will be metabolised to innocuous products.

Subgroup 4: 1,2-Dihydrolinalool [FL-no: 02.140] can be expected to be directly conjugated to glucuronic acid like the supporting substance linalool, and excreted. After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep, dihydrolinalool and tetrahydrolinalool are formed as metabolites. *In vivo* metabolism studies in rats on ¹⁴C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucuronides. Additionally, it might be oxidised at the methyl groups, introducing new hydroxyl groups that also can be conjugated and excreted.

Subgroup 5: From the metabolism studies of alpha-terpineol and menthol it is anticipated that the candidate substances terpineol [FL-no: 02.230], p-menthane-1,8-diol [FL-no: 02.054], bisabola-1,12-dien-8-ol [FL-no: 02.129] and p-menthan-8-ol [FL-no: 02.171] (also an hydrolysis product of candidate substance [FL-no: 09.617]) may undergo allylic oxidation of the exocyclic methyl group. This could be further oxidised to a carboxylic acid group. Alternative or subsequent metabolism may occur by conjugation to glucuronic acid, followed by excretion in the urine.

Subgroup 6: A metabolism study on elemol indicate that the substance is absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of elemol [FL-no: 02.149] and sclareol [FL-no: 02.206], which have the same structural features as elemol, would not be anticipated.

Subgroup 7: Two metabolism studies on cedrol [FL-no: 02.120] indicate that the candidate substances 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197], cedrol [FL-no: 02.120] and cedryl acetate [FL-no: 09.171] and guaiyl acetate [FL-no: 09.808] will be further hydroxylated and excreted in urine as such or as conjugates.

Subgroup 8: In metabolism studies, the supporting substance 1-isopropyl-4-methylbenzene [FL-no: 01.002] (synonym: p-cymene) was oxidised at the isopropyl side chain yielding 2-(p-tolyl)-2-propanol, which is not further oxidised, but excreted unchanged or as a glucuronic acid conjugate. It is anticipated that the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203] will follow the same pathway and be excreted unchanged or in conjugation with glucuronic acid.

In summary, 28 of the candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.808, 09.171, 09.356, 09.614, 09.617 and 09.671] are anticipated to be metabolised to innocuous products.

For the remaining candidate substance [FL-no: 02.146] (subgroup 3), which contains a conjugated terminal double bond, data from the supporting substance myrcene [FL-no: 01.008] indicate that this terminal double bond may be oxidised, giving rise to epoxide intermediates. Thus, it cannot be anticipated that this candidate substance will be metabolised to innocuous products. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies.

Appendix E. Toxicity

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Rat	NR	Oral	3500	(Schaffarzick and Brown, 1952)	
	Rat	M, F	Gavage	3046	(Johnson, 1981a)	
	Rat	M, F	Gavage	2733	(Johnson, 1981b)	
	Rat	NR	Oral	> 3800	(Eastman Kodak Co., 1994)	
	Rabbit	M, F	Gavage	3560	(Munch, 1972)	
2-Methylbutan-2-ol [02.041]	Rat	NR	Oral	1000	(Schaffarzick and Brown, 1952)	
	Rat	NR	Oral	1000 – 2000	(Dow Chemical Company, 1982)	
	Rabbit	M, F	Gavage	2027	(Munch, 1972)	
3-Methylpentan-3-ol [02.184]	Rat	NR	Oral	710	(Brown et al., 1955)	
2,6-Dimethyl-2-heptanol [02.219]	Rat	NR	Oral	6800	(BASF, 1979)	
	Rat	NR	Oral	> 5000	(Moreno, 1976a)	
3,6-Dimethyloctan-3-ol [02.147]	Rat	NR	Oral	> 5000	(Moreno, 1973a)	
2-Methylbut-3-en-2-ol [02.123]	Rat	NR	Oral	1800	(BASF, 1972)	
(Linalool [02.013])	Rat	M, F	Gavage	2790	(Jenner et al., 1964)	
	Mouse	M, F	Oral	2200	(Rhône-Poulenc, Inc., 1992)	
	Mouse	M, F	Oral	3918	(Hoffman-LaRoche, Inc., 1967)	
	Mouse	M, F	Oral	3000 ¹ 1700 ²	(Rhône-Poulenc, Inc., 1992)	
(3,7-Dimethyloctan-3-ol [02.028])	Rat	NR	Oral	> 5000	(Moreno, 1976b)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat	NR	Oral	3600	(Moreno, 1973b)	
Myrcenol [02.185]	Rat	NR	Oral	5300	(Moreno, 1972a)	
Ocimenol [02.191]	Rat	NR	Oral	1700	(Wohl, 1974)	
Geranyl linalool [02.150]	Rat	NR	Oral	> 5000	(Moreno, 1982a)	
	Mouse	M, F	Oral	14,632	(Hoffman-LaRoche Inc., 1967)	
(Linalyl formate [09.080])	Rat	NR	Oral	> 5000	(Russell, 1973)	
	Mouse	M	Oral	5389	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl acetate [09.013])	Rat	M, F	Gavage	14,550	(Jenner et al., 1964)	
	Rat	NR	Oral	10,000	(Zeller, 1969)	
	Mouse	NR	Gavage	13,360	(Jenner et al., 1964)	
	Mouse	M	Oral	13,539	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl propionate [09.130])	Rat	NR	Oral	> 5000	(Moreno, 1973c)	
	Mouse	M	Oral	13,874	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl butyrate [09.050])	Rat	NR	Oral	> 5000	(Levenstein, 1975)	
	Mouse	M	Oral	> 8907	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl isobutyrate [09.423])	Rat	M, F	Gavage	> 36,300	(Jenner et al., 1964)	
	Mouse	NR	Gavage	15,100	(Jenner et al., 1964)	
	Mouse	M	Oral	> 17,698	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl isovalerate [09.454])	Rat	NR	Oral	> 5000	(Moreno, 1975)	
	Mouse	M, F	Oral	25,165	(Hoffman-LaRoche, Inc., 1967)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Linalyl hexanoate [09.068])	Mouse	M, F	Oral	37,869	(Hoffman-LaRoche Inc., 1967)	
Myrcenyl acetate [09.669]	Rat	NR	Oral	6300	(Moreno, 1972b)	
(Linalyl octanoate [09.116])	Mouse	M, F	Oral	48,849	(Hoffman-LaRoche Inc., 1967)	
Nerolidyl acetate [09.671]	Rat	NR	Oral	> 5000	(Moreno, 1976c)	
(alpha-Terpineol [02.014])	Rat	NR	Oral	4300 ³	(Moreno, 1971)	
	Mouse	M	Gavage	2830	(Yamahara et al., 1985)	
(alpha-Terpinyol formate [09.081])	Rat	NR	Oral	> 5000	(Moreno, 1976d)	
(Terpinyl acetate [09.830])	Rat	M, F	Gavage	5075	(Jenner et al., 1964)	
(Terpinyl propionate [09.142])	Rat	NR	Oral	> 5000	(Moreno, 1973d)	
(Terpinyl 2-methylpropionate [09.425])	Rat	NR	Oral	> 5000	(Moreno, 1982b)	
(4-Terpinenol [02.072])	Rat	NR	Oral	1300	(Moreno, 1977)	
Guaiyl acetate [09.808]	Rat	NR	Oral	> 5000	(Moreno, 1973e)	
2-Phenylpropan-2-ol [02.203]	Rat	NR	Oral	1037 ⁴	(Patty, 1982)	
(beta-Terpineol) [02.097])	Rat	NR	Oral	4300 ³	(Moreno, 1971)	
<i>p</i> -Menthan-8-ol [02.171]	Rat	NR	Oral	> 5000	(Moreno, 1973f)	
(1-Acetoxy-1-acetylcyclohexane [09.293])	Rat	M, F	Gavage	2150	(Piccirillo and Hartman, 1980)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Menthol [02.015])	Mouse	M	Gavage	2652	(Food and Drug Research Laboratories, Inc., 1975)	
	Mouse	M	Gavage	4384	(Food and Drug Research Laboratories, Inc., 1975)	
	Mouse	NR	Gavage	3100	(Wokes, 1932)	
	Rat	M, F	Gavage	3180	(Jenner et al., 1964)	
	Rat	M	Gavage	940	(Food and Drug Research Laboratories, Inc., 1975)	
Bisabola-1,12-dien-8-ol [02.129]	Rat	NR	Oral	> 5000	(CIR, 1999)	
	Mice	NR	Oral	0.633	(CIR, 1999)	
	Rat	M, F	Oral	M: 14,9 ml/kg bw F: 15.6 ml/kg bw	(Habersang et al., 1979)	
	Mice	M, D	Oral	15.1 ml/kg bw	(Habersang et al., 1979)	

¹Value represents calculated LD₅₀ when substance was administered in peanut oil.

²Value represents calculated LD₅₀ when substance was administered as an emulsion in an aqueous Arabic gum solution at 10 %.

³Reported for a mixture of alpha- and beta-terpineol.

⁴LD₅₀ value reported as 1.07 ml; conversion based on a density of 0.97 g/ml.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Rat; M 5-6	Drinking water	0 or 0.5 %, equivalent to 0 or 500 mg/kg bw per day	10 weeks	None	(Acharya et al., 1997)	Histological examination of liver and kidney only.
	Rat; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	90 days	None	(Lindamood et al., 1992); (NTP, 1995)	Fully described NTP study. No NOAEL for males or females.
	Rat; M 10	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	90 days	None	(Takahashi et al., 1993)	Good quality investigative study using extra renal samples from the above 90-day study; indicates nephropathy in male F344 rats is due to alpha-2 μ -globulin. No NOAELs.
	Rat; M, F 120 ^(d)	Drinking water	Males: 0, 0.125, 0.25 or 0.5 %, equivalent to 0, 90, 200 or 420 mg/kg bw per day Females: 0, 0.25, 0.5 or 1 %, equivalent to 0, 180, 330 or 650 mg/kg bw per day	2 years	None	(Cirvello et al., 1995); (NTP, 1995)	Fully described NTP study. No NOAELs. NTP conclusions on carcinogenicity: some evidence of carcinogenic activity in males, no evidence of carcinogenicity in females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
	Rat; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	90 days	Male: 0.5 %, equivalent to 500 mg/kg bw per day Female: 1 %, equivalent to 1000 mg/kg bw per day	(Brown and Wheeler, 1979)	The report is incomplete as it is lacking tables.
	Mouse; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 625, 1250, 2500, 5000 or 10,000 mg/kg bw per day	90 days		(Brown and Wheeler, 1979)	The report is incomplete as it is lacking tables.
	Mouse; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 625, 1250, 2500, 5000 or 10,000 mg/kg bw per day	90 days	Male: 1 %, equivalent to 2500 mg/kg bw per day Female: 2 %, equivalent to 5000 mg/kg bw per day	(Lindamood et al., 1992); (NTP, 1995)	Fully described NTP study.
	Mouse; M, F 120	Drinking water	0, 0.5, 1 or 2 %, equivalent to 0, 540, 1040 or 2070 mg/kg bw per day in males and 0, 510, 1020 or 2100 mg/kg bw per day in females	2 years	M: None F: 0.5 %, equivalent to 510 mg/kg bw per day	(Cirvello et al., 1995); (NTP, 1995)	Fully described NTP study. NTP conclusions on carcinogenicity: equivocal evidence of carcinogenic activity in males, some evidence of carcinogenic activity in females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
2-Methylbut-3-en-2-ol [02.123]	Rat; M, F 10	Gavage	0, 30, 150 or 750 mg/kg bw per day 5 days/week	4 weeks	150	(BASF, 1994)	Full study details provided. Study considered valid. Dosing only 5 days/week but a clear NOAEL.
(Linalool [02.013])	Rat; M, F NR	Diet ^(b)	0 or 50 mg/kg bw per day ^(a)	84 days	50 ^(a)	(Oser, 1967)	Unpublished report. Administered as 50 % mixture of linalool and citronellol. Slight retardation of growth in males at 50 mg/kg bw per day, not related to food intake.
	Mouse; F 30	Gavage	0 or 365 mg/kg bw per day	5 days	375 ^{(a),(c)}	(Gaworski et al., 1994)	Immunotoxicity study only.
	Rat; F 100	Gavage		21 days	500	(Lewis, 2006)	
(Linalyl acetate [09.013])	Rat; M, F NR	Diet ⁵	0 or 24 mg/kg bw per day ^(a)	84 days	24 ^(a)	(Oser, 1967)	Unpublished report. Administered as mixture of linalyl acetate (24 mg/kg bw per day), linalyl isobutyrate (27 mg/kg bw per day) and geranyl acetate (48 mg/kg bw per day). Slight growth retardation of females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
(Linalyl isobutyrate [09.423])	Rat; M, F NR	Diet ⁶	0 or 27 mg/kg bw per day ^(a)	84 days	27 ^(a)	(Oser, 1967)	
	Rat; NR 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw per day	18 weeks	500 (10,000 ppm)	(Hagan et al., 1967)	Very limited details provided.
(Linalyl cinnamate [09.736] ⁷)	Rat; M, F 10	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw per day	17 weeks	500 ^(a) (10,000 ppm)	(Hagan et al., 1967)	Very limited details provided.
(Geranyl acetate [09.011] /Citronellyl acetate[09.012] ⁸)	Mouse; M, F 50	Gavage		103 weeks	- ⁹	(NTP, 1987)	
	Rat; M, F 50	Gavage		103 weeks	1000 (710; 290) ⁸	(NTP, 1987)	
(Myrcene [01.008])	Rat; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	13 weeks	None	(NTP, 2004)	Draft results only available. Evaluated by the JECFA. No NOAEL as most sensitive adverse effect (nephropathy) present at lowest dose tested (JECFA, 2006).
	Mouse; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day, 5 days/week	13 weeks	Male: None Female: 250 mg/kg bw per day	(NTP, 2004)	Draft results only available. Evaluated by the JECFA. Liver hypertrophy in males at all doses (JECFA, 2006).

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
	Rats; M, F 10	Diet	0, 8.0, 40 and 44 mg/kg bw per day (males) 0, 9.6, 48 and 53 mg/kg bw per day (females)	90 days	Male: 44 mg/kg bw per day Female: 53 mg/kg bw per day	(Bauter, 2013)	Compliant with OECD Guideline 408.
(Menthol [02.015])	Mouse; M, F 50	Diet	0, 2000 or 4000 ppm, equivalent to 0, 300 or 600 mg/kg bw per day	103 weeks	600 ^(a)	(National Cancer Institute, 1979)	Good quality.
	Mouse; F 30	I.P. injection	0, 500 or 2000 mg/kg, 3 times/week	24 weeks	None	(Stoner et al., 1973)	Good quality.
	Rat; M, F 20	Gavage	0, 200, 400 or 800 mg/kg bw day	28 days	None	(Thorup et al., 1983)	Relative good quality.
	Rat; M, F 80	Diet	0, 100 or 200 mg/kg bw	5.5 weeks	200 ^(a)	(Herken, 1961)	Limited information.
	Rat; M, F 50	Diet	0, 3750 or 7500 ppm, equivalent to 0, 188 or 375 mg/kg bw per day	103 weeks	375 ^(a)	(National Cancer Institute, 1979)	Good quality.
(Terpinyl acetate [09.830])	Rat; M, F 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw per day	20 weeks	500 ^(a)	(Hagan et al., 1967)	Very limited details provided.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
Sclareol [02.206]	Rat; M, F 20	Gavage	8.8 mg/kg bw per day	28 days	8.8	(IOFI, 2006)	
Cedrol [02.120]	Rat; M, F 20	Gavage	8.4 mg/kg bw per day	28 days	8.4	(IOFI, 2006)	
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat; M, F 40	Gavage	10, 50, 500, 1000 mg/kg bw per day	90 days	50 (F) 10 (M)	(Dunster et al., 2007)	
Bisabola-1,12-dien-8-ol [02.129]	Dog, NR 20	Gavage	1 ml/kg bw	14 days	850	(Habersang et al., 1979)	
	Rat, M,F 20	Gavage	2, 3 ml/kg bw	28 days	850	(Habersang et al., 1979)	

NR Not Reported

(a): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(b): Administered with citronellol as part of a 50/50 mixture.

(c): Immunotoxicity study.

(d): Three treatment groups per sex. Each dose group included 60 male and female animals.

⁵ Administered with linalyl isobutyrate and geranyl acetate as a part of a mixture.

⁶ Administered with linalyl acetate and geranyl acetate as a part of a mixture.

⁷ Structurally related ester of linalool not evaluated as part of the supporting chemicals group.

⁸ Structurally related terpenoid esters administered as a mixture: geranyl acetate, 71 %; citronellyl acetate, 29 %.

⁹ A NOAEL could not be established due to a high incidence of gavage errors and low survival associated with pneumonia.

Table 18: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Duration	Species/Species ex No/group	Route	Dose levels	NOAEL (mg/kg per day)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Developmental Toxicity Gestation Days 6 - 20	Mouse; F 15	Liquid Diet	0, 0.5, 0.75 or 1 % (equivalent to approximately 0, 3 400, 4 900 and 6 400 mg/kg bw per day)	Maternal: 0.5 % Foetal: None	(Daniel and Evans, 1982)	Study considered valid. Dose related reduction of offspring performance in neurobehavioural tests (righting reflex, negative geotaxis, open field behaviour, cliff avoidance, roto-rod performance).
	Developmental Toxicity: Gestation Days 6– 18	Mouse; F 9 - 12	Gavage ^(a)	0 or 10.5 mmol/kg bw per day, equivalent to 1 557 mg/kg bw per day	Maternal: None Foetal: None	(Faulkner et al., 1989)	Study considered valid. Increased foetal resorptions and decreased births per litter in treated group.
(Linalool [02.013])	Reproductive & Developmental Toxicity: 33 - 39 days ^(c)	Rat; F 10	Gavage	0, 250, 500 or 1 000 mg/kg bw per day coriander oil, equivalent to 0, 182.3, 364.5 or 729 mg/kg bw per day linalool	Maternal: None Foetal: 364.5	(Hoberman and Christian, 1989)	Test substance was coriander oil. Linalool content 72.9 %.
(Menthol [02.015])	Teratology Gestation days 6 - 15	Mouse; F 22	Gavage	0, 1.85, 8.59, 39.9, 185	185 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	
	Teratology Gestation days 6 - 15	Rat; F 22 - 23	Gavage	0, 2.18, 10.15, 47.05, 218	218 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	

Table 18: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Duration	Species/Species ex No/group	Route	Dose levels	NOAEL (mg/kg per day)	Reference	Comments
	Teratology Gestation days 6 - 15	Hamster; F 20 - 22	Gavage	0, 4.05, 21.15, 98.2, 405	405 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	
	Teratology Gestation days 6 - 18	Rabbit; F 9 - 11	Gavage	0, 4.25, 19.75, 91.7, 425	425 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	
(Myrcene [01.008])	Teratology Gestation days 6 - 15	Rat; F 16 - 29	Gavage	0, 250, 500 and 1 200 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal: 500 mg/kg bw per day	(Delgado et al., 1993a)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Gestation day 15 to postnatal day 21	Rat; F 12 - 18	Gavage	0, 250, 500, 1 000 and 1 500 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal/neonatal: 250 mg/kg bw per day	(Delgado et al., 1993b)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Prior to mating until postnatal day 21 ^(d)	Rat; M, F 60	Gavage	0, 100, 300, 500 mg/kg bw per day	Maternal/paternal: 500 mg/kg bw per day Foetal: 300 mg/kg bw per day	(Paumgartten et al., 1998)	Study considered valid. Evaluated by the JECFA.
2,6-Dimethyloct-7-en-2-ol [02.144]	Developmental Toxicity: Gestation Days 7 - 17	Rat, M, F 25	Gavage	0, 250, 500 and 1 000 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal: 500 mg/kg bw per day	(Politano et al., 2008)	

(a): Test substance was administered to two strains of mice at a dose level of 778 mg/kg (10.5 mmoles/kg) twice daily.

(b): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(c): Animals were dosed for seven days prior to mating, during mating (maximum of seven days), during gestation, delivery and four days post parturition.

(d): Males were dosed for 91 days prior to mating and during mating; females were dosed from 21 days prior to mating to 21 days after birth

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	0.1, 0.5, 2.5, 5, 10 μl/plate (7800 μg/plate)	Questionable ^(a)	(Haworth et al., 1981a)	Unpublished GLP study. According to the conclusion of the report, the test substance “did cause a weak but significant increase in TA1535 revertants per plate in both the presence and absence of rat liver microsomes.” However, this result cannot be re-evaluated because the corresponding page with results on TA1535 in Table format is lacking.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	probably 1 to 10 μl/plate (7800 μg/plate) (corresponding pages of the report are lacking)	Questionable ^(a)	(Haworth et al., 1981b)	Unpublished GLP study. According to the conclusion of the report, the test substance (purity 99.9 %) “did not cause a significant increase in the number of revertants per plate in any of the tester strains with or without metabolic activation. It should be noted, however, that there was a slight increase in TA1535 revertants per plate observed in the presence and absence of rat liver microsomes.” However, this result cannot be re-evaluated because 15 pages with all results in Table format are lacking.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	10000 μg/plate	Negative ^(a)	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD guideline 471. There was a slight increase in TA1535 revertants per plate observed in the presence and absence of rat and hamster liver microsomes. This effect is dose-related only with hamster liver S9. Overall, the effects were less than twice compared to control. The study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Yeast mitochondrial mutation assay	Several <i>Saccharomyces</i> strains	4 %	Positive	(Jiménez et al., 1988)	This non-GLP study was not in accordance with OECD guideline 480 (1986), and the study protocol does not belong to standard protocols used in routine testing. However, the result is considered valid since main details of method and results are reported. Endpoint not relevant for genotoxicity.
	Forward mutation assay	Mouse lymphoma L5178Y TK +/-	0, 1000, 2000, 3000, 4000, 5000 µg/ml	Negative ^(a)	(McGregor et al., 1988)	Non-GLP study in accordance with OECD guideline 476 (1984). Study is considered valid.
	Forward mutation assay	Mouse lymphoma L5178Y TK +/-	1.3 to 100 µl/ml (78000 µg/ml)	Negative	(Kirby et al., 1981)	Unpublished GLP study. According to the report's summary, test substance of high (99.9 %) purity did not induce any detectable increases in the mutant frequencies in the presence and absence of S9-mix. When cultures were tested in the presence of S9-mix with less pure test substance none of the cultures exhibited increases in mutant frequency. Without S9-mix this test substance did appear to induce an increase in the mutant frequency of cultures treated with the higher doses, but a dose-related response was not evident. In addition, in only one of two experiments was a greater than two-fold increase in mutant frequency observed. However, this result cannot be re-evaluated because 47 pages with all results in Table format are lacking in the report submitted.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration	Chinese hamster ovary cells	160 - 5000 µg /ml	Negative	(NTP, 1984)	Limited validity. This non-GLP study was in accordance with OECD guideline 473 (1983) except that only a single harvest time was used, however, the study protocol does not fully meet the criteria of the revised guideline from 1997. According to the version from 1997, a single sampling time should be equivalent to about 1.5 normal cell cycle lengths, duplicate cultures should be used at each concentration and 200 metaphases should be scored per concentration.
	Sister chromatid exchange	Chinese hamster ovary cells	6 concentrations ranging from 0.625 to 20 µl/ml (15 600 µg/ml)	Negative	(Putman, 1985)	Unpublished GLP study. According to the report's summary, test substance of high (99.9 %) purity caused a significant increase in sister chromatid exchanges at the high dose only in the assay without S9 and at the two highest doses in the assay with S9, however, the test article did not meet the criteria for a positive response (at least two-fold increase or a significant positive dose response over at least three doses). However, this result cannot be re-evaluated because pages with all results in Table format are lacking in the report submitted.
	Sister chromatid exchange	Chinese hamster ovary cells	20 µl/ml (15 600 µg/ml)	Negative	(Thilagar et al., 1981)	Unpublished GLP study is considered valid. A marginal increase in SCE frequency was observed in the tests with and without S9, while only the highest concentration without S9 resulted in a significant increase. Thus, the test article did not meet the criteria for a positive response (at least two-fold increase or a significant positive dose response over at least three doses). (All relevant tables

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
2-Methylbutan-2-ol [02.041]	Mutagenicity assays	<i>S. typhimurium</i> TA1535; TA1537; TA1538; <i>S. cerevisiae</i>	NR	Negative	(Dow Chemical Company, 1982)	were submitted). Very short abstract only.
	Sister chromatid exchange	Chinese hamster ovary cells	160, 500, 1600, 5 000 µg/ml	Positive ^(c) Negative ^(b)	(NTP, 1997)	
	Sister chromatid exchange	Chinese hamster ovary cells	2 000, 3 000, 4 000, 5 000 µg/ml	Negative ^(a)	(NTP, 1997)	
2-Methylbut-3-en-2-ol [02.123]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	20 – 5 000 µg/plate	Negative ^(a)	(BASF, 1989)	Summary in IUCLID data set only. According to this summary, the assay was not in compliance with GLP but in accordance with OECD guideline 471. The unpublished study report is not available for re-evaluation.
	Liquid suspension assay	<i>S. typhimurium</i> TA98; TA100	20 - 5 000 µg/plate	Negative ^(a)	(BASF, 1991)	Summary in IUCLID data set only. According to this summary, the assay was not in compliance with GLP but in accordance with OECD guideline 471. The unpublished study report is not available for re-evaluation.
Isophytol [02.168]	Ames test	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535	100, 333, 1 000, 3 333, 10 000 µg/plate	Equivocal ^(a)	(NTP, 1994)	This non-GLP study is considered valid. It is in accordance with OECD guideline 471 (1983). The study is published in the Web and the report contains sufficient details.
	Ames test	<i>S. typhimurium</i> TA97, TA98, TA100, and TA1535	100 - 10000 µg/plate	Negative	(NTP, 2000)	This non-GLP study is considered valid. It is in accordance with OECD guideline 471 (1983). The study is published in the Web and the report contains sufficient details.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Linalool [02.013])	Ames test (modified)	<i>S. typhimurium</i> TA100	3 µl/2 ml (2 610 µg/2ml) incubation volume 1 mg/plate	Negative ^(a)	(Eder et al., 1980)	
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537	(1 000 µg/plate)	Negative ^(a)	(Ishidate et al., 1984)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	100 µl (87 000 µg)	Negative ^(a)	(Rockwell and Raw, 1979)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 nl/plate (8700 µg/plate)	Negative ^(a)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster fibroblasts	250 µg/ml	Negative ^(a)	(Ishidate et al., 1984)	
	Unscheduled DNA synthesis	Rat hepatocytes	50 nl/ml (43.6 µg/ml)	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	1 mg/plate (1 000 µg/plate)	Negative	(Yoo, 1986)	In Japanese (only summary and tables in English). Thus, the validity cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17(rec+); M45(rec-)	17 µg	Negative	(Oda et al., 1979)	
	Rec assay	<i>B. subtilis</i> H17(rec+); M45(rec-)	10 µl/disk (8700 µg/disk)	Positive	(Yoo, 1986)	In Japanese (only summary and tables in English). Thus, the validity cannot be evaluated.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell mutation	Mouse lymphoma L5178Y TK+/-	3.9 - 300 nl/ml	Negative ^(b) Positive ^(c)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	1 000 µM (15 4250 µg)	Negative ^{(a),(d)}	(Sasaki et al., 1989)	
(Linalyl acetate [09.013])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	25 000 nl/plate (22 575 µg/plate)	Negative ^(a)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Unscheduled DNA synthesis	Fischer or SD rat hepatocytes	300 nl/ml (271 µg/ml)	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17 (rec+); M45 (rec-)	18 µg	Negative	(Oda et al., 1979)	
	Chromosome aberration	Peripheral human lymphocytes	180 µg/ml	Negative ^(a)	(Bertens and van de Waart, 2000)	
(alpha-Terpineol [02.014])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 µg/plate	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA97a; TA98; TA100; TA102	2 500 µg/plate 2 500 µg/plate	Negative Weakly positive ^(e)	(Gomes-Carneiro et al., 1998)	The study is considered valid. A slight but dose-related response was noted with TA102 with and without the use of metabolic activation.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	1 000 µg/plate	Negative ^(a)	(National Cancer Institute, 1983)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 µg/plate	Negative ^(a)	(Lorillard, 1983)	
	Spot test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmol/plate (463 µg/plate)	Negative ^(a)	(Florin et al., 1980)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	0.5 µl/ml (467 µg/ml) 0.75 µl/ml (700 µg/ml)	Negative ^(c) Negative ^(b)	(Kirby et al., 1984)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	300 nl/ml (280 µg/ml) 250 nl/ml (233 µg/ml)	Negative ^(a)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	15.6 - 250 nl/ml 15.6 - 300 nl/ml	Negative ^(b) Negative ^(c)	(Lorillard, 1982)	
	Rec assay	<i>S. cerevisiae</i>	NR	Negative	(Oda et al., 1979)	
(Terpinyl acetate [09.830])	Rec assay	<i>B. subtilis</i> H17; M45	19 µg	Negative	(Oda et al., 1979)	
(beta-Terpineol) [02.097])	Ames	<i>S. typhimurium</i> TA98; TA100	0.05 µl (100 µl)	Negative ^(a)	(Rockwell and Raw, 1979)	
	Rec assay	<i>S. cerevisiae</i>	NR	Negative ^(a)	(Oda et al., 1979)	Article does not specify alpha- or beta-terpineol.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(1-Isopropyl-4-methylbenzene [01.002])	<i>In vivo/in vitro</i> Ames test	<i>S. typhimurium</i> TA98 and TA100	0.5 ml (equivalent to 1 706 mg/kg bw) administered to Sprague-Dawley rats, urine collected and tested <i>in vitro</i>	Negative ^(e)	(Rockwell and Raw, 1979)	
(Myrcene [01.008])	Ames test	<i>S. typhimurium</i> TA100; TA1535; TA97; TA98	0, 33, 100, 333, 1 000, 3 333 and 10 000 µg/plate	Negative ^(a)	(NTP, 1999)	
	Chromosome aberration	Human lymphocytes	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	Sister chromatid exchange	Human lymphocytes	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	HPRT assay	V79 Chinese hamster cells	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	Sister chromatid exchange	V79 Chinese hamster cells	0, 100, 250 and 500 µg/ml	Negative ^(a)	(Röscheisen et al., 1991)	
	Sister chromatid exchange	HTC cells	0, 100, 250 and 500 µg/ml	Negative	(Röscheisen et al., 1991)	
(Menthol [02.015])	Ames test	<i>S. typhimurium</i> , TA92, TA100, TA94, TA98, TA1535, TA1537	0 – 5 000 µg/plate	Negative ^(a)	(Ishidate et al., 1984)	d,l-Menthol was used. The study is considered valid.
	Ames test (preincubation method)	<i>S. typhimurium</i> , TA1535, TA97, TA100, TA98	3 - 666 µg/plate	Negative ^(a)	(Zeiger et al., 1988)	d,l-Menthol was used. The study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> , TA2637, TA100, TA98	0 and 5 - 500 µg/plate	Negative ^(a)	(Nohmi et al., 1985)	d,l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> , TA2637, TA100, TA98	0 and 20 - 500 µg/plate	Negative ^(a)	(Nohmi et al., 1985)	l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> , TA1537, TA1535, TA100, TA98	0, 6.4, 32, 160 and 800 µg/plate	Negative ^(a)	(Andersen and Jensen, 1984)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.
	Ames test	<i>E. coli</i> WP2 <i>uvrA</i> (Trp)	100 - 800 µg/plate	Negative	(Yoo, 1986)	l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.
	Ames test	<i>S. typhimurium</i> TA97A; TA98; TA100; TA102	0, 5 - 800 µg/plate	Negative ^(a)	(Gomes-Carneiro et al., 1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.
	Rec assay	<i>B. subtilis</i> H17, M45	Up to 10 000 µg/disk	Positive	(Yoo, 1986)	l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method (Rec-assay) has poor predictive value.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay	<i>B. subtilis</i> H17, M45	20 µg/disk	Negative	(Oda et al., 1979)	l-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (Rec-assay) has poor predictive value.
	Alkaline elution assay	Rat hepatocytes	0 and 0.1 - 1.3 mM (203.2 µg/ml ^(d))	Negative	(Storer et al., 1996)	The experiment employed d-Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.
	Sister chromatid exchange	Chinese hamster ovary cells	5 – 50; 0 and 2 - 25 µg/ml ^(c) 0 and 16 - 167 µg/ml ²	Negative ^(a)	(Ivett et al., 1989)	d,l-Menthol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1 and 10 mM (1 563 µg/ml ^(d))	Negative ^(a)	(Murthy et al., 1991)	The study is considered valid.
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1 and 10 µg/ml	Negative	(Food and Drug Research Laboratories, Inc., 1975)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 - 200 µg/ml	Negative ^(c)	(Ishidate et al., 1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.
	Chromosome aberration	Chinese hamster ovary cells	0 and 50 - 250 µg/ml	Negative ^(a)	(Ivett et al., 1989)	d,l-Menthol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosome aberration	Human lymphocytes	0, 0.1, 1 and 10 mM (1 563 µg/ml ^(d))	Negative ^(a)	(Murthy et al., 1991)	The study is considered valid.
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0 and 12.5 - 200 µg/ml	Negative ^(a)	(Myhr and Caspary, 1991)	d,l-Menthol was used. The maximum concentration was selected by a preliminary test The study is considered valid.
Bisabola-1,12-dien-8-ol [02.129]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA102, TA1535, TA1537	0 and 0.5 – 1 500 µg/plate	Negative ^(a)	(King and Harnasch, 2002)	
	Ames test	<i>S. typhimurium</i> , TA97a, TA98, TA100, TA1535	0 and 1 - 400 µg/plate in ethanol	Negative ^(a)	(Gomes-Carneiro et al., 2005)	Publication in peer-reviewed journal. No reference to OECD and GLP-guidelines being made, but study is of good quality and considered valid.
	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537	0 and 20 – 5 000 µg/plate in DMSO	Negative ^(a)	(CIR, 1999)	Review.
	Chromosome aberration	Chinese hamster fibroblast cells V79	0 and 0.78 - 40 µg/ml in DMSO	Negative ^(a)	(CIR, 1999)	Review.
Cedrol [02.120]	Ames test	<i>S. typhimurium</i> , TA97a, TA98, TA102, TA1535	0 – 5 000 µg/plate	Negative ^(a)	(Scheerbaum, 2001)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.
1,2-Dihydrolinalool [02.140]	Ames test	<i>S. typhimurium</i> , TA97, TA98, TA100, TA102, TA1535	0 – 1 000 µg/plate	Negative ^(a)	(Gocke, 1999)	Research report according to OECD and GLP-guidelines, the study is considered valid.
2,6-Dimethyloct-7-en-2-ol [02.144]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA102, TA1535, TA1537	0 – 5 000 µg/plate	Negative ^(a)	(King, 2000)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol [02.197]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537 <i>E. coli</i> WP2 <i>uvrA</i>	0 - 250 µg/plate ^(c) 0 - 500 µg/plate ^(b)	Negative	(Watanabe, 2002)	Research report according to OECD guideline 471, the study is considered valid.

- NR: Not Reported.
(a): With and without metabolic activation.
(b): With metabolic activation.
(c): Without metabolic activation.
(d): With and without pre-treatment with mitomycin C at 0.15 microM for 21 hours.
(e): With and without presence of beta-glucuronidase.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	Chromosomal Aberration assay	Male rats	Once via gavage	0.2xLD50	Positive ¹	(Barilyak and Kozachuk, 1988)	Validity questionable. This study was not in compliance with GLP and not in accordance with OECD Guideline No. 475 (1983). Some main details of method and results are not available ^(b) .
	Micronucleus assay	Mouse bone marrow erythrocytes	I.P. x 3 at 24 hours intervals (=72 hours)	312.5, 625, and 1250 mg/kg bw	Negative	(NTP, 1996)	Valid. It was not in compliance with GLP but in accordance with OECD Guideline No. 474 (1983/1997) except that only 5 male animals were tested. The study is published in the Web and the report contains sufficient details. Due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance has reached the bone marrow. Relevance of the result is limited.
	Micronucleus assay	Rat bone marrow cells	I.P. x 3 at 24 hours intervals (=72 h)	0, 39 – 1250 mg/kg bw,	Negative	(NTP, 1997)	
	Micronucleus assay	Mouse peripheral blood cells	Drinking water	3000 - 40000	Negative	(NTP, 1995)	
2-Methylbut-3-en-2-ol [02.123]	Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	500, 1000, 1500 mg/kg	Negative	(BASF, 1992)	Summary in IUCLID data set only. According to this summary, the assay was performed in compliance with GLP and in accordance with OECD guideline 474. One thousand PCEs were counted per animal. The unpublished study report is not available for re-evaluation.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Linalool [02.013])	Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	1500 mg/kg	Negative	(Meerts and van de Waart, 2001)	Valid. It was in compliance with GLP and in accordance with OECD Guideline 474 (1997). However, due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance reached the bone marrow. Thus, the relevance of the result is limited.
(Myrcene [01.008])	Micronucleus assay	Rat bone marrow cells	Gavage	0, 100, 500 or 1000 mg/kg bw	Negative	(Zamith et al., 1993)	
	Micronucleus assay	Mouse peripheral blood cells	Gavage	Up to 2000 mg/kg bw per day for 13 weeks	Negative	(NTP, 2001)	
(Menthol [02.015])	Host mediated mutation assay	<i>S. typhimurium</i> TA1530 and G46; <i>S. cerevisiae</i> D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Equivocal	(Food and Drug Research Laboratories, Inc., 1975)	Negative results, with exception of the combination <i>S. typhimurium</i> TA1530 – 5000 mg/kg bw and <i>S. cerevisiae</i> D3 – 1150 mg/kg bw per day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels.
	Cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	Oral DL ₅₀ was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
	Micronucleus assay	B6C3F1 male mouse bone marrow cells	I.P.	0, 250 - 1000 mg/kg bw per day, during 3 days	Negative	(Shelby et al., 1993)	d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	Dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	Valid.

- (a): Cytogenetic analysis indicated the following results for controls and 2-methylpropan-2-ol, respectively: % polyploid cells, 0.5 ± 0.3 / 0.8 ± 0.4 ; % cells with gaps 0.3 ± 0.2 / 0.4 ± 0.2 ; % cells with aberrations, 0 / 1.6 ± 0.5 . Statistical comparisons were not performed.
- (b): The authors report the results of tests on a series of monohydric alcohols (from C1 to C16, 18 compounds) in rat bone marrow cytogenetic tests. All compounds were claimed positive compared to the untreated control group, even though no statistics is shown. It is noted that a single control group, with 0.0 % of cells with aberrations was used throughout the study. Lacking historical control data, it is not possible to establish whether the alleged positive results were due to and uniformly positive response elicited by all chemicals, or rather by an incidental very low frequency of aberrations in the group of rats (8 animals) used as control. In this respect it is noted that the incidence of chromosomal aberrations observed with some “positive” compounds, including 2-methylpropan-2-ol (1.6 ± 0.5 %), are close to background incidences commonly observed. Even the lack of a concurrent raise in gaps in treated animals casts doubts on an induced genotoxic effect. Moreover, the lack of a positive control group in the study is noted. For these reasons, the results of this study are considered inconclusive.

ABBREVIATIONS

ADI	Acceptable Daily Intake
bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
CPN	Chronic progressive nephropathy
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EC	European Commission
EFFA	European Flavours and Fragrances Association
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practise
ID	Identity
IOFI	International Organization of the Flavor Industry
Ip	Intraperitoneal
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest Observed Adverse Effect Level
MSDI	Maximised Survey-derived Daily Intake

mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA Synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 9, Revision 6 (FGE.09Rev6): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 22 flavouring substances in the Flavouring Group Evaluation 9, Revision 6, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances was considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure–activity relationships, intake from current uses, toxicological threshold of concern and available data on metabolism and toxicity. The present revision of FGE.09 includes the assessment of one additional flavouring substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219]. The Panel concluded that the 22 substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. However, based on mTAMDI calculations, for 11 flavouring substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949] more reliable exposure data are required for a re-evaluation. For two substances [FL-nos: 07.219 and 09.929] no use levels are available and these should be submitted. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have been considered. Specifications, including complete purity criteria and identity for the materials of commerce, have been provided for all candidate substances.

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KEY WORDS

flavourings, alcohols, ketones, esters, secondary alicyclic, saturated, unsaturated, food safety, FGE.09

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 22 flavouring substances in the Flavouring Group Evaluation (FGE) 9, Revision 6 (FGE.09Rev6), using the Procedure referred to in Commission Regulation (EC) No 1565/2000 “(hereinafter ‘the Procedure’)”. These flavouring substances belong to chemical groups 8, 25 and 30 of Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.09, FGE.09Rev6, includes the assessment of one additional candidate substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], which was not included in FGE.09Rev5. This substance is an α,β -unsaturated carbonyl, for which concern for genotoxicity was ruled out in FGE.212Rev3.

FGE.09Rev6 deals with 22 candidate substances: secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols.

Two candidate substances [FL-nos: 07.203 and 07.255] possess one chiral centre and 15 substances [FL-nos: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] possess two or more chiral centres.

Fourteen candidate substances belong to structural class I, seven substances belong to structural class II and one to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Fifteen flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Flavour Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by the Flavour Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, intakes in Europe of the 14 flavouring substances belonging to structural class I range from 0.0012 to 830 μg per capita per day, intakes of the seven substances from structural class II range from 0.0085 to 530 μg per capita per day and intake of the substance from structural class III is 1.2 μg per capita per day. These intakes are all below the threshold of concern values for structural classes I, II and III of 1 800, 540 and 90 μg per person per day, respectively. For one substance [FL-no: 09.520] from structural class II the MSDI is 770 μg per capita per day, which is above the threshold of concern of 540 μg per person per day. For this substance a No

Observed Adverse Effect Level (NOAEL) is available, providing a sufficient margin of safety based on the MSDI approach.

The total combined intakes of candidate and supporting substances from structural classes I and II do not give rise to a safety concern.

For five of the candidate substances [FL-nos: 07.109, 07.202, 07.219, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances. Genotoxicity data are available for only a limited number of the remaining flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

All candidate substances are expected to be metabolised to innocuous products at the estimated levels of use as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that the 22 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances based on the default MSDI approach.

In order to determine whether the conclusion for the 22 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications, including complete purity criteria and information on identity for the materials of commerce, have been provided for all flavouring substances.

Thus, for 22 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949].

The estimated intakes of 13 candidate substances in structural class I, based on the mTAMDI approach, ranged from 420 to 63 000 µg per person per day. For six substances [FL-nos: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949], the mTAMDI is above the threshold of concern of 1 800 µg per person per day. For seven substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold. The mTAMDI of five substances assigned to structural class II [FL-nos: 07.059, 07.109, 07.202, 07.203 and 09.520] range from 1 600 to 8 700 µg per person per day, which are above the threshold of concern for structural class II substances of 540 µg per person per day. The mTAMDI estimates for one substance from structural class II [FL-no: 07.255] and for the one candidate substance in class III [FL-no: 06.136] are 320 and 0.075 µg per person per day, respectively, which are below the thresholds of their structural classes (540 and 90 µg per person per day). For all substances with mTAMDI values below their structural class thresholds, the Panel noted that they have been evaluated using the A-side of the Procedure.

For one flavouring substance [FL-no: 09.929] from structural class I and one flavouring substance [FL-no: 07.219] from structural class II, use levels are missing and an mTAMDI cannot be calculated for these two substances.

In conclusion, for 11 candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], for which the mTAMDI is above the thresholds for their structural class, and for another two substances [FL-nos: 07.219 and 09.929], for which use levels are missing, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavouring is regulated under Regulation (EC) No 1334/2008⁴ of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of article 9(a) of this Regulation an evaluation and approval are required for flavouring substances.

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012.⁵ The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000.⁶

On 25 November 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 212, Revision 1 (FGE.212Rev1): α,β -unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19.⁷

The Panel concluded that the argumentation of Industry to expand its conclusion for the six-carbon ring members of subgroup 2.6 also to the cyclopentenyl derivatives in this subgroup [FL-no: 07.033, 07.094, 07.112 and 07.140] was considered too limited, given the lack of support from experimental data. Therefore, additional genotoxicity tests are still required for the representative substance [FL-no: 07.112] already chosen by the Panel. Alternatively, a more thorough explanation (physico-chemical parameters; experimental underpinning) of the proposed similar reactivity of six- and five-membered ring substances should be provided by Industry.

The requested data have been submitted by the applicant.

In addition, the flavouring substance [FL-no: 07.219], trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, was put in FGE.212 (former FGE.19, subgroup 2.6b: α,β -unsaturated aldehydes and ketones and precursors) because of its structure relationship with this group. Although the substance as such is not mentioned in the data submitted by the applicant, the submitted data are likely to be relevant for [FL-no: 07.219] as well.

Therefore, this request covers as well the re-evaluation of trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment of these flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.⁶

INTERPRETATION OF THE TERMS OF REFERENCE

As additional genotoxicity data have been submitted to support evaluation of trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219] with respect to genotoxic potential in FGE.212rev3. Subsequently, the European Commission requests EFSA to carry out a safety assessment in accordance with Commission Regulation (EC) No 1565/2000 for trans-3-methyl-2-(2-pentenyl)-2-

⁴ Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

⁵ EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

⁶ Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8–16.

⁷ EFSA Journal 2011;9(3):1923.

cyclopenten-1-one [FL-no: 07.219]. The Panel concluded that [FL-no: 07.219] does not give rise to concern with respect to genotoxicity and can accordingly now be evaluated through the Procedure in FGE.09Rev6.

ASSESSMENT

1. History of the evaluation of the substances in the present Flavouring Group Evaluation

The Flavouring Group Evaluation (FGE) 09, FGE.09, dealt with 10 candidate substances, nine secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols, and an ester of a phenol carboxylic acid and a secondary alicyclic alcohol.

The first revision of FGE.09, FGE.09Rev1, included the assessment of five additional flavouring substances [FL-nos: 06.136, 09.154, 09.520, 09.929 and 09.935]. No new toxicity or metabolism data were provided for four of the five substances. For one substance [FL-no: 09.520] acute and short-term toxicity data and *in vitro* and *in vivo* genotoxicity data were provided. Additional data on five substances [FL-nos: 02.075, 02.167, 09.355, 09.619 and 09.621] were made available since FGE.09 was published.

The second revision of FGE.09, FGE.09Rev2, included the assessment of one additional substance, carvyl-3-methylbutyrate [FL-no: 09.870]. No toxicity and/or metabolism data were provided for this substance. Carvyl-3-methylbutyrate has initially been considered in FGE.212 with respect to genotoxicity, together with other α,β -unsaturated substances from subgroup 2.6 of FGE.19, where the Panel concluded that “*d*-Carvone [FL-no: 07.146] was found genotoxic *in vitro*. However, *d*-carvone was not carcinogenic in mice. Therefore, the Panel concluded that this substance together with the structurally related *l*-carvone as well as carveol and the carvyl derivatives [FL-nos: 02.062, 07.147, 09.143, 09.215 and 09.870] could be evaluated through the Procedure”.

The third revision of FGE.09, FGE.09Rev3, included the assessment of one additional substance, L-menthyl (S)-3-hydroxybutyrate [FL-no: 09.949]. No toxicity and/or metabolism data were provided for this substance.

The fourth revision of FGE.09, FGE.09Rev4, included the assessment of four additional substances [FL-nos: 07.059, 07.202, 07.255 and 09.843]. Two of these substances [FL-nos: 07.202 and 07.255] are α,β -unsaturated ketones originally allocated to FGE.212Rev1. The two substances were considered with respect to genotoxicity (EFSA CEF Panel, 2011) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the two substances could be evaluated through the Procedure. No toxicity or metabolism data were provided for the new substances. Since the publication of FGE.09Rev3, one substance (former candidate substance [FL-no: 07.207]) is no longer supported for use as a flavouring substance in Europe by the flavour industry and will therefore not be considered any further (EFFA, 2009). Information from the previous version of FGE.09 on this substance is to be kept in the main text only if relevant for the remaining candidate substances.

The fifth revision of FGE.09, FGE.09Rev5, included the assessment of one additional substance, 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109]. This substance is an α,β -unsaturated ketone originally allocated to FGE.213. The substance was considered with respect to genotoxicity in FGE.213Rev1 (EFSA CEF Panel, 2014) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the substance could be evaluated through the Procedure. No toxicity or metabolism data were provided for the substance.

FGE	Opinion adopted by EFSA	Link	No of candidate substances
FGE.09	9 December 2004	http://www.efsa.eu.int/science/afc/afc_opinions/814_en.html	10
FGE.09Rev1	1 April 2008	http://www.efsa.europa.eu/en/efsajournal/doc/927.pdf	15
FGE.09Rev2	13 May 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1454.htm	16
FGE.09Rev3	28 September 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2396.htm	17
FGE.09Rev4	9 July 2012	http://www.efsa.europa.eu/en/efsajournal/pub/2836.htm	21

FGE	Opinion adopted by EFSA	Link	No of candidate substances
FGE.09Rev5	25 September 2014	http://www.efsa.europa.eu/en/efsajournal/doc/3865.pdf	21
FGE.09Rev6	9 September 2015	http://www.efsa.europa.eu/en/efsajournal/doc/4243.pdf	22

The present revision of FGE.09, FGE.09Rev6, includes the assessment of one additional substance [FL-no: 07.219]. This substance is an α,β -unsaturated ketone originally allocated to FGE.212. The substance has been considered with respect to genotoxicity in FGE.212Rev3 (EFSA CEF Panel, 2015) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the substance can be evaluated through the Procedure. No toxicity or metabolism data were provided for this candidate substance. A search in the open literature for the substance did not provide any further data on toxicity or metabolism.

2. Presentation of the substances in Flavouring Group Evaluation 09, Revision 6

2.1. Description

The present FGE (FGE.09Rev6), using the Procedure referred to in Commission Regulation (EC) No 1565/2000 (for a schematic form see Appendix A), deals with 10 secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and 11 esters containing secondary alicyclic alcohols. These 22 flavouring substances (candidate substances) belong to chemical groups 8, 25 and 30 of Annex I of Regulation (EC) No 1565/2000.

The candidate substances under consideration, with their chemical register names, FLAVIS (FL), Chemical Abstract Service (CAS), Council of Europe (CoE) and Flavor and Extract Manufacturers Association (FEMA) numbers, structure and specifications, are listed in Table 1.

A summary of the outcome of the safety evaluation of the candidate substances is listed in Table 6.

The hydrolysis products of the candidate substances and their evaluation status as flavouring substances are listed in Table 7.

The candidate substances are structurally related to 27 flavouring substances (supporting substances) evaluated at the 51st, 59th and 63rd meetings of the Joint (Food and Agriculture Organization of the United Nations (FAO)/(World Health Organization (WHO) Expert Committee on Food Additives (JECFA, 2000a, 2003, 2005a, b) in the groups “Substances structurally related to menthol”, “Carvone and structurally related substances”, “Alicyclic ketones, secondary alcohols and related esters” and “Monocyclic and bicyclic secondary alcohols, ketones and related esters” (JECFA, 1999a, 2003, 2006). In addition the racemate of menthyl-3-hydroxybutyrate has been evaluated by the JECFA at the 69th meeting (JECFA, 2009) in the group of “Substances structurally related to menthol”.

The names and structures for the 27 supporting substances are listed in Table 8, together with their evaluation status.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the FGE.09Rev6

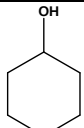
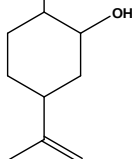
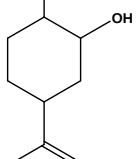
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.070	Cyclohexanol		2138 108-93-0	Solid C ₆ H ₁₂ O 100.16	Slightly soluble Freely soluble	158 25 MS 95 %	1.462–1.468 0.942–0.948	
02.075	(1R,2S,5S)-neo-Dihydrocarveol		2296 18675-33-7	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	107 (33 hPa) MS 95 %	1.476–1.482 0.920–0.926	
02.135	Cyclopentanol		10193 96-41-3	Liquid C ₅ H ₁₀ O 83.13	Slightly soluble Freely soluble	140 MS 95 %	1.449–1.455 0.945–0.951	
02.167	(1R,2R,5S)-Isodihydrocarveol		18675-35-9	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	90 (6.7 hPa) MS 95 %	1.475–1.481 0.918–0.924	

Table 1: Specification Summary of the Substances in the FGE.09Rev6

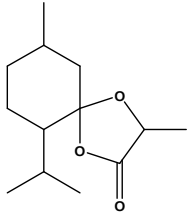
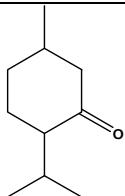
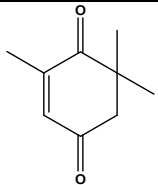
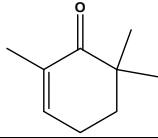
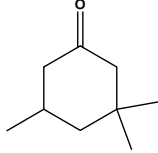
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		4285 831213-72-0	Liquid C ₁₃ H ₂₂ O ₃ 226.32	Slightly soluble Soluble	259 IR NMR MS 98.9 %	1.4606–1.4609 1.017–1.021	Mixture of isomers: (3 <i>S</i> , 5 <i>R</i> , 6 <i>S</i> ,9 <i>R</i>)-isomer: 65.6 % and (3 <i>S</i> , 5 <i>S</i> , 6 <i>S</i> ,9 <i>R</i>)-isomer: 27.4 %, mixture of other diastereomers: 5.86 % (Flavour Industry, 2006b)
07.059	<i>p</i> -Menthan-3-one		2667 2035 10458-14-7	Liquid C ₁₀ H ₁₈ O 154.25	Soluble Soluble	207 MS 96 %	1.448–1.453 0.888–0.895	Mixture of diastereomers, approximately 25 % of each (EFFA, 2012)
07.109 1857	2,6,6-Trimethylcyclohex-2-en-1,4-dione		3421 11200 1125-21-9	Solid C ₉ H ₁₂ O ₂ 152.2	Slightly soluble Soluble	222 23–28 IR NMR 98 %	n.a. n.a.	
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		20013-73-4	Liquid C ₉ H ₁₄ O 138.21	Slightly soluble Freely soluble	63 (16 hPa) MS 95 %	1.470–1.476 0.924–0.930	
07.203	3,3,5-Trimethylcyclohexan-1-one		873-94-9	Liquid C ₉ H ₁₆ O 140.22	Practically insoluble or insoluble Freely soluble	189 MS 95 %	1.442–1.448 0.888–0.894	Racemate (EFFA, 2010a, b)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

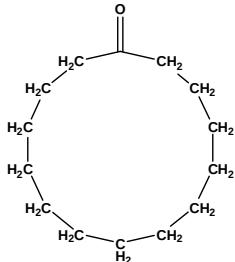
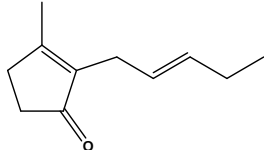
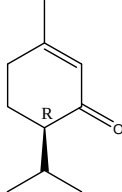
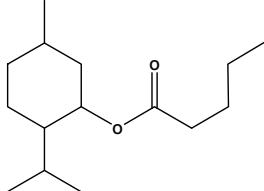
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
07.207	Cyclotetradecanone		3603-99-4	Solid C ₁₄ H ₂₆ O 210.36	Practically insoluble or insoluble Freely soluble	159 (16 hPa) 53 NMR 95 %	n.a. n.a.	No longer supported by the Flavour Industry (EFFA, 2009)
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		3196 11786 6261-18-3	Liquid C ₁₁ H ₁₆ O 164.25	Soluble Soluble	248 MS 98 %	1.495–1.501 0.942–0.948	
07.255 1856	l-Piperitone		4200 4573-50-6	Liquid C ₁₀ H ₁₆ O 152.24	Slightly soluble Freely soluble	246 MS 99 %	1.482–1.488 0.929–0.935	
09.154 1852	Menthyl valerate		4156 472 89-47-4	Liquid C ₁₅ H ₂₈ O ₂ 240.39	Practically insoluble or insoluble Freely soluble	261 NMR 95 %	1.445–1.451 0.903–0.909	Register name to be changed to (1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>)-5- methyl-2-(1- methylethyl)cyclo- hexyl valerate (EFFA, 2010a)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

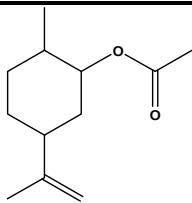
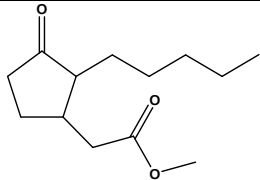
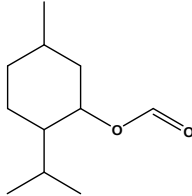
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
09.355	neo-Dihydrocarvyl acetate		10859 56422-50-5	Liquid C ₁₂ H ₂₀ O ₂ 196.29	Practically insoluble or insoluble Freely soluble	266 MS 95 %	1.453–1.459 0.925–0.931	According to EFFA: Mixture of the two racemic forms (1 <i>S</i> ,2 <i>R</i> ,5 <i>R</i>) and (1 <i>R</i> ,2 <i>S</i> ,5 <i>S</i>), which is specified by the name (EFFA, 2005)
09.520 1898	Methyl 3-oxo-2- pentyl-1- cyclopentylacetate		3408 10785 24851-98-7	Liquid C ₁₃ H ₂₂ O ₃ 226.32	Slightly soluble Freely soluble	111 (0.1 hPa) NMR MS 98 %	1.458–1.462 0.997–1.006	According to EFFA: Mixture of the four stereoisomeric forms (<i>RR</i> , <i>RS</i> , <i>SR</i> and <i>SS</i>) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a)
09.618	Menthyl formate		10751 2230-90-2	Liquid C ₁₁ H ₂₀ O ₂ 184.28	Practically insoluble or insoluble Freely soluble	95 (13 hPa) 9 MS 95 %	1.446–1.452 0.933–0.939	According to EFFA: Mixture of the two racemic forms (1 <i>S</i> ,2 <i>R</i> ,5 <i>S</i>) and (1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>), which is specified by the name (EFFA, 2010b)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

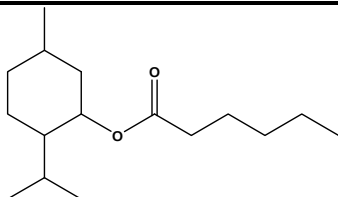
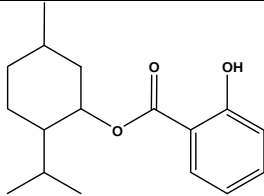
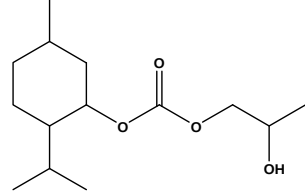
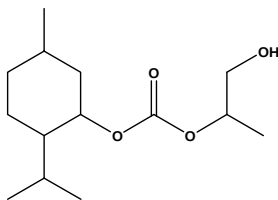
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
09.619	(1R,2S,5R)-Menthyl hexanoate		6070-16-2	Liquid C ₁₆ H ₃₀ O ₂ 254.14	Practically insoluble or insoluble Freely soluble	153 (20 hPa) MS 95 %	1.445–1.451 0.898–0.906	
09.621	(1R,2S,5R)-Menthyl salicylate		89-46-3	Liquid C ₁₇ H ₂₄ O ₃ 276.37	Practically insoluble or insoluble Freely soluble	175 (13 hPa) MS 95 %	1.509–1.515 1.047–1.053	
09.843	Menthol 1- and 2- propylene glycol carbonate	 + 	3806 30304-82-6	Liquid C ₁₄ H ₂₆ O ₄ 258.36	Insoluble Soluble	143 IR MS 98 %	1.458–1.458 1.013–1.014	According to EFFA: [FL-no: 09.843] is a mixture of 60 % menthol 1- propylene glycol carbonate (which is a mixture of four stereoisomers, 15 % of each) and 40 % menthol 2- propylene glycol carbonate (which is a mixture of four stereoisomers 10 % of each) (EFFA, 2012)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

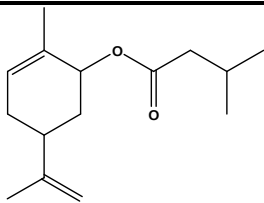
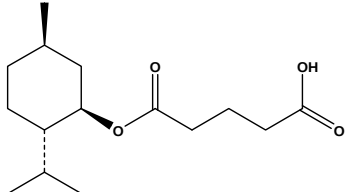
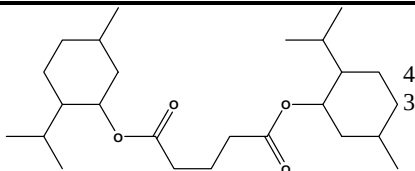
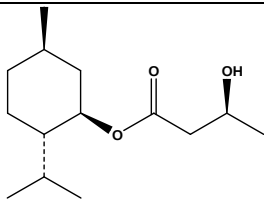
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
09.870	Carvyl-3-methylbutyrate		94386-39-7	Liquid C ₁₅ H ₂₄ O ₂ 236.37	Practically insoluble or insoluble Freely soluble	343 MS 95 %	1.462–1.468 0.932–0.938	According to EFFA: mixture of the four stereoisomeric forms (<i>RR</i> , <i>RS</i> , <i>SR</i> and <i>SS</i>) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a)
09.929	L-Monomenthyl glutarate		4006 220621-22- 7	Liquid C ₁₅ H ₂₆ O ₄ 270	Sparingly soluble Soluble	390 (decomp) n.a. IR NMR 95 %	1.462–1.470 1.026–1.036	

Table 1: Specification Summary of the Substances in the FGE.09Rev6

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
09.935	Dimenthyl glutarate		406179-71-3	Solid C ₂₅ H ₄₄ O ₄ 408	Insoluble Soluble	48–50 NMR MS 98 %	n.a. n.a.	According to EFFA: Menthyl moiety mixture of the two racemic forms (1 <i>S</i> ,2 <i>R</i> ,5 <i>S</i>) and (1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>), which is specified by the name. Since there are two menthyl moieties, three combinations exist, approximately 25 % (+/+), 25 % (–/–) and 50 % (+/–) (EFFA, 2010a)
09.949	L-Menthyl (<i>S</i>)-3-hydroxybutyrate		4308 115869-76-6	Liquid C ₁₄ H ₂₆ O ₃ 242.35	Slightly soluble Soluble	95–97 (0.7 hPa) IR MS 98 %	1.454–1.464 0.969–0.979	Stereoisomeric composition of (<i>S</i>)-form: > 80 % ee and (<i>R</i>)-form < 20 % ee (ee = enantiomeric excess)

ID, identity; IR, infrared spectroscopy; MS, mass spectrometry; n.a., not applicable; NMR, nuclear magnetic resonance.

(a): Solubility in water, if not otherwise stated.

(b): Solubility in 95 % ethanol, if not otherwise stated.

(c): At 1 013.25 hPa, if not otherwise stated.

(d): At 20 °C, if not otherwise stated.

(e): At 25 °C, if not otherwise stated.

2.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, and they may have different chemical properties, resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

Two candidate substances possess one chiral centre [FL-nos: 07.203 and 07.255] and 15 substances possess two or more chiral centres [FL-nos: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] (Table 1).

2.3. Natural occurrence in food

Fifteen candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618 and 09.619] have been reported to occur in fruits, spices, butter, chicken, wine, drinks, tea, juice and essential oils. Quantitative data on the natural occurrence of these substances in food have been reported for four substances (TNO, 2000, 2011, 2014, 2015) (Table 2).

Table 2: Candidate substances for which quantitative information on occurrence in food is available

FL-no	Name	Quantitative data reported
02.070	Cyclohexanol	Up to 0.1 mg/kg in fruits (passionfruit) and 0.006 mg/kg in white wine
02.135	Cyclopentanol	0.01–0.1 mg/kg in passiflora juice, 0.01–0.1 mg/kg in <i>Passiflora mollissima</i> , 0.01–0.02 mg/kg in oysters and 0.01 mg/kg in Chinese quince flesh
07.109	2,6,6-Trimethylcyclohex-2-en-1,4-dione	Up to 8 mg/kg in honey for consumption and up to 9 mg/kg in tea
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	2 000 mg/kg in maize, 1 mg/kg in tea and up to 0.23 mg/kg in citrus fruits

According to Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO) the remaining seven candidate substances have not been reported to occur naturally in any food items (TNO, 2000, 2010, 2011) (Table 3).

Table 3: Candidate substances not reported to occur naturally in food

FL-no	Name
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one
09.621	Menthyl salicylate
09.843	Menthol 1-and 2-propylene glycol carbonate
09.870	Carvyl-3-methylbutyrate
09.929	L-Monomenthyl glutarate
09.935	Dimenthyl glutarate
09.949	L-Menthyl (S)-3-hydroxybutyrate

3. Specifications

Purity criteria for the 22 candidate substances have been provided by the Flavour Industry (EFFA, 2003a, 2010a, 2011; Flavour Industry, 2004, 2006a, b, 2007, 2010a).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000, the specifications are adequate for all substances (see Section 2.2 and Table 1).

4. Intake data

Annual production volumes of the flavouring substances as surveyed by the Flavour Industry can be used to calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production figure represents only 60 % of the use in food because of underreporting and that 10 % of the total European Union (EU) population are consumers (SCF, 1999).

However, the Panel noted that because of year-to-year variability in production volumes, uncertainties in the underreporting correction factor and uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that, in contrast to the generally low per capita intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds, below which exposures are not considered to present a safety concern, might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the Scientific Committee on Food (SCF) recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

4.1. Estimated daily per capita intake (MSDI Approach)

The intake estimation is based on the MSDI approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavor Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population⁸ (Eurostat, 1998). This is derived for candidate substances from

⁸ EU figure: 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

estimates of annual volume of production provided by the Flavour Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Flavour Industry surveys (SCF, 1999).

In the present FGE.09Rev6, the total annual volume of production of the candidate substances from use as flavouring substances in Europe has been reported to be approximately 19 600 kg (EFFA, 2003a, b, 2011; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b). For 26 of the 27 supporting substances, for which production figures are available for Europe, the total annual volume of production is approximately 138 500 kg (JECFA, 1999a, 2003, 2006).

On the basis of the annual volumes of production reported for the candidate substances, the MSDI values for each of these flavourings have been estimated (Table 6).

Ninety-six per cent of the total annual volumes of production for the candidate substances is accounted for by four of these flavourings, *p*-menthan-3-one [FL-no: 07.059], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], menthol 1- and 2-propylene glycol carbonate [FL-no: 09.843] and *L*-monomenthyl glutarate [FL-no: 09.929]. The estimated daily intakes from use as flavouring substances are 530, 770, 830 and 110 µg per capita per day, respectively. The intakes for each of the remaining substances are 50 µg per capita per day or below (Table 6).

4.2. Intake estimated on the basis of the modified TAMDI (mTAMDI)

The method for calculation of the modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the candidate substances, information on food categories and normal and maximum use levels^{9,10,11} were submitted by the Flavour Industry (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b). For 20 candidate substances, the use in flavoured food products divided into the food categories as outlined in Annex III of the Commission Regulation (EC) No 1565/2000, is shown in Table 4. For trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219] and *L*-monomenthyl glutarate [FL-no: 09.929] the use levels have not been reported in accordance with the Commission Regulation.

For the present calculation of the mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 4: Use of 20 candidate substances for which data on use have been provided

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except one [FL-no: 07.059]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except four [FL-nos: 07.059, 07.255, 09.843 and 09.949]
03.0	Edible ices, including sherbet and sorbet	All

⁹ “Normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002).

¹⁰ The normal and maximum use levels in different food categories [Reg. (EC) No 1565/ 2000] have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

¹¹ The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007).

Table 4: Use of 20 candidate substances for which data on use have been provided

Food category	Description	Flavourings used
04.1	Processed fruits	All except four [FL-nos: 06.136, 07.059, 07.255 and 09.843]
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	Only one [FL-no: 09.935]
05.0	Confectionery	All
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery	All except four [FL-nos: 07.059, 07.109, 07.255 and 09.935]
07.0	Bakery wares	All
08.0	Meat and meat products, including poultry and game	All except five [FL-nos: 06.136, 07.059, 07.255, 09.843 and 09.949]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except five [FL-nos: 06.136, 07.059, 07.255, 09.843 and 09.949]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products, etc.	All except two [FL-nos: 06.136 and 07.059]
13.0	Foodstuffs intended for particular nutritional uses	All except five [FL-nos: 07.059, 07.255, 09.843, 09.935 and 09.949]
14.1	Non-alcoholic (“soft”) beverages, excluding dairy products	All except one [FL-no: 09.870]
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	All
15.0	Ready-to-eat savouries	All except two [FL-nos: 06.136 and 07.059]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 1–15	All except two [FL-nos: 07.059 and 09.843]

For the candidate substances for which the Flavour Industry has provided data on food categories, normal use levels are in the range of 0.0001–500 mg/kg food, and the maximum use levels are in the range of 0.0001–2000 mg/kg food (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b).

The mTAMDI values for 13 candidate substances from structural class I (see Section 7) for which exposure data have been submitted range from 420 to 63 000 µg per person per day. For the six candidate substances from structural class II, the mTAMDI values range from 320 to 8 700 µg per person per day. For the remaining substance from structural class III the mTAMDI is 0.075 µg per person per day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 7 and Appendix C.

5. Absorption, distribution, metabolism and elimination

The 11 esters [FL-nos: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the data available for the supporting substances (White et al., 1990; Emberger, 1994a, b). The resulting carboxylic acids are either metabolised through common physiological pathways, such as beta-oxidation and the citric acid cycle, or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994) (see Table 7 and Appendix D).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, *p*-menthan-3-one [FL-no: 07.059], and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-nos: 02.070, 02.075, 02.135, 02.167, 07.059, 07.109, 07.202, 07.203, 07.219 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, the hydrolysis products of 11 substances [FL-nos: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are also metabolised via this pathway. Menthol, carveol and dihydrocarveol are not anticipated to be oxidised to the corresponding ketone (for detailed discussion, see Appendix D).

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion (see Appendix D). Thus, it may be anticipated that these 22 substances will be metabolised to innocuous products.

A more detailed description of the metabolism is given in Appendix D.

6. Application of the procedure for the safety evaluation of flavouring substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment, using the mTAMDI approach, is not carried out using the Procedure. In these cases, the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 7.

For the safety evaluation of the candidate substances the Procedure was applied. The stepwise evaluations are summarised in Tables 5 and 6.

Step 1

Fourteen of the candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are classified into structural class I, seven candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219, 07.255 and 09.520] into structural class II and one substance [FL-no: 06.136] is classified into structural class III according to the decision tree approach presented by Cramer et al. (1978).

Step 2

Step 2 requires consideration of whether detoxification pathways are available to metabolise the substances, at the estimated levels of intake, to innocuous products.

All the candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are expected to be metabolised to innocuous products and, accordingly, they proceed via the A-side of the Procedure scheme (Section 5 and Appendix D).

Step A3

For the 14 candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] that have been assigned to structural class I, estimated European daily per capita intakes range from 0.0012 to 830 µg (Table 6). These intakes are below the threshold of concern of 1 800 µg per person per day for structural class I.

For the seven candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219, 07.255 and 09.520] assigned to structural class II, European daily per capita intakes range from 0.0085 to 770 µg. For six of these [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219 and 07.255] the intakes are below the threshold of concern of 540 µg per person per day for structural class II. For one substance [FL-no: 09.520] the daily per capita intake of 770 µg is above the threshold of concern for a substance assigned to structural class II. The substance therefore proceeds to step A4.

One candidate substance [FL-no: 06.136] has been assigned to structural class III and its European daily per capita intake is 1.2 µg. This intake is below the threshold of concern of 90 µg per person per day for structural class III.

Based on the results of the safety evaluation sequence, 21 candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] proceeding via the A-side of the Procedure do not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substances.

Step A4

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] and its metabolites are not endogenous; therefore, this substance proceeds to step A5.

Step A5

A 90-day study in rats has been performed for one substance [FL-no: 09.520] from which a No Observed Adverse Effect Level (NOAEL) of 100 mg/kg body weight (bw) per day could be derived. This NOAEL provides a margin of safety of 7 700 compared with the daily intake of 0.013 mg/kg bw per day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Therefore, this substance [FL-no: 09.520] does not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substance.

7. Comparison of the intake estimations based on the MSDI and the mTAMDI approach

The estimated intakes, based on the mTAMDI, range from 420 to 63 000 µg per person per day for 13 candidate substances in structural class I. For seven of these substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870] the mTAMDI is below the threshold of concern of 1 800 µg per person per day and for six substances [FL-nos: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] the mTAMDI is above the threshold. For L-monomenthyl glutarate [FL-no: 09.929] no mTAMDI could be calculated due to lack of information on use levels in the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For six substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.255 and 09.520] assigned to structural class II, the estimated intakes, based on the mTAMDI, range from 320 to 8 700 µg per person per day, which are all above the threshold of concern for structural class II substances of 540 µg per person per day, except for one substance [FL-no: 07.255]. For the seventh substance assigned to class II, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], no mTAMDI could be calculated due to lack of information on use levels in the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For one substance [FL-no: 06.136], assigned to structural class III, the estimated intake based on the mTAMDI is 0.075 µg per person per day, which is below the threshold of concern for a structural class II substance of 90 µg per person per day.

Thus, for 10 candidate substances [FL-nos: 07.059, 07.202, 07.203, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219] and L-monomenthyl glutarate [FL-no: 09.929] use levels are needed in accordance with the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 5.

Table 5: Estimated Intakes based on the MSDI Approach and the mTAMDI Approach

FL-no	EU Register name	MSDI (µg per capita per day)	mTAMDI (µg per person per day)	Structural class	Threshold of concern (µg per person per day)
02.070	Cyclohexanol	3.7	1 600	Class I	1 800
02.075	(1R,2S,5S)-neo-Dihydrocarveol	2.4	1 600	Class I	1 800
02.135	Cyclopentanol	0.012	1 600	Class I	1 800
02.167	(1R,2R,5S)-Isodihydrocarveol	2.4	1 600	Class I	1 800
09.154	Menthyl valerate	1	3 900	Class I	1 800
09.355	neo-Dihydrocarvyl acetate	0.012	1 600	Class I	1 800
09.618	Menthyl formate	0.73	3 900	Class I	1 800
09.619	(1R,2S,5R)-Menthyl hexanoate	0.37	3 900	Class I	1 800
09.621	(1R,2S,5R)-Menthyl salicylate	0.012	420	Class I	1 800
09.843	Menthol 1-and 2-propylene glycol carbonate	830	63 000	Class I	1 800
09.870	Carvyl-3-methylbutyrate	0.0012	1 000	Class I	1 800
09.929	L-Monomenthyl glutarate	110		Class I	1 800
09.935	Dimenthyl glutarate	30	38 000	Class I	1 800
09.949	L-Menthyl (S)-3-hydroxybutyrate	37	10 600	Class I	1 800
07.059	<i>p</i> -Menthan-3-one	530	8 700	Class II	540
07.109	2,6,6-Trimethylcyclohex-2-en-1,4-dione	50	1 900	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	0.12	1 600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	0.0085	1 600	Class II	540
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	4.7		Class II	540
07.255	l-Piperitone	12	320	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate	770	3 900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one	1.2	0.075	Class III	90

8. Considerations of combined intakes from use as flavouring substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be

considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. At present, the combined intake estimates are based only on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily per capita intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003a, b, 2011; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b), the combined estimated daily per capita intake as flavourings of the 14 candidate substances assigned to structural class I is 1 000 µg, which does not exceed the threshold of concern of 1 800 µg per person per day.

The candidate substances from structural class I are structurally related to 15 supporting substances for which European intake data are available (European intake data are available for only 15 of the 16 supporting substances from structural class I). The total combined intake of the 14 candidate and 15 supporting substances is approximately 17 000 µg per capita per day, which is above the threshold for structural class I substances of 1 800 µg per person per day. The major contribution (92 %) is provided by one supporting substance, menthol [FL-no: 02.015] (16 mg per capita per day), for which an Acceptable Daily Intake (ADI) of 0–4 mg/kg bw was allocated by the JECFA at its 51st meeting (JECFA, 2000a). The ADI is 15 times higher than the MSDI of 16 mg per capita per day. The total combined intake for the remaining substances from structural class I is approximately 1 400 µg per capita per day, which is below the threshold of 1 800 µg per person per day.

On the basis of the reported annual production volumes in Europe, the combined estimated intake as flavourings of the candidate substances assigned to structural class II is 1 300 µg per capita per day, which exceeds the threshold of concern for a compound belonging to structural class II of 540 µg per person per day. A combined intake of 1 300 µg per capita per day corresponds to 22 µg/kg bw per day. A NOAEL of 100 mg/kg bw per day has been established for one candidate substance in this group, namely methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]. This NOAEL provides a margin of safety of more than 4 500 for the combined intake of 1 300 µg per capita per day. The Panel noted that this candidate substance [FL-no: 09.520] accounts for 60 % of this combined intake estimate.

The total combined intake from the seven candidate and 15 supporting substances from structural class II is approximately 2 200 µg per capita per day, which exceeds the threshold for structural class II substances of 540 µg per person per day. Also in the case of the total combined intake for structural class II substances, the NOAEL of 100 mg/kg bw per day, for methyl 3-oxo-2-pentyl-1-cyclopentylacetate, provides a margin of safety of 2 700 to the combined intake of 2 200 µg per capita per day, corresponding to 37 µg/kg bw per day.

The daily per capita intake of the only candidate substance from structural class III, 6-isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [FL-no: 06.136], is 1.2 µg, which does not exceed the threshold of 90 µg per person per day. There are no supporting substances from structural class III.

9. Toxicity

9.1. Acute toxicity

Data are available for five candidate substances [FL-nos: 02.070, 02.135, 06.136, 09.520 and 09.355]. Oral median Lethal Dose (LD₅₀) values from studies in the rat range from 625 mg/kg bw to > 5 000 mg/kg bw.

Ten supporting substances [FL-nos: 02.015, 02.061, 02.062, 02.209, 07.111, 07.148, 07.176, 09.016, 09.215 and 09.216] were tested for acute toxicity in the mouse, rat, rabbit, dog and guinea pig. The LD₅₀ values ranged from 930 mg/kg bw to > 5 000 mg/kg bw.

The magnitudes of the LD₅₀ values indicate that the oral acute toxicity is rather low for the candidate substances and supporting substances.

The acute toxicity data are summarised in Table 14.

9.2. Subacute, subchronic, chronic and carcinogenicity studies

Only one conventional subchronic oral study has been conducted on one candidate substance [FL-no: 09.520]. For cyclohexanol [FL-no: 02.070] data were available from a study designed to investigate only peripheral neuropathy in which rats were given intraperitoneal doses of 200 mg cyclohexanol once or twice daily for up to six weeks. No effects on the peripheral nervous system were observed but the experiment was terminated early because the animals were in poor condition, there was a decrement of weight gain and two animals died prematurely. No general gross or histopathological examinations were reported and no NOAEL was established. This study was not considered applicable to the evaluation of the oral toxicity of cyclohexanol (Perbellini et al., 1981).

For methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] a study, following the current Organisation for Economic Co-operation and Development (OECD) guidelines, was performed in rats. Male and female Sprague–Dawley CD rats were given, in their diets, daily doses of 0 (basal diet), 10, 50 or 100 mg/kg bw of methyl 3-oxo-2-pentyl-1-cyclopentylacetate over a 90-day period (10 males and 10 females per dose group). No treatment-related changes were observed in mortality, expanded clinical observations, ophthalmic examination, body weight gain, body weight change, food consumption, haematology, clinical chemistry, urinalysis, organ weights and macroscopic examination. There were no treatment-related histopathological changes in the tissues from rats of any of the treatment groups. The NOAEL was therefore considered to be 100 mg/kg bw per day (the highest dose tested) (Kelly and Bolte, 2000).

Carcinogenicity studies are available for the two supporting substances, cyclohexanone and menthol [FL-nos: 07.148 and 02.015].

Cyclohexanone [FL-no: 07.148] was given to male and female rats in drinking water (doses of 3 300 and 6 500 mg/kg) and male and female mice [doses of 6 500, 13 000 and 25 000 mg/kg (only female)] for two years. A reduction in weight gain (15–20 %) was observed in all groups at the highest doses. An increase in the incidence of lymphomas was observed at a lower dose level, but it was not dose related (Lijinsky and Kovatch, 1986).

In two other studies, two doses of DL-menthol were given to rats in the diet (3 750 and 7 500 mg/kg) and mice (2 000 and 4 000 mg/kg) for 103 weeks. A small reduction in survival was seen in the treated female mice. An increase of incidence of mammary gland fibroadenomas or mammary adenocarcinomas was observed in female rats at the lower dose level, but this was not dose related. In male rats, dl-menthol was not considered toxic or carcinogenic. In mice, a small increase in the incidence of hepatocellular carcinomas was observed. However, this increase was within the normal range of tumour incidence in the historical control groups, and the authors concluded that dl-menthol was not carcinogenic in rats and mice in the performed studies (National Cancer Institute, 1979).

For five supporting substances [FL-nos: 02.015, 07.095, 07.111, 07.176 and 07.148] there are further oral subchronic toxicity data.

Repeated-dose toxicity data are summarised in Table 15.

9.3. Developmental/reproductive toxicity studies

There is a study available for one candidate substance [FL-no: 02.070], with a NOAEL of < 1 500 mg/kg bw per day. For one supporting substance [FL-no: 02.015] there are several studies.

The developmental/reproductive toxicity study is summarised in Table 16.

9.4. Genotoxicity studies

Owing to the presence of a structural alert for genotoxicity (“ α,β -unsaturated carbonyl moiety”) for three candidate substances [FL-nos: 07.202, 07.255 and 09.870] in the current revision of FGE.09, the genotoxicity of these substances was further assessed in FGE.212 and FGE.212Rev1. In FGE.212 (EFSA, 2009) the concern for carvyl-3-methylbutyrate [FL-no: 09.870] was alleviated and the Panel concluded that this substance could be evaluated through the Procedure. Since it was concluded in FGE.212Rev1 (EFSA CEF Panel, 2011) that, based on additional information, the concern for genotoxic potential for isophorone [FL-no: 07.126] has been alleviated, a genotoxic potential can also be ruled out for substances structurally related to isophorone (including [FL-nos: 07.202 and 07.255]). Therefore, these two substances [FL-nos: 07.202 and 07.255] can be evaluated using the Procedure.

In FGE.212Rev3 (EFSA CEF Panel, 2015) it was concluded, that, based on additional information on the supporting substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112], the concern for genotoxicity for the candidate substance [FL-no: 07.219] could be ruled out. Therefore, this candidate substance can be evaluated using the Procedure.

Owing to the presence of a structural alert for genotoxicity (“ α,β -unsaturated ketone”) for the candidate substance 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109], the genotoxicity of this substance was assessed in FGE.213 and FGE.213Rev1. In FGE.213Rev1 the concern was alleviated and the Panel concluded that this substance could be evaluated through the Procedure (EFSA CEF Panel, 2014).

Genotoxicity data are available for only three of the remaining candidate substances—cyclohexanol [FL-no: 02.070], cyclopentanol [FL-no: 02.135], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]—and for nine supporting substances and one structurally related substance.

Cyclohexanol [FL-no: 02.070] was not genotoxic in two Ames tests (Barsky, 1976; Haworth et al., 1983) and in an *in vivo* micronucleus assay (Gelbke, 1991), which are all considered valid studies. However, the results of the *in vivo* study are of limited relevance, because of the lack of evidence that the substance did reach the bone marrow. Inconclusive results were reported in an *in vitro* chromosomal aberration assay with human leucocytes (Collin, 1971) and negative results were reported in a dominant lethal mutations assay with *Drosophila melanogaster* (Goncharova, 1970); both studies were considered inadequate.

Cyclopentanol [FL-no: 02.135] was studied in a valid Ames test (McMahon et al., 1979). No mutagenicity was found.

A battery of *in vitro* and *in vivo* genotoxicity studies were conducted on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] including valid negative reverse mutation tests in *E. coli* (Wagner and Klug, 2000) and *S. typhimurium* (Thompson, 2000).

In a mouse lymphoma test on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], pre-dating Good Laboratory Practice (GLP), a more than twofold increase in the mutant frequency over the solvent-treated control values was found at the highest tested cytotoxic concentration of 300 $\mu\text{g/ml}$ in the presence of metabolic activation, and at the two highest tested cytotoxic concentrations of 200 and 300 $\mu\text{g/ml}$ in the absence of metabolic activation. Only limited documentation is provided in the study report; together with the fact that several cultures were infected and a lack of a confirmatory test, it is impossible to assess the reliability of these results (Ross and Harris, 1979).

No induction of forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells were found in a study performed in compliance with the current OECD test guidelines, both in the absence and in the presence of metabolic activation, up to and including cytotoxic concentrations (Cifone, 2001).

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was tested in a bone marrow micronucleus test in mice following a single intraperitoneal administration of 0, 280, 560 or 1 120 mg/kg bw in corn oil. The study was performed in compliance with the current OECD test guidelines. The two highest doses chosen induced clear signs of toxicity; slight reductions (up to 12 %) in the ratio of polychromatic erythrocytes to total erythrocytes were found, indicating that the test material had reached the target cells. No increase in micronucleated cells was found in the groups treated with the test material. The positive control induced the expected increases (Gudi and Krsmanovic, 1998).

In an Unscheduled DNA Synthesis (UDS) study, the ability of methyl 3-oxo-2-pentyl-1-cyclopentylacetate to induce DNA repair was studied in isolated rat hepatocytes after administration *in vivo*. The study was performed in compliance with the current OECD Guideline 486 (OECD, 1997c). Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was administered to male Sprague–Dawley CD rats by intraperitoneal injection in doses of 333.3 and 1 000 mg/kg bw (the latter dose was the maximum tolerated dose) followed by liver perfusion at 2 or 16 hours after dosing. No marked increase in the incidence of UDS was observed at either dose level or perfusion time. Statistically significant differences were revealed in the positive control groups when compared with the negative control group and the test article (Durward, 2001).

Genotoxicity data are available for nine supporting substances [FL-nos: 02.015, 02.062, 07.045, 07.148, 07.149, 07.176, 09.027, 09.215 and 09.230].

Cyclohexanone [FL-no: 07.148], structurally related to the alicyclic ketones and secondary alcohols in this FGE, was not mutagenic in an Ames test, which was considered to be valid (Haworth et al., 1983). Negative and positive results were reported in several other *in vitro* studies at gene and chromosomal level, as well as a negative result in a sex-linked recessive lethal mutations in *D. melanogaster*. However, these studies were considered inadequate.

Menthol [FL-no: 02.015] gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes (Storer et al., 1996). With the same substance equivocal results in an *in vivo* host-mediated mutation assay were observed at high-dose levels (Food and Drug Research Laboratories, Inc., 1975) and negative results in several Ames tests, a TK+/- mouse lymphoma assay (Myhr and Caspary, 1991), sister chromatid exchange (SCE) tests in Chinese hamster ovary (CHO) cells (Ivett et al., 1989) and human lymphocytes (Murthy et al., 1991), and chromosomal aberration assays with human embryonic lung cells (Food and Drug Research Laboratories, Inc., 1975), human lymphocytes (Murthy et al., 1991) and CHO cells (Ivett et al., 1989). Negative results were also reported in two *in vivo* micronucleus (Shelby et al., 1993) and chromosomal aberration assays (Food and Drug Research Laboratories, Inc., 1975). However, the results of these studies have a limited relevance, owing to the lack of bone marrow toxicity. In addition, an *in vivo* dominant lethal assay was available, from which negative results were also obtained.

trans-Menthone [FL-no: 07.176] was genotoxic in an Ames test (Andersen and Jensen, 1984) and in a somatic mutation and recombination test (SMART) with *D. melanogaster* (Franzios et al., 1997). The observed effects were not very pronounced. Further, *trans*-menthone is easily converted to menthol, which is estimated to be, overall, negative in genotoxicity tests.

Carveol and carvyl acetate [FL-nos: 02.062 and 09.215] were tested in an Ames test at various doses from 10 to 560 µg/plate in the *S. typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without S9 mix in dimethyl sulphoxide. Positive and negative controls were used. No mutagenicity was observed (Mortelmans et al., 1986).

Conclusion on genotoxicity

For five of the candidate substances [FL-nos: 07.109, 07.202, 07.219, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances.

Some genotoxicity data are available for only three of the remaining candidate substances, and for these three mainly negative results were obtained. For the supporting substances, mainly negative, but also some positive results were obtained. The positive results were obtained from poorly reported tests, or tests that are difficult to interpret with respect to their relevance for genotoxicity.

Overall, the genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

Data on genotoxicity are summarised in Tables 17 (*in vitro*) and 18 (*in vivo*).

CONCLUSIONS

The present revision of FGE.09, FGE.09Rev6, includes the assessment of one additional candidate substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], which was not included in FGE.09Rev5.

FGE.09Rev6 deals with 22 candidate substances; secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols. These flavouring substances belong to chemical groups 8, 25 and 30 of Annex I of Regulation (EC) No 1565/2000.

Two candidate substances [FL-nos: 07.203 and 07.255] possess one chiral centre and 15 substances [FL-nos: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] possess two or more chiral centres.

Fourteen candidate substances belong to structural class I, seven substances belong to structural class II and one to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Fifteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, intakes in Europe of the 14 flavouring substances belonging to structural class I range from 0.0012 to 830 µg per capita per day, intakes of the seven substances from structural class II range from 0.0085 to 530 µg per capita per day and intake of the substance from structural class III is 1.2 µg per capita per day, which are all below the threshold of concern values for structural classes I, II or III of 1 800, 540 or 90 µg per person per day, respectively. For one substance [FL-no: 09.520] from structural class II, the MSDI is 770 µg per capita per day, which is above the threshold of concern of 540 µg per person per day. For this substance a NOAEL is available, providing a sufficient margin of safety based on the MSDI approach.

The total combined intakes of candidate and supporting substances from structural classes I and II do not give rise to a safety concern.

For five candidate substances [FL-nos: 07.109, 07.202, 07.219, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances. Genotoxicity data are available for only a limited number of the remaining flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

All 22 candidate substances are expected to be metabolised to innocuous products at the estimated levels of use as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that the 22 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances based on the default MSDI approach.

In order to determine whether the conclusion for the 22 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications, including complete purity criteria and information on identity for the materials of commerce, have been provided for all flavouring substances.

Thus, for 22 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949].

The estimated intakes for 13 candidate substances in structural class I, based on the mTAMDI approach, ranged from 420 to 63 000 µg per person per day. For six substances [FL-nos: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949], the mTAMDI is above the threshold of concern of 1 800 µg per person per day. For seven substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold. The mTAMDI of five substances assigned to structural class II range from 1 600 to 8 700 µg per person per day, which are above the threshold of concern for structural class II substances of 540 µg per person per day. The mTAMDI estimates for one substance from structural class II [FL-no: 07.255] and for the one candidate substance in class III [FL-no: 06.136] are 320 and 0.075 µg per person per day, respectively, which are below the thresholds of their structural classes (540 and 90 µg per person per day). For all substances with mTAMDI values below their structural class thresholds, the Panel noted that they have been evaluated via the A-side of the Procedure.

For one flavouring substance [FL-no: 09.929] from structural class I and one flavouring substance [FL-no: 07.219] from structural class II, use levels are missing and an mTAMDI cannot be calculated for these two substances.

In conclusion, for 11 candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], for which the mTAMDI is above the thresholds for their structural class, and for another two substances [FL-nos: 07.219 and 09.929], for which use levels are missing, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

DOCUMENTATION PROVIDED TO EFSA

1. Barsky FC, 1976. *In vitro* microbial mutagenicity studies of cyclohexanol, with cover letter dated 10/3/1995. Cyclohexanol. E. I. Dupont De Nemour & Co. Lab. no. 9822, study no. 755-75. EPA Doc 86960000143S, microfiche no. OTS0558283. January 7, 1977. Unpublished report submitted by EFFA to FLAVIS Secretariat.
2. Birch MD, Evans MJ, Birch RE and Woods WR, 1981. Initial submission: Toxicological investigation of: RAKA with cover letter dated 081392. Cyclohexanol–cyclohexanone mixture. Monsanto Co. Project no. Y0-81-049. EPA Doc 88-920007866, microfiche no. OTS0546011. July 7, 1981. Unpublished data submitted by EFFA to SCF.
3. Birch MD, 1978. Toxicological investigation of: crude cyclohexanol with cover letter dated 09/29/95. Cyclohexanol. Monsanto Co. Project no. Y-78-73. EPA Doc 869600000003, microfiche no. OTS0572833. June 28, 1978. Unpublished data submitted by EFFA to FLAVIS Secretariat.
4. Cifone MA, 2001. ST 08 C 99: L5178Y TK +/- mouse lymphoma forward mutation assay with a confirmatory assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Covance Laboratories Inc, Vienna, Virginia. Study no. 21997-0-431 ICH. February 27, 2001. Unpublished report submitted by EFFA to FLAVIS Secretariat.
5. Durward R, 2001. ST 41 C 00: *In vivo* Liver Unscheduled DNA Synthesis (UDS) assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Safepharm Laboratories Limited, Derby, U.K. Project no. 161/266. 08 August 2001. Unpublished report submitted by EFFA to the FLAVIS Secretariat.
6. EFFA (European Flavour and Fragrance Association), 2002. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
7. EFFA (European Flavour and Fragrance Association), 2003a. Submission 2002-2. Flavouring group evaluation of 14 flavouring substances (candidate chemicals) of the chemical group 8 (Annex I of 1565/2000/EC), structurally related to menthol, carvone and ionones [FAO/WHO JECFA 42/51], and alicyclic ketones, secondary alcohols and related esters [considered during the 59th meeting of JECFA] used as flavouring substances 31. December 2002. SCOOP/FLAV/8.18.
8. EFFA (European Flavour and Fragrance Association), 2003b. Submission 2002-2. Flavouring group evaluation of 14 flavouring substances (candidate chemicals) of the chemical group 8 (Annex I of 1565/2000/EC), structurally related to menthol, carvone and ionones [FAO/WHO JECFA 42/51], and alicyclic ketones, secondary alcohols and related esters [considered during the 59th meeting of JECFA] used as flavouring substances 31. December 2002. SCOOP/FLAV/8.18. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Unpublished report submitted by EFFA to SCF.
9. EFFA (European Flavour and Fragrance Association), 2004. Intake – Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.
10. EFFA (European Flavour and Fragrance Association), 2005. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
11. EFFA (European Flavour and Fragrance Association), 2007. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages. FLAVIS/8.70.

12. EFFA (European Flavour Association), 2009. Supplement list of EU-only Footnote-10 materials for Commission. Unpublished communication submitted by EFFA to the FLAVIS secretariat. 14 December 2009.
13. EFFA (European Flavour Association), 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
14. EFFA (European Flavour Association), 2010b. E-mail from EFFA to EFSA/CEF Secretariat, dated 22 June 2012. Information on chirality of two substances evaluated in FGE.09 [FL-no: 07.203 and 09.618]. FLAVIS/8.166.
15. EFFA (European Flavour Association), 2011. Specifications and poundage data for 42 Register substances submitted by EFFA/Industry to FLAVIS Secretariat. August 2011. FLAVIS/8.124.
16. EFFA (European Flavour Association), 2012. Private Communication forwarded to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark. Dated 17 January 2012, 14, 23 and 24 February 2012 and 19 March 2012. Specification data related to substances in FGE.29.Rev1: [FL-no: 01.015]; FGE.09Rev4 [FL-no: 07.059, 09.843 and 09.920] and FGE.51Rev1 [FL-no: 07.034, 07.035, 07.095, 07.129, 07.172, 07.257 and 09.930]. FLAVIS/8.143.
17. Emberger D, 1994a. *In vitro* hydrolysis test. Menthyl glycarbonate (MGC). Flavor and Extract Manufacturers' Association of the United States. Unpublished data submitted by EFFA to FLAVIS Secretariat.
18. Emberger D, 1994b. *In vitro* hydrolysis test. Menthyl propyleneglycol carbonate (MPC). Flavor and Extract Manufacturers' Association of the United States. Unpublished data submitted by EFFA to FLAVIS Secretariat.
19. Flavour Industry, 2004. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09Rev1.
20. Flavour Industry, 2006a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09.
21. Flavour Industry, 2006b. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09Rev1.
22. Flavour Industry, 2007. Addendum of one Flavouring Substances (candidate chemicals) to the Flavouring Group Evaluation of Chemical Group 8 (Annex I of 1565/2000/EC), structurally related to menthol, carvone and ionones [FAO/WHO JECFA 42/51], and alicyclic ketones, secondary alcohols and related esters [considered during the 59th meeting of JECFA] used as flavouring substances. Addendum to FGE.09 (EFFA submission 2002-2). 10 January 2007. Unpublished data submitted by Flavour Industry to FLAVIS Secretariat. A-09Rev4.
23. Flavour Industry, 2010a. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-09Rev3 [FL-no: 09.949].
24. Flavour Industry, 2010b. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-09Rev3 [FL-no: 06.136].
25. Food and Drug Research Laboratories, Inc., 1973. Teratologic evaluation of FDA 71-57 (menthol natural, Brazilian). Food and Drug Research Laboratories, Inc. Morgareidge K Lab. no. 1573k, study no. FDABF-GRAS-134, June 1, 1973. Unpublished report submitted by EFFA to FLAVIS Secretariat.

26. Food and Drug Research Laboratories, Inc., 1975. Mutagenic evaluation of compound FDA 71-57, menthol. Litton Bionetics, Inc. Weir, R.J. January 14, 1975. Unpublished report submitted by EFFA to FLAVIS Secretariat.
27. Gelbke H-P, 1991. Cytogenetic study *in vivo* of cyclohexanol in mice: Micronucleus test: Single oral administration of cyclohexanol, with cover letter dated 9/11/95. BASF Abteilung Toxikologie. Engelhardt, G. Project no. 26H0843/894490. EPA Doc 86950000355, microfiche no. OTS0557795. September 11, 1995. Unpublished report submitted by EFFA to SCF.
28. Gudi R and Krsmanovic L, 1998. Mammalian erythrocyte micronucleus test. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. MA BioServices, Inc, Rockville, MD. Lab no. G98AN94.123. August 4, 1998. Unpublished report submitted by EFFA to FLAVIS Secretariat.
29. Hummler, 1969. Private communication. Submitted to WHO by Flavor and Extract Manufacturers' Association of the United States.
30. IOFI (International Organization of the Flavor Industry), 1995. European inquiry on volume of use.
31. Keating JW, 1972. Acute oral toxicity (rat-5 gms/kg body weight dose). Dermal toxicity (rabbit-5 gms/kg body weight dose). Amyris acetylated, Bois de rose acetylated, Cadinene, Castoreum, Lavandin acetylated, Dihydrojasnone, Trans-2-hexenol, Methyl isoeugenol, Methyl eugenol, Santalyl acetate, Phenyl propyl cinnamate, Phenylacetic acid, 1-Carveol, Santatol, Methyl heptenone. Biological Science Laboratories. Unpublished report submitted by EFFA to SCF.
32. Kelly CM and Bolte HF, 2000. A 3-month dietary toxicity study in rats. Final report. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Huntingdon Life Sciences, East Milestone, New Jersey. Project no. 99-2643. 15 December 2000. Unpublished report submitted by EFFA to FLAVIS Secretariat.
33. Levenstein I, 1973a. To determine the oral LD50, in fasted rats, of the test material as submitted. Menthone racemic pure. Leberco Laboratories, Inc. Assay no. 30969. January 10, 1973. Unpublished report submitted by EFFA to FLAVIS Secretariat.
34. Levenstein I, 1973b. To determine the oral LD50, in fasted rats, of the test material as submitted. Menthyl acetate racemic. Leberco Laboratories, Inc. Assay no. 30970. January 10, 1973. Unpublished report submitted by EFFA to SCF.
35. Levenstein I, 1976. Acute oral toxicity (rats 5 gms./kg body weight dose). Dermal toxicity (rabbits 5 gms./kg. body weight dose). 1-Carvyl acetate. Leberco Laboratories, Inc. Assay no. 62970. August 18, 1976. Unpublished report submitted by EFFA to FLAVIS Secretariat.
36. Miller L and Sherman H, 1965. Oral LD50 test of cyclohexanol with cover letter dated 10/3/95. p-tert-butylpyrocatechol. Haskell Laboratory. Report no. 103-65. EPA Doc 86960000139S, microfiche no. OTS0558279. July 28, 1965. Unpublished report submitted by EFFA to FLAVIS Secretariat.
37. Moreno OM, 1977. Acute toxicity study in rats. Dermal toxicity in rabbits. Dihydro carveol. MB Research Laboratories, Inc. Project no. MB 77-1748. August 22, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

38. Moreno OM, 1980. Oral toxicity in rats. Dermal toxicity in rabbits. Dihydro carvyl acetate. Project no. MB 80-4888, date 12/02/80. Test for oral toxicity in rats. Dihydro carvyl acetate, project no. MB 80-4888A, date 9/05/80. Test for acute dermal toxicity in rabbits. Dihydro carvyl acetate, project no. MB 80-4888B, date 10/08/80. MB Research Laboratories, Inc. Study director: Cerven, D.R. Unpublished data submitted by EFA to FLAVIS Secretariat.
39. Morimoto T, 2005. Bacterial reverse mutation study of menthyl 3-hydroxybutyrate. Study No. 235. February 21, 2005. Private communication to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavour Industry, Brussels, Belgium.
40. Myers RC, Homan ER, Weil CS and Frank FR, 1980. Initial submission: Cyclopentanol: Range finding toxicity studies (final report) with attachment and cover letter dated 121091. Bushy Run Research CTR. Kuryla, W.C. EPA Doc 88-920000476, microfiche no. OTS0534929. February 2, 1980. Unpublished report submitted by EFA to FLAVIS Secretariat.
41. Ross C and Harris WJ, 1979. Testing of compound 0478/5 in the mouse lymphoma specific locus mutation assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Inveresk Research International, Edinburgh, Scotland. Project no. 410917. October 1979. Unpublished report submitted by EFA to FLAVIS Secretariat.
42. Smyth HF Jr, Carpenter CP, Shaffer CB and Weil CS, 1946. Letter from Union Carbide Corp to USEPA regarding toxicology studies of cyclohexanol, with attachments dated 08/25/95. Cyclohexanol. EPA Doc 86950000304, microfiche no. OTS0557744. August 25, 1995. Unpublished report submitted by EFA to FLAVIS Secretariat.
43. Thompson PW, 2000. ST 41 C 00: Reverse mutation assay "Ames test" using *Salmonella typhimurium*. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Safepharm Laboratories Limited, Derby, U.K. Project no. 161/265. 11 October 2000. Unpublished report submitted by EFA to FLAVIS Secretariat.
44. Wagner VO and Klug ML, 2000. ST 08 C 99: Bacterial reverse mutation assay. Methyl 3-oxo-2-pentyl-1-cyclopentylacetate. BioReliance, Rockville, MD. Study no. AA31NK.502.BTL. August 28, 2000. Unpublished report submitted by EFA to FLAVIS Secretariat.

REFERENCES

- Aaron CS, Brewen JG, Stetka DG, Bleicher WT and Spahn MC, 1985. Comparative mutagenesis in mammalian cells (CHO) in culture: multiple genetic endpoint analysis of cyclohexanone *in vitro*. *Environmental Mutagenesis*, 7 (Suppl. 3), 60–61.
- Anders MW, 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: *Intermediary Xenobiotic Metabolism in Animals*. Eds Hutson DH, Caldwell J and Paulson GD. Taylor and Francis, New York, NY, USA, 81–97.
- Andersen PH and Jensen NJ, 1984. Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. *Mutation Research*, 138, 17–20.
- Bär F and Griepentrog F, 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. [Where we stand concerning the evaluation of flavouring substances from the viewpoint of health]. *Ernährung und Medizin*, 8, 244–251.
- Burdock GA (Ed.), 1995. *Fenaroli's Handbook of Flavour Ingredients*. 3rd Ed. Vol I + II. CRC Press, Inc., Florida, USA.
- Bär F and Griepentrog F, 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. [Where we stand concerning the evaluation of flavouring substances from the viewpoint of health]. *Ernährung und Medizin* 8, 244–251.
- CoE (Council of Europe), 1992. *Flavouring substances and natural sources of flavourings*, 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg, France.
- Collin J-P, 1971. Effet cytogénétique du cyclamate de soude, de la cyclohexanone et du cyclohexanol. [Cytogenetic effect of cyclamate, cyclohexanone and cyclohexanol]. *Le Diabete*, 19, 215–221. (In French)
- Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. *Food and Cosmetics Toxicology* 16(3), 255–276.
- Deichmann WB and LeBlanc TJ, 1943. Determination of the approximate lethal dose with about six animals. *Journal of Industrial Hygiene and Toxicology* 25(9), 415–417.
- Dyshlovoi VD, Boiko NL, Shemetun AM and Kharchenko TI, 1981. [Cytogenetic action of cyclohexanone]. *Gigiena i sanitariia*, 5, 76–77. (In Russian)
- EFSA (European Food Safety Authority), 2004. Minutes of the 7th Plenary Meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12–13 July 2004. Brussels, 28 September 2004. Available online: <http://www.efsa.europa.eu/sites/default/files/event/afc040712-m.pdf>
- EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food related to Flavouring Group Evaluation 212 (FGE.212): α,β -unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). *The EFSA Journal* 2009, 878, 1–28.
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on Flavouring Group Evaluation 212 Revision 1 (FGE.212Rev1): α,β -unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19. *EFSA Journal* 2011;9(3):1923, 29 pp. doi:10.2903/j.efsa.2011.1923
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 213, Revision 1 (FGE.213Rev1): α,β -unsaturated alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19. *EFSA Journal* 2014;12(5):3661, 46 pp. doi:10.2903/j.efsa.2014.3661

- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 212, Revision 3 (FGE.212Rev3): α,β -unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19. EFSA Journal 2015;13(5):4116, 39 pp. doi:10.2903/j.efsa.2015.4166
- Elliott TH, Parke DV and Williams RT, 1959. Studies in detoxication. The metabolism of cyclo[14C]hexane and its derivatives. Biochemical Journal, 72, 193–200.
- Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008. Available online: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL
- Fischer FG and Bielig HJ, 1940. Über die hydrierung ungesättigter stoffe im tierkörper. [On the hydrogenation of unsaturated materials in the animal body]. Hoppe-Seyler's Zeitschrift für physiologische Chemie, 266, 73–98.
- Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology, 18, 219–232.
- Franzios G, Mirosou M, Hatzia Apostolou E, Kral J, Scouras ZG and Mavragani-Tsipidou P, 1997. Insecticidal and genotoxic activities of mint essential oils. Journal of Agricultural and Food Chemistry, 45(7), 2690–2694.
- Gomes-Carneiro MR, Felzenszwalb I and Paumgarten FJ, 1998. Mutagenicity testing ($\pm$)-camphor, 1,8-cineole, citral, citronellal, (–)-menthol and terpineol with the *Salmonella*/microsome assay. Mutation Research, 416, 129–136.
- Goncharova RI, 1970. [Genetic activity of some cyclohexane derivatives]. Tsitologiya i genetika, 137–142. (In Russian)
- Gondry E, 1972. [Research on the toxicity of cyclohexamin, cyclohexanone and cyclohexanol, metabolites of cyclamate]. Toxicologie expérimentale, 4, 227–238. (In French)
- Governa M, Calisti R, Coppa G, Tagliavento G, Colombi A and Troni W, 1987. Urinary excretion of 2,5-hexanedione and peripheral polyneuropathies in workers exposed to hexane. Journal of Toxicology and Environmental Health, 20, 219–228.
- Gupta PK, Lawrence WH, Turner JE and Autian J, 1979. Toxicological aspects of cyclohexanone. Toxicology and Applied Pharmacology, 49, 525–533.
- Hämäläinen J, 1912. [The conduct of the alicyclic compounds in the glucuronic acid matching in the organism]. Skandinavisches Archiv für Physiologie, 27, 141–226. (In German)
- Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environmental Mutagenesis, 5(Suppl. 1), 3–142.
- Herken H, 1961. Pharmakologisches gutachten über die verträglichkeit von natürlichem (*l*-) und synthetischem (*d,l*-) menthol. Unpublished report from the director, Pharmakologischen Institute der Freien Universität, Berlin-Duhlem, submitted to the World Health Organisation by Schering AG. Cited in JECFA, 1976.
- Heymann E, 1980. Carboxylesterases and amidases. In: Jakoby WB (Ed.). Enzymatic Basis of Detoxication. 2nd Ed. Academic Press, New York, pp. 291–323.
- Igimi H and Ide H, 1974. Improvements in or relating to substances for use in the treatment of gallstones. Patent 1343561. Application no 13606/72. Filed 23 March 1972. Complete specification published 9 January 1974. International Classification A61K 27/00.
- Ishidate Jr M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22(8), 623–636.

- Ivett JL, Brown BM, Rodgers C, Anderson BE, Resnick MA and Zeiger E, 1989. Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*. IV. Results with 15 chemicals. *Environmental and Molecular Mutagenesis*, 14, 165–187.
- James SP and Waring RH, 1971. The metabolism of alicyclic ketones in the rabbit and rat. *Xenobiotica*, 1, 573–580.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1976. Toxicological evaluation of certain food additives. Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 21–29 April, 1976. Food Additive Series 10, pp. 64–69.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14–23 February 1995. WHO Technical Report Series, no 859. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series, 35. IPCS, WHO, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6–15 February 1996. WHO Technical Report Series, no. 868. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1998. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9–18 June 1998. FAO Food and Nutrition paper 52 Add. 6.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999a. Safety evaluation of certain food additives. Fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 42. IPCS, WHO, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17–26 June 1997. WHO Technical Report Series, no. 884. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000a. Evaluation of certain food additives. Fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9–18 June 1998. WHO Technical Report Series, no. 891. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000b. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Committee of Food Additives. Fifty-fifth Meeting. Geneva, 6–15 June 2000. FAO Food and Nutrition paper 52 Add. 8.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002a. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 909. Geneva, 5–14 June 2001.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002b. Evaluation of certain food additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 913. Geneva, 4–13 June 2002.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002c. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4–13 June 2002. FAO Food and Nutrition paper 52 Addition 10.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Safety evaluation of certain food additives. Fifty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005a. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8–17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005b. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8–17 June 2004.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Safety evaluation of certain food additives and contaminants. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2009. Safety evaluation of certain food additives and contaminants. Sixty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 60. IPCS, WHO, Geneva 2009. http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf (May 2009).
- Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh OG, 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. *Food and Cosmetics Toxicology* 2, 327–343.
- Keefer LK, Streeter AJ, Leung LY, Perry WC, Hu HS-W and Baillie TA, 1987. Pharmacokinetic and deuterium isotope effect studies on the metabolism of formaldehyde and formate to carbon dioxide in rats *in vivo*. *Drug Metabolism and Disposition* 15(3), 300–304.
- Kohli RP, Kishor K, Dua PR and Saxena RC, 1967. Anticonvulsant activity of some carbonyl containing compounds. *Indian Journal of Medical Research* 55(11), 1221–1225.
- Lake BG, Foster JR, Collins MA, Stubberfield CR, Gangolli SD and Srivastava SP, 1982. Studies on the effects of orally administered dicyclohexyl phthalate in the rat. *Acta Pharmacology & Toxicology* 51, 217–226.
- Lijinsky W and Kovatch RM, 1986. Chronic toxicity study of cyclohexanone in rats and mice. *Journal of the National Cancer Institute* 77(4), 941–949.
- McMahon RE, Cline JC and Thompson CZ, 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. *Cancer Research*, 39, 682–693.
- Madsen C, Wurtzen G and Carstensen J, 1986. Short-term toxicity study in rats dosed with menthone. *Toxicology Letters* 32(1–2), 147–152.
- Madyastha KM and Srivatsan V, 1988. Studies on the metabolism of l-menthol in rats. *Drug Metabolism and Disposition* 16(5), 765–772.
- Massoud A, Aly A and Shafik H, 1980. Mutagenicity and carcinogenicity of cyclohexanone. *Mutation Research* 74(3), 174.
- Messiha FS and Lox CD, 1985. Effect of selected organic solvents on hepatic alcohol and aldehyde-dehydrogenase. *Neurobehavioral Toxicology and Teratology* 7(2), 207–208.
- Miyazawa M and Nakanishi K, 2006. Biotransformation of (-)-Menthone by human liver microsomes. *Bioscience, Biotechnology and Biochemistry* 70(5), 1259–1261.
- Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E, 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. *Environmental and Molecular Mutagenesis* 8(Suppl. 7), 1–119.
- Mráz J, Gálová E, Nohová H and Vitková D, 1994. Uptake, metabolism and elimination of cyclohexanone in humans. *International Archives of Occupational and Environmental Health* 66(3), 203–208.
- Mráz J, Gálová E, Nohová H and Vitková D, 1998. 1,2- and 1,4-cyclohexanediol: major urinary metabolites and biomarkers of exposure to cyclohexane, cyclohexanone, and cyclohexanol in humans. *International Archives of Occupational and Environmental Health* 71(8), 560–565.
- Murthy PBK, Ahmed MM and Regu K, 1991. Lack of genotoxicity of menthol in chromosome aberration and sister chromatid exchange assays using human lymphocytes *in vitro*. *Toxicology In Vitro* 5(4), 337–340.

- Myhr BC and Caspary WJ, 1991. Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program. *Environmental and Molecular Mutagenesis*, 18, 51–83.
- National Cancer Institute, 1979. Bioassay of dl-menthol for possible carcinogenicity. U.S. Department of Health, Education, and Welfare. April 1978. NCI Technical Report Series, no 98.
- Nohmi T, Miyata R, Yoshikawa K and Ishidate M, 1985. [Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests]. *Eisei Shikenjo hokoku. Bulletin of National Institute of Hygienic Sciences*, 103(60), 60–64. (In Japanese)
- NTP (National Toxicology Program), 2007. Search Result on cyclohexanone. Available online: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.searchresults&searchterm=cyclohexanone. [14th September, 2007].
- Oda Y, Hamono Y, Inoue K, Yamamoto H, Niihara T and Kunita N, 1979. [Mutagenicity of food flavours in bacteria]. *Osaka Furitsu Kosho Eisei Kenkyusho kenkyu hokoku. Shokuhin eisei hen* 9, 177–181. (In Japanese)
- OECD (The Organisation for Economic Co-operation and Development), 1997a. Test No 471. Bacteria Reverse Mutation Test. OECD Guideline for Testing of Chemicals. Section 4. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en
- OECD (The Organisation for Economic Co-operation and Development), 1997b. Test No 476. *In vitro* mammalian cell gene mutation test. OECD Guideline for Testing of Chemicals. Section 4. Available online: http://www.oecd-ilibrary.org/environment/test-no-476-in-vitro-mammalian-cell-gene-mutation-test_9789264071322-en
- OECD (The Organisation for Economic Co-operation and Development), 1997c. Test No 486. Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo*. OECD Guideline for Testing of Chemicals. Section 4. Available online: http://www.oecd-ilibrary.org/environment/test-no-486-unscheduled-dna-synthesis-uds-test-with-mammalian-liver-cells-in-vivo_9789264071520-en
- Oh S-M, Yeon J-D, Nam H-Y, Park D-K, Cho M-H and Chang K-H, 1997. [Acute and subacute toxicity studies of l-muscone in rats]. *Korean Journal of Toxicology* 13(4), 435–447. (In Korean)
- Perbellini L, De Grandis D, Semenzato F and Bongiovanni LG, 1981. Studio sperimentale sulla neurotossicità del cicloesanol e del cicloesanone. *La Medicina del Lavoro* 2, 102–106. (In Italian)
- Price TD, 1951. Studies on the metabolism of acetone. A Dissertation. Univ. Microfilms Pub. No. 2549. 82.
- SCF (Scientific Committee for Food), 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.
- SCF (Scientific Committee on Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health and Consumer Protection Directorate-General.
- Shelanski MV, 1972. Report to RIFM, 14 July. *l*-Menthyl acetate. Cited in Opdyke DLJ (Ed.). *Fragrance raw materials monographs*. Pergamon Press, p. 477.
- Shelby MD, Erexson GL, Hook GJ and Tice RR, 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environmental and Molecular Mutagenesis*, 21(2), 160–179.

- Shimada T, Shindo M and Miyazawa M, 2002. Species differences in the metabolism of (+) and (-)-limonenes and their metabolites, carveols and carvones, by cytochrome P450 enzymes in liver microsomes of mice, rats, guinea pigs, rabbits, dogs, monkeys, and humans. *Drug Metabolism and Pharmacokinetics* 17(6), 507–515.
- Smyth Jr HF and Carpenter CP, 1948. Further experience with the range-finding test in the industrial toxicology laboratory. *Journal of Industrial Hygiene and Toxicology* 30, 63–68.
- Smyth Jr HF, Carpenter CP, Weil CS, Pozzani UC, Striegel JA and Nycum JS, 1969. Range-finding toxicity data: List VII. *American Industrial Hygiene Association Journal* 30(5), 470–476.
- Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK and Gori GB, 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumour response in strain mice. *Cancer Research* 33(12), 3069–3085.
- Storer RD, McKelvey TW, Kraynak AR, Elia MC, Barnum JE, Harmon LS, Nichols WW and DeLuca JG, 1996. Revalidation of the *in vitro* alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. *Mutation Research* 368(2), 59–101.
- Thorup I, Wurtzen G, Carstensen J and Olsen P, 1983. Short-term toxicity study in rats dosed with pulegone and menthol. *Toxicology Letters* 19(3), 207–210.
- TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2000. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database. Zeist, The Netherlands. TNO Triskelion, 1963–2000.
- TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2010. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 12.2/12.3. Zeist, The Netherlands. TNO Triskelion, 1963–2010.
- TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2011. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 13.1. Zeist, The Netherlands. TNO Triskelion, 1963–2011.
- TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2014. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 15.1. Zeist, The Netherlands. TNO Triskelion, 1963–2014.
- TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2014. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 15.2. Zeist, The Netherlands. TNO Triskelion, 1963–2015.
- Treon JF, Crutchfield WE and Kitzmiller KV, 1943. The physiological response of rabbits to cyclohexane, methylcyclohexane and certain derivatives of these compounds. *Journal of Industrial Hygiene and Toxicology*, 25, 199–214.
- Truhaut R, Dutertre-Catella H and Phu-Lich N, 1970. Biochemical toxicology. First results of a study of metabolism in rabbits, which were administered isophorone, an industrial solvent. *Comptes Rendus de l'Académie des Sciences*, 271, 1333–1336.
- Truhaut R, Lich NP, Cluet JL and Dutertre-Catella H, 1979. [Dismutation as a metabolic pathway: Transformation of trimethyl-3,5,5-cyclohexanone]. *Toxicological European Research* 2(2), 71–76. (In French)
- Vree TB, Kolmer EWJV, Verweyvanwissen CPWG and Hekster YA, 1994. Effect of urinary pH on the pharmacokinetics of salicylic-acid, with its glycine and glucuronide conjugates in human. *International Journal of Clinical Pharmacology and Therapeutics* 32, 550–558.
- White DA, Heffron F, Miciak A, Middleton B, Knights S and Knight D, 1990. Chemical synthesis of dual radiolabelled cyclandelate and its metabolism in rat hepatocytes and mouse J774 cells. *Xenobiotica* 20(1), 71–79.

- Wokes F, 1932. The antiseptic value and toxicity of menthol isomers. *Quarterly Journal of Pharmacy and Pharmacology* 5, 233–244.
- Yamaguchi T, Caldwell J and Farmer PB, 1994. Metabolic fate of [3H]-*l*-menthol in the rat. *Drug Metabolism and Disposition* 22(4), 616–624.
- Yoo YS, 1986. Mutagenic and antimutagenic activities of flavouring agents used in foodstuffs. *Osaka City Medical Journal* 34(3–4), 267–288.
- You A-S, Kweon O-K, Sung H-J, Kwak H-I, Fang M-Z, Park D-K, Chung K-H, Yoon H-I and Cho M-H, 1997. Acute and subacute toxicity of *l*-muscone in beagle dogs. *Korean Journal of Toxicology* 13(4), 449–460.
- Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environmental and Molecular Mutagenesis* 11(Suppl. 12), 1–158.

ABBREVIATIONS

ADI	Acceptable Daily Intake
bw	body weight
CAS	Chemical Abstract Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
	Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
GLP	Good Laboratory Practice
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavor Industry
IP	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear magnetic resonance
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SCE	sister chromatid exchange
SCF	Scientific Committee on Food
SMART	somatic mutation and recombination test
TAMDI	Theoretical Added Maximum Daily Intake
TK	Thymidine Kinase
UDS	Unscheduled DNA Synthesis
WHO	World Health Organization

APPENDICES

Appendix A. Summary of safety evaluation

Table 6: Summary of safety evaluation applying the procedure

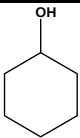
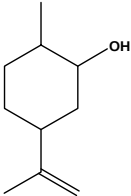
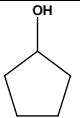
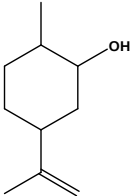
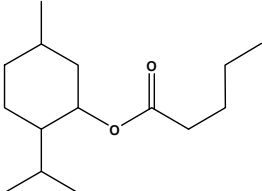
FL-no	EU Register name	Structural formula	MSDI ^(a) (µg per capita per day)	Class ^(b) Evaluation procedure path ^(c)	Outcome on the named compound ^{(d)(e)}	Outcome on the material of commerce ^{(f)(g)(h)}	Evaluation remarks
02.070	Cyclohexanol		3.7	Class I A3: Intake below threshold	d	f	
02.075	(1R,2S,5S)-neo-Dihydrocarveol		2.4	Class I A3: Intake below threshold	d	f	
02.135	Cyclopentanol		0.012	Class I A3: Intake below threshold	d	f	
02.167	(1R,2R,5S)-Isodihydrocarveol		2.4	Class I A3: Intake below threshold	d	f	
09.154 1852	Menthyl valerate		1	Class I A3: Intake below threshold	d	f	

Table 6: Summary of safety evaluation applying the procedure

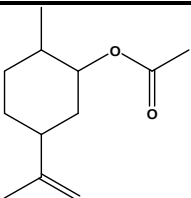
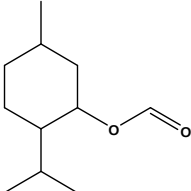
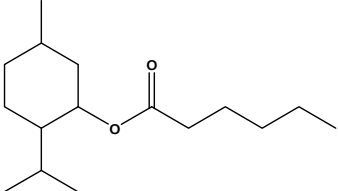
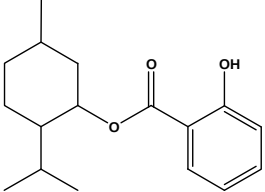
FL-no	EU Register name	Structural formula	MSDI ^(a) (µg per capita per day)	Class ^(b) Evaluation procedure path ^(c)	Outcome on the named compound ^{(d)(e)}	Outcome on the material of commerce ^{(f)(g)(h)}	Evaluation remarks
09.355	neo-Dihydrocarvyl acetate		0.012	Class I A3: Intake below threshold	d	f	
09.618	Menthyl formate		0.73	Class I A3: Intake below threshold	d	f	
09.619	(1R,2S,5R)-Menthyl hexanoate		0.37	Class I A3: Intake below threshold	d	f	
09.621	(1R,2S,5R)-Menthyl salicylate		0.012	Class I A3: Intake below threshold	d	f	

Table 6: Summary of safety evaluation applying the procedure

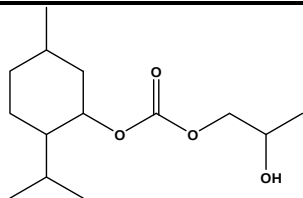
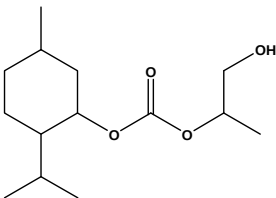
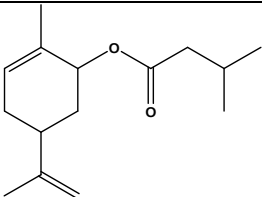
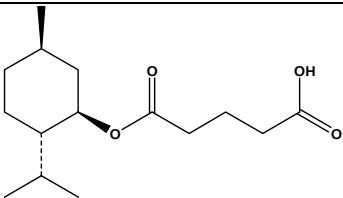
FL-no	EU Register name	Structural formula	MSDI ^(a) (µg per capita per day)	Class ^(b) Evaluation procedure path ^(c)	Outcome on the named compound ^{(d)(e)}	Outcome on the material of commerce ^{(f)(g)(h)}	Evaluation remarks
09.843	Menthol 1- and 2-propylene glycol carbonate	 + 	830 380	Class I A3: Intake below threshold	d	f	
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: Intake below threshold	d	f	Evaluated in FGE.212, genotoxic concern could be ruled out
09.929	L-Monomenthyl glutarate		110	Class I A3: Intake below threshold	d	f	

Table 6: Summary of safety evaluation applying the procedure

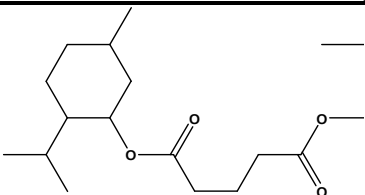
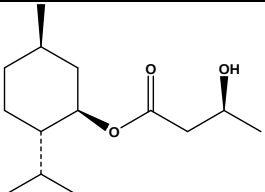
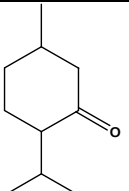
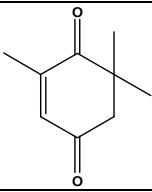
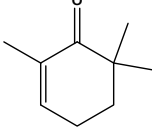
FL-no	EU Register name	Structural formula	MSDI ^(a) (µg per capita per day)	Class ^(b) Evaluation procedure path ^(c)	Outcome on the named compound ^{(d)(e)}	Outcome on the material of commerce ^{(f)(g)(h)}	Evaluation remarks
09.935	Dimethyl glutarate		30	Class I A3: Intake below threshold	d	f	
09.949	L-Menthyl (S)-3-hydroxybutyrate		37	Class I A3: Intake below threshold	d	f	
07.059	<i>p</i> -Menthan-3-one		530 2500	Class II A3: Intake below threshold	d	f	
07.109 1857	2,6,6-Trimethylcyclohex-2-en-1,4-dione		50	Class II A3: Intake below threshold	d	f	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		0.12	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out

Table 6: Summary of safety evaluation applying the procedure

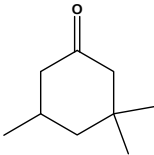
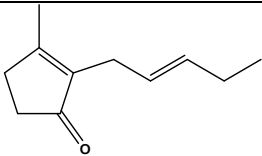
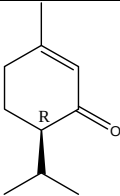
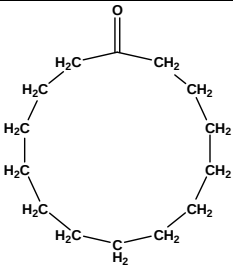
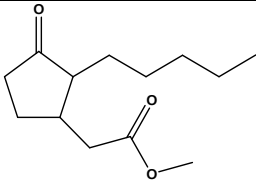
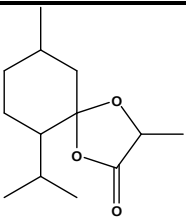
FL-no	EU Register name	Structural formula	MSDI ^(a) (µg per capita per day)	Class ^(b) Evaluation procedure path ^(c)	Outcome on the named compound ^{(d)(e)}	Outcome on the material of commerce ^{(f)(g)(h)}	Evaluation remarks
07.203	3,3,5-Trimethylcyclohexan-1-one		0.0085	Class II A3: Intake below threshold	d	f	
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		4.7	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev3, genotoxicity concern could be ruled out
07.255 1856	l-Piperitone		12	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out
07.207	Cyclotetradecanone		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Flavour Industry (EFFA, 2009)
09.520 1898	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		770	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	f	

Table 6: Summary of safety evaluation applying the procedure

FL-no	EU Register name	Structural formula	MSDI ^(a) (µg per capita per day)	Class ^(b) Evaluation procedure path ^(c)	Outcome on the named compound ^{(d)(e)}	Outcome on the material of commerce ^{(f)(g)(h)}	Evaluation remarks
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		1.2	Class III A3: Intake below threshold	d	f	

(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg per capita per day.

(b): Thresholds of concern: Class I = 1 800 µg per person per day, Class II = 540 µg per person per day, Class III = 90 µg per person per day.

(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

(d): No safety concern based on intake calculated by the MSDI approach of the named compound.

(e): Data must be available on the substance or closely related substances to perform a safety evaluation.

(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).

(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

(h): No conclusion can be drawn because of the lack of information on the purity of the material of commerce.

Table 7: Evaluation status of hydrolysis products of candidate esters in FGE.09Rev6

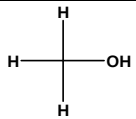
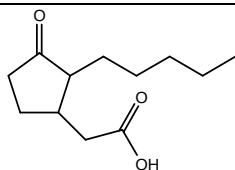
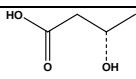
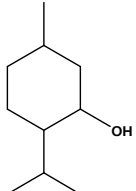
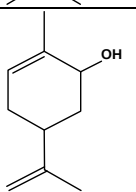
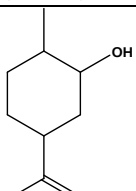
FL-no	EU Register name JECFA no	Structural formula	SCF status ^(a) JECFA status ^(b) CoE status ^(c) EFSA status	Structural class ^(d) Procedure path (JECFA) ^(e)	Comments
Not in Register	Methanol		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	3-Oxo-2-pentyl-1-cyclopentyl acetic acid		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	(S)-3-Hydroxybutyric acid		Not evaluated as flavouring substance		Not a Register substance.
02.015	Menthol 427		No safety concern (JECFA, 2000a) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	NOAEL: 380 mg/kg bw/day
02.062	Carveol 381		No safety concern (JECFA, 2000a) Category B (CoE, 1992)	Class I A3: Intake below threshold	
02.075	(1R,2S,5S)-neo-Dihydrocarveol		Category B (CoE, 1992) FGE.09	Class I A3: Intake below threshold	

Table 7: Evaluation status of hydrolysis products of candidate esters in FGE.09Rev6

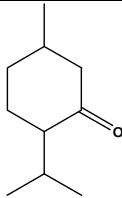
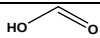
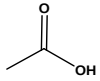
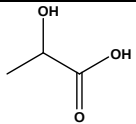
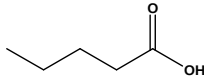
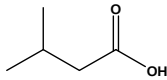
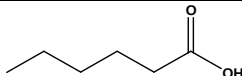
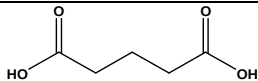
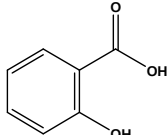
FL-no	EU Register name JECFA no	Structural formula	SCF status ^(a) JECFA status ^(b) CoE status ^(c) EFSA status	Structural class ^(d) Procedure path (JECFA) ^(e)	Comments
07.059	<i>p</i> -Menthan-3-one		Category B (CoE, 1992) FGE.09	Class II A3: Intake below threshold	
08.001	Formic acid 79		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Deleted (CoE, 1992)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern (JECFA, 2002a) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	

Table 7: Evaluation status of hydrolysis products of candidate esters in FGE.09Rev6

FL-no	EU Register name JECFA no	Structural formula	SCF status ^(a) JECFA status ^(b) CoE status ^(c) EFSA status	Structural class ^(d) Procedure path (JECFA) ^(e)	Comments
08.009	Hexanoic acid 93		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.082	Glutaric acid			Class I A3: Intake below threshold	
08.112	Salicylic acid 958		FGE.10 No safety concern (JECFA, 2002a)	Class I A3: Intake below threshold	

(a): Category 1: Considered safe in use. Category 2: Temporarily considered safe in use. Category 3: Insufficient data to provide assurance of safety in use. Category 4: Not acceptable because of evidence of toxicity.

(b): No safety concern at estimated levels of intake.

(c): Category A: Flavouring substance which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

(d): Threshold of concern: Class I = 1 800 µg per person per day, Class II = 540 µg per person per day, Class III = 90 µg per person per day.

(e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

SUPPORTING SUBSTANCES SUMMARY

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

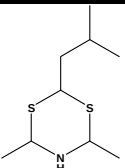
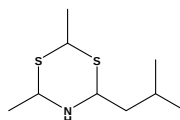
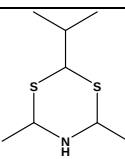
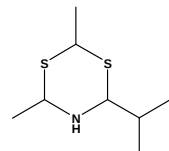
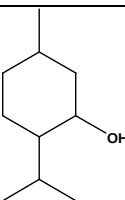
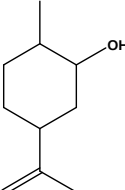
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^(a) (µg per capita per day)	SCF status ^(b) JECFA status ^(c) CoE status ^(d)	Comments
	2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	  (Mixture)	3781 101517-87-7 and 101517-86-6	1046 JECFA specification (JECFA, 2002c)	0.1	No safety concern	Not in the EU Register
	2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	  (Mixture)	3782 104691-41-0 and 104691-40-9	1047 JECFA specification (JECFA, 2002c)	ND	No safety concern	Not in the EU Register
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998)	16 000	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	ADI: 0–4 mg/kg bw (JECFA, 2000a)
02.061	Dihydrocarveol		2379 2025 619-01-2	378 JECFA specification (JECFA, 1998)	0.37	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

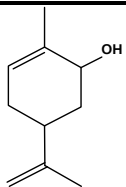
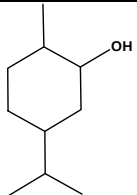
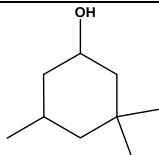
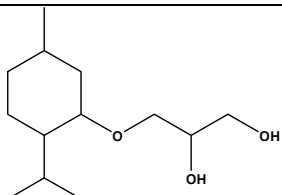
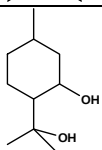
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^(a) (µg per capita per day)	SCF status ^(b) JECFA status ^(c) CoE status ^(d)	Comments
02.062	Carveol		2247 2027 99-48-9	381 JECFA specification (JECFA, 1998)	9.5	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.071	<i>p</i> -Menthan-2-ol		3562 2228 499-69-4	376 JECFA specification (JECFA, 2000b)	0.012	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.209	3,3,5-Trimethylcyclohexan-1-ol		3962 116-02-9	1099 JECFA specification (JECFA, 2002c)	0.12	No safety concern (JECFA, 2002b)	JECFA name: 3,3,5-Trimethyl cyclohexanol
02.224	3-(1-Menthoxy)propane-1,2-diol		3784 87061-04-9	1408 JECFA specification (JECFA, 2005a)	4.1	No safety concern (JECFA, 2005b)	JECFA name: 3-L-Menthoxypropa ne-1,2-diol
02.246	<i>p</i> -Menthane-3,8-diol		4053 42822-86-6	1416 JECFA specification (JECFA, 2005a)	39	No safety concern (JECFA, 2005b)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

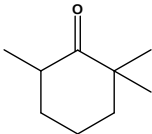
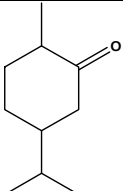
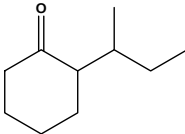
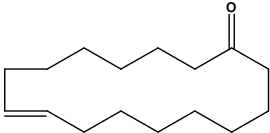
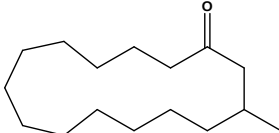
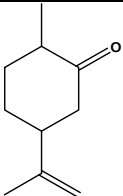
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^(a) (µg per capita per day)	SCF status ^(b) JECFA status ^(c) CoE status ^(d)	Comments
07.045	2,2,6-Trimethylcyclohexanone		3473 686 2408-37-9	1108 JECFA specification (JECFA, 2002c)	2.1	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
07.092	<i>p</i> -Menthan-2-one		3176 11128 499-70-7	375 JECFA specification (JECFA, 1998)	0.012	No safety concern (JECFA, 2000a)	
07.095	2-(sec-Butyl)cyclohexanone		3261 11044 14765-30-1	1109 JECFA specification (JECFA, 2002c)	5.1	No safety concern (JECFA, 2002b)	
07.110	Cycloheptadec-9-en-1-one		3425 11744 542-46-1	1401 JECFA specification (JECFA, 2005a)	0.24	No safety concern (JECFA, 2005b)	
07.111	3-Methylcyclopentadecan-1-one		3434 11135 541-91-3	1402 JECFA specification (JECFA, 2005a)	0.37	No safety concern (JECFA, 2005b)	JECFA name: 3-Methyl-1- cyclopentadecan one.
07.128	Dihydrocarvone		3565 11703 7764-50-3	377 JECFA specification (JECFA, 2000b)	0.012	No safety concern (JECFA, 2000a)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

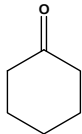
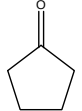
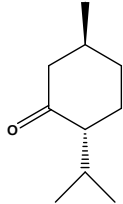
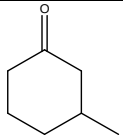
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^(a) (µg per capita per day)	SCF status ^(b) JECFA status ^(c) CoE status ^(d)	Comments
07.148	Cyclohexanone		3909 11047 108-94-1	1100 JECFA specification (JECFA, 2002c)	0.12	No safety concern (JECFA, 2002b)	
07.149	Cyclopentanone		3910 11050 120-92-3	1101 JECFA specification (JECFA, 2002c)	0.018	No safety concern (JECFA, 2002b)	
07.176	trans-Menthone	 (-)-menthone shown	2667 2035 89-80-5	429 JECFA specification (JECFA, 1998)	890	No safety concern (JECFA, 2000a)	JECFA name: Menthone. CAS No in register refers to cyclohexanone, 5-methyl-2-(1- methylethyl)-, (2 <i>R</i> ,5 <i>S</i>)-rel-
07.180	3-Methylcyclohexanone		3947 591-24-2	1103 JECFA specification (JECFA, 2002c)	0.12	No safety concern (JECFA, 2002b)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

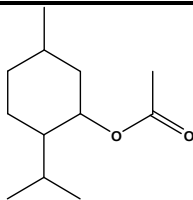
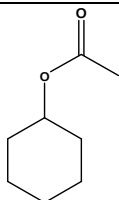
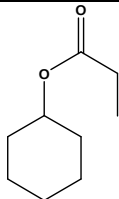
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^(a) (µg per capita per day)	SCF status ^(b) JECFA status ^(c) CoE status ^(d)	Comments
09.016	Menthyl acetate		2668 206 29066-34-0	431 JECFA specification (JECFA, 1998)	270	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	JECFA evaluated menthyl acetate (CAS No 16409-45-3, which does not specify isomer). CAS No in register replaced by 89-48-5, which refers to Cyclohexanol, 5-methyl-2-(1- methylethyl)-, acetate, (1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>) (SciFinder)
09.027	Cyclohexyl acetate		2349 217 622-45-7	1093 JECFA specification (JECFA, 2002c)	12	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
09.140	Cyclohexyl propionate		2354 421 6222-35-1	1097 JECFA specification (JECFA, 2002c)	0.012	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

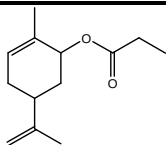
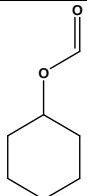
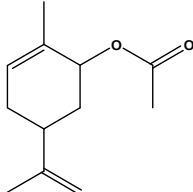
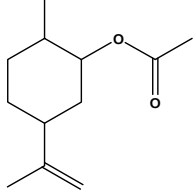
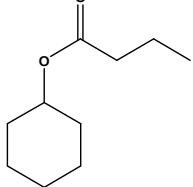
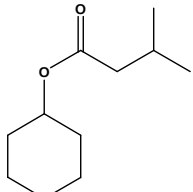
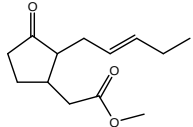
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^(a) (µg per capita per day)	SCF status ^(b) JECFA status ^(c) CoE status ^(d)	Comments
09.143	Carvyl propionate		2251 424 97-45-0	383 JECFA specification (JECFA, 2000b)	0	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.160	Cyclohexyl formate		2353 498 4351-54-6	1095 JECFA specification (JECFA, 2002c)	0.012	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
09.215	Carvyl acetate		2250 2063 97-42-7	382 JECFA specification (JECFA, 1998)	4	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.216	Dihydrocarvyl acetate		2380 2064 20777-49-5	379 JECFA specification (JECFA, 1998)	9.7	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.230	Cyclohexyl butyrate		2351 2082 1551-44-6	1094 JECFA specification (JECFA, 2002c)	0.89	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^(a) (µg per capita per day)	SCF status ^(b) JECFA status ^(c) CoE status ^(d)	Comments
09.464	Cyclohexyl isovalerate		2355 459 7774-44-9	1096 JECFA specification (JECFA, 2002c)	0.28	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
09.521	Methyl 3-oxo-2-pent-2-enyl-1-cyclopentylacetate		3410 10821 39924-52-2	1400 JECFA specification (JECFA, 2005a)	26	No safety concern (JECFA, 2005b)	JECFA evaluated methyl jasmonate (CAS No 1211-29-6). (R)- or (S)- nor (E)- or (Z)- not specified by register CAS No

ND, No intake data reported.

(a): EU MSDI: Amount added to food as flavouring substance in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.

(b): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

(c): No safety concern at estimated levels of intake.

(d): Category A: Flavouring substance which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

Appendix B. Procedure for the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, named the “Procedure”, is shown in schematic form in Figure B.1. The Procedure is based on the Opinion of the SCF expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by JECFA at its 44th, 46th and 49th meetings (JECFA, 1995, 1996, 1997, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure–activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern of 1 800, 540 or 90 µg per person per day for classes I, II and III, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- Can the flavourings be predicted to be metabolised to innocuous products¹² (Step 2)?
- Do their exposures exceed the threshold of concern for the structural class (Steps A3 and B3)?
- Are the flavourings or their metabolites endogenous¹³ (Step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (Steps A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

¹² “Innocuous metabolic products”: Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent” (JECFA, 1997a).

¹³ “Endogenous substances”: Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

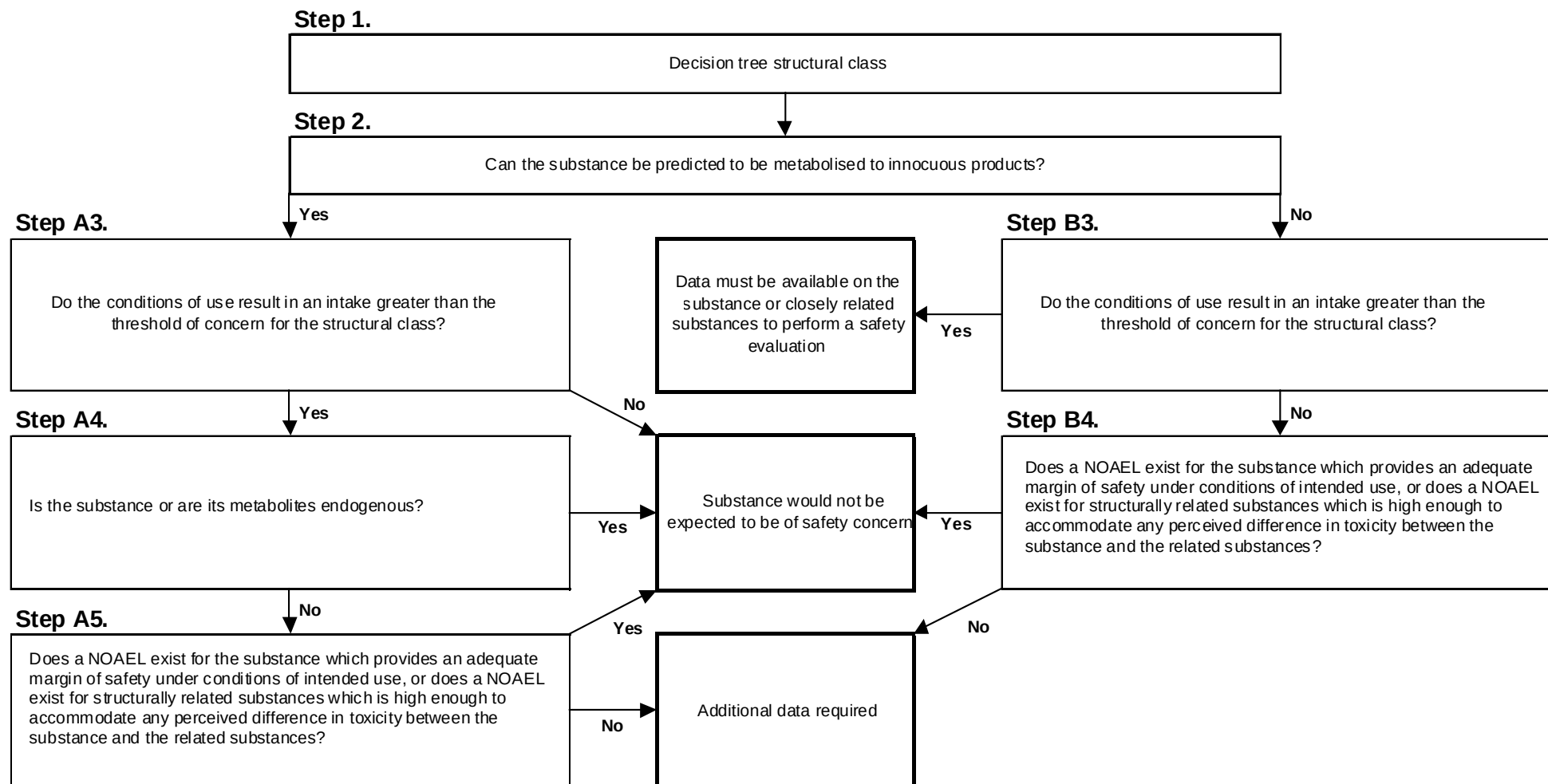


Figure 1: Procedure for safety evaluation of chemically defined flavouring substances

Appendix C. Use levels/mTAMDI

C.1 Normal and maximum use levels

For each of the 18 Food categories (Table 9) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level”. According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

Table 9: Food categories according to Commission Regulation (EC) No 1565/2000

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds
05.0	Confectionery
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic (“soft”) beverages, excluding dairy products
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 01.0–15.0

The “normal and maximum use levels” are provided by the Flavour industry (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b) for 20 of the 22 candidate substances in the present flavouring group (Table 10).

Table 10: Normal and maximum use levels (mg/kg) for the candidate substances FGE.09Rev5 (Burdock, 1995; ECHA, 2003a, 2007; Flavour Industry, 2004, 2006a, 2006b, 2007, 2010a, 2010b).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.070	3	2	3	2	–	4	2	5	1	1	–	–	2	3	2	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	10	20	25	10
02.075	3	2	3	2	–	4	2	5	1	1	–	–	2	3	2	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	10	20	25	10
02.135	3	2	3	2	–	4	2	5	1	1	–	–	2	3	2	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	10	20	25	10
02.167	3	2	3	2	–	4	2	5	1	1	–	–	2	3	2	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	10	20	25	10
06.136	0.0001	0.0001	0.0001	–	–	0.001	0.0001	0.0001	–	–	–	–	–	0.0001	0.0001	0.0001	–	0.0001
	0.0005	0.0001	0.0008	–	–	0.005	0.0005	0.0005	–	–	–	–	–	0.0005	0.0005	0.0008	–	0.0005
07.059	–	–	15.32	–	–	33.27	–	47.89	–	–	–	–	–	–	4.22	0.87	–	–
	–	–	22.99	–	–	52.97	–	68.1	–	–	–	–	–	–	5.86	2.59	–	–
07.109	7	2	3	2	–	4	–	5	1	1	–	–	2	3	2	4	5	2
	35	10	15	10	–	20	–	25	5	5	–	–	10	15	10	20	25	10
07.202	3	2	3	2	–	4	2	5	1	1	–	–	2	3	2	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	10	20	25	10
07.203	3	2	3	2	–	4	2	5	1	1	–	–	2	3	2	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	10	20	25	10
07.255	0.5	–	0.2	–	–	0.5	–	1	–	–	–	–	0.2	–	0.2	0.2	5	0.2
	5	–	2	–	–	5	–	10	–	–	–	–	2	–	1	2	50	2
09.154	7	5	10	7	–	10	5	10	2	2	–	–	5	10	5	10	20	5
	35	25	50	35	–	50	25	50	10	10	–	–	25	50	25	50	100	25
09.355	3	2	3	2	–	4	2	5	1	1	–	–	2	3	2	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	10	20	25	10
09.520	7	5	10	7	–	10	5	10	2	2	–	–	5	10	5	10	20	5
	35	25	50	35	–	50	25	50	10	10	–	–	25	50	25	50	100	25
09.618	7	5	10	7	–	10	5	10	2	2	–	–	5	10	5	10	20	5
	35	25	50	35	–	50	25	50	10	10	–	–	25	50	25	50	100	25
09.619	7	5	1	7	–	10	5	10	2	2	–	–	5	10	5	10	20	5
	35	25	50	35	–	50	25	50	10	10	–	–	25	50	25	50	100	25

Table 10: Normal and maximum use levels (mg/kg) for the candidate substances FGE.09Rev5 (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, 2006b, 2007, 2010a, 2010b).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
09.621	0.5	0.2	0.5	0.4	–	1	0.2	2	0.2	0.2	–	–	0.3	0.5	0.2	1	2	0.4
	2.5	1	2.5	2	–	5	1	10	1	1	–	–	1.5	2.5	1	5	10	2
09.843	200	–	100	–	–	500	15	60	–	–	–	–	25	–	30	100	25	–
	800	–	400	–	–	2 000	60	250	–	–	–	–	100	–	120	400	100	–
09.870	3	2	3	2	–	4	2	5	1	1	–	–	2	3	–	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	–	20	25	10
09.935	1	1	10	1	1	100	–	10	1	1	–	–	1	–	100	100	1	1
	15	15	150	15	15	1 500	–	150	15	15	–	–	15	–	1 500	1 500	15	15
09.949	30	–	10	20	–	50	5	20	–	–	–	–	10	–	10	10	30	10
	150	–	50	100	–	200	20	100	–	–	–	–	30	–	50	50	150	30

The candidate substances [FL-nos: 07.059, 09.843 and 09.949] are also used in chewing gum, which is not covered by any of the above food categories. The normal/maximum use levels for these substances in chewing gum are reported to be 14.34/14.34 mg/kg [FL-no: 07.059], 5 000/20 000 mg/kg [FL-no: 09.843] and 500/1 000 mg/kg [FL-no: 09.949]. Under the assumptions that all of the flavouring substances are released from the chewing gum and that the intake estimate is 2 g chewing gum per day, the calculation of the mTAMDI of the candidate substance based on the 16 food categories and the use of chewing gum sum up to 8 700, 63 000 and 10 600 µg per person per day, respectively. These figures are presented in Tables 5 and 9.

C.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table 11. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table 11: Estimated amount of flavourable foods, beverages and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g per day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by the Flavour Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 and reported by the Flavour Industry in the following way (see Table 12):

- Beverages correspond to food category 14.1.
- Foods correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16.
- Exception a corresponds to food categories 5 and 11.
- Exception b corresponds to food category 15.
- Exception c corresponds to food category 14.2.
- Exception d corresponds to food category 12.
- Exception e corresponds to others, e.g. chewing gum.

Table 12: Distribution of the 16 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic (“soft”) beverages, excluding dairy products		Beverages	
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)— foods that could not be placed in categories 01.0–15.0	Food		

The mTAMDI values (see Table 13) are presented for each of the flavouring substances in the present flavouring group, for which the Flavour Industry has provided use and use levels (Burdock, 1995; EFSA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b). The mTAMDI values are only given for highest reported normal use levels (see Table 13).

Table 13: Estimated intakes based on the mTAMDI approach.

FL-no	EU Register name	mTAMDI (µg per person per day)	Structural class	Threshold of concern (µg per person per day)
02.070	Cyclohexanol	1 600	Class I	1 800
02.075	(1R,2S,5S)-neo-Dihydrocarveol	1 600	Class I	1 800
02.135	Cyclopentanol	1 600	Class I	1 800
02.167	(1R,2R,5S)-Isodihydrocarveol	1 600	Class I	1 800
09.154	Menthyl valerate	3 900	Class I	1 800
09.355	neo-Dihydrocarvyl acetate	1 600	Class I	1 800
09.618	Menthyl formate	3 900	Class I	1 800
09.619	(1R,2S,5R)-Menthyl hexanoate	3 900	Class I	1 800
09.621	(1R,2S,5R)-Menthyl salicylate	420	Class I	1 800
09.843	Menthol 1- and 2-propylene glycol carbonate	63 000	Class I	1 800
09.870	Carvyl-3-methylbutyrate	1 000	Class I	1 800
09.929	L-Monomenthyl glutarate		Class I	1 800
09.935	Dimenthyl glutarate	38 000	Class I	1 800
09.949	L-Menthyl (S)-3-hydroxybutyrate	10 600	Class I	1 800
07.059	p-Menthan-3-one	8 700	Class II	540
07.109	2,6,6-Trimethylcyclohex-2-en-1,4-dione	1 900	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	1 600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	1 600	Class II	540
07.219	trans-3-Methyl-2-(2-pentenyl)-2- cyclopenten-1-one		Class II	540
07.255	l-Piperitone	320	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1- cyclopentylacetate	3 900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4- dioxyspiro[4.5]decan-2-one	0.075	Class III	90

Appendix D. Metabolism

D.1 Absorption, distribution and elimination

The candidate substances of secondary alicyclic saturated and unsaturated alcohols, ketones and esters of the present flavouring group evaluation are expected to be rapidly absorbed from the gastrointestinal tract. Supporting substances evaluated by the JECFA sustain this view (JECFA, 1999a, 2003).

D.2 Biotransformation

The candidate substances are expected to be metabolised through several alternative metabolic pathways. Depending on their chemical structure, the possible metabolic reactions are the following:

D.2.1 Ester hydrolysis

D.2.2 Reduction of ketone groups and oxidation of alcohol groups

D.2.3 Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

D.2.4 Metabolism to glucuronides

D.2.5 Metabolism to sulphates

D.2.1. Ester hydrolysis

The esters included in this FGE are expected to be hydrolysed enzymatically to carboxylic acids and alcohols via carboxylesterases found in most tissues throughout the body, the most important of which are the β -esterases (Heymann, 1980). For the one hemiketal ester [FL-no: 06.136] hydrolysis to the corresponding cyclic ketone, p-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004] is expected.

The supporting substances, menthyl acetate [FL-no: 09.016] and dihydrocarvyl acetate [FL-no: 09.216] were previously evaluated by JECFA (1999a), but no metabolism studies were available for these supporting substances, structurally related to the candidate substances menthyl valerate, neo-dihydrocarvyl acetate, menthyl formate and menthyl hexanoate [FL-nos: 09.154, 09.355, 09.618 and 09.619]. The JECFA evaluation was based on a study demonstrating about 75 % and 85 % hydrolysis of 1-menthol propylene glycol carbonate and 1-menthol ethylene glycol carbonate, respectively, after four hours in liver homogenate. Less than 20 % of these two substances were hydrolysed in gastric juice and intestinal fluid (Emberger, 1994a, b). More than 80 % of a radioactively labelled mandelic acid of 3,3,5-trimethylcyclohexanol, a cyclohexyl ester structurally related to the candidate substance menthyl salicylate [FL-no: 09.621], was hydrolysed after 15 minutes of incubation with rat hepatic microsomes (White et al., 1990).

Based on these data, it is anticipated that candidate esters and the one hemiketal ester [FL-nos: 06.136, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949], after intestinal absorption are hydrolysed to the corresponding alcohols/ketone and their corresponding carboxylic acids (see Table 7). The simple mono- and di-carboxylic acids [FL-nos: 08.001, 08.002, 08.004, 08.007, 08.008, 08.009 and 08.082] and 3-hydroxybutyric acid are expected to be completely metabolised through common routes of biotransformations. The acids salicylic acid [FL-no: 08.112] and 3-oxo-2-pentyl-1-cyclopentyl acetic acid (formed from [FL-no: 09.520]) are anticipated to be conjugated and excreted with the urine.

D.2.2. Reduction of ketone groups and oxidation of alcohol groups

Seven of the candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219, 07.255 and 09.520] contain a ketone group, which may be metabolically reduced to a hydroxyl group. This may also be expected for the hemiketal ester [FL-no: 06.136] after hydrolysis to ketone.

Incubation of human liver microsomes with the supporting substance trans-menthone resulted in formation of two metabolites. The major metabolite was a reduction product, (+)-neomenthol and a hydroxylation product, 7-hydroxymenthone was a minor metabolite (Miyazawa and Nakanishi, 2006)

Metabolism of the supporting substance carveol [FL-no: 02.062], the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870], was studied *in vitro*. (+)-Carveol and (+)-carvone were incubated with liver microsomes from dogs, rabbits, guinea pigs, mice, rats, monkeys and humans. (+)-Carveol was oxidised to (+)-carvone by liver microsomes of dogs, rabbits and guinea pigs, but not by those of humans, monkeys, rats and mice. On the other hand, the (+)-carvone was reduced to (+)-carveol by liver microsomes of all animals examined. These results suggest a species-specific metabolism of (+)-carveol, and shows that carveol is not converted to carvone in the liver of humans (Shimada et al., 2002).

In vivo metabolism of *l*-menthol was studied in adult male rats by giving the rats 800 mg/kg bw *l*-menthol solved in 1 % methyl cellulose solution by gavage every day for 20 days. Control rats were given vehicle only. The following metabolites of *l*-menthol were found in the urine: *p*-menthane-3,8-diol, *p*-menthane-3,9-diol, 3,8-oxy-*p*-menthane-7-carboxylic acid and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. The main urinary metabolites were *p*-menthane-3,9-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. Menthone was not detected (Madyastha and Srivatsan, 1988).

D.2.3. Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

Oxidation of alkyl groups have been observed for menthol and for the hydrolysis product of the candidate esters neo-dihydrocarvyl acetate [FL-no: 09.355] and menthyl formate [FL-no: 09.618], and for the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] (Truhaut et al., 1970; Yamaguchi et al., 1994).

D.2.4. Metabolism to glucuronides

The hydrolysis product menthol, as such or after the oxidation of the alkyl ring substituents, is mainly conjugated with glucuronic acid and excreted via the bile in rats. Low levels of oxidation products were found in the urine, but no unchanged menthol was detected in the urine, faeces or bile after oral administration of radioactive labelled menthol (Yamaguchi et al., 1994).

The candidate substances isodihydrocarveol and neo-dihydrocarveol [FL-nos: 02.167 and 02.075] are also anticipated to be conjugated with glucuronic acid, since dihydrocarveol after application by gavage to rabbits was found in the urine as the glucuronide (Hämäläinen, 1912; JECFA, 1999a). However, dihydrocarveol was also found to be excreted unchanged (Fischer and Bieligi, 1940; JECFA, 1999a). In rabbits, carvone is reduced to yield carveol, which then is converted to the glucuronic acid conjugate and excreted in the urine (Fischer and Bieligi, 1940). Carveol, the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870] is therefore anticipated to be conjugated with glucuronic acid and excreted in the urine.

Salicylic acid (resulting from hydrolysis of menthyl salicylate [FL-no: 09.621]) is excreted either unchanged or as salicyluric acid and salicylic glucuronide (Vree et al., 1994).

The candidate alicyclic ketones (*p*-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203], trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], *l*-piperitone [FL-no: 07.255] and methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]) are anticipated to be reduced to the corresponding secondary alcohols. These secondary alcohols and the candidate secondary alcohols cyclohexanol [FL-no: 02.070], neo-dihydrocarveol [FL-no: 02.075], isodihydrocarveol [FL-no: 02.167] and cyclopentanol [FL-no: 02.135] are mainly excreted as conjugates with glucuronic acid. Studies in rabbits with the supporting substance cyclohexanone [FL-no: 07.148] and with cyclopentanone and cycloheptanone show that 50–70 % of these substances are reduced to the corresponding alcohols (the candidate substances cyclohexanol [FL-no: 02.070] and cyclopentanol [FL-no: 02.135] and

cycloheptanol), which are conjugated with glucuronic acid and excreted (Elliott et al., 1959; James and Waring, 1971). Workers employed in a shoe factory were exposed to small amounts of cyclohexane in the air. Cyclohexanol and cyclohexanone were found in the urine of these workers, indicating that the same metabolic pathways are also found in humans (Governa et al., 1987). A recent study in humans shows that the main metabolite in urine after cyclohexanone or cyclohexanol exposure is not cyclohexanol-glucuronide as in rabbit and rats, but 1,2-cyclohexanediol-glucuronide (Mráz et al., 1994, 1998).

When the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] was given to rats and rabbits glucuronides of 3,5,5-trimethylcyclohexanol were detected in the urine (Truhaut et al., 1979).

D.2.5. Metabolism to sulphates

A small fraction of the two candidate substances, cyclopentanol and cyclohexanol [FL-nos: 02.135 and 02.070], is anticipated to be conjugated with sulphate and excreted in the urine. This is based on studies on the structurally related substances cyclopentanone, cyclohexanone and cycloheptanone, which were given by gavage to rabbits (1.7–2.3 mmol/kg) and rats (1.8–2.5 mmol/kg), and 1–3 % of the dose was found in the urine as sulphate conjugates (James and Waring, 1971).

D.3 Summary and conclusions

The 11 esters [FL-nos: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the evaluation of supporting substances (Heymann, 1980; Anders, 1989; White et al., 1990; Emberger, 1994a, b). The resulting carboxylic acids are either metabolised through common physiological pathways, such as beta-oxidation and the citric acid cycle, or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, p-menthan-3-one [FL-no: 07.059], and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-nos: 02.070, 02.075, 02.135, 02.167, 07.059, 07.109, 07.202, 07.203, 07.219 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, hydrolysis products of [FL-nos: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929 and 09.935], are also metabolised via this pathway. Neither menthol nor carveol or dihydrocarveol is anticipated to be oxidised to the corresponding ketone.

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl-substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion.

Thus, it may be anticipated that these 22 substances will be metabolised to innocuous products.

Appendix E. Toxicity data

Table 14: Acute toxicity

Chemical Name [FL-no] ^(a)	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
Menthol [02.015]	Mouse	M	Gavage	2 652	Food and Drug Research Laboratories, Inc. (1975)	
	Mouse	M	Gavage	4 384	Food and Drug Research Laboratories, Inc. (1975)	
	Mouse	NR	Gavage	3 100	Wokes (1932)	
	Rat	M, F	Gavage	3 180	Jenner et al. (1964)	
	Rat	M	Gavage	940	Food and Drug Research Laboratories, Inc. (1975)	
trans-Menthone [07.176]	Rat	M, F	Oral	1 600–1 950	(Levenstein (1973a), Igimi and Ide (1974)	Test material = racemic menthone
Menthyl acetate [09.016]	Rat	M, F	Gavage	> 7 000	Levenstein (1973b)	Test material = racemic menthyl acetate
	Rat	M, F	Oral	> 5 000	Shelanski (1972)	Test material = <i>l</i> -menthyl acetate
Dihydrocarveol [02.061]	Rat	NR	Oral	> 5 000	Moreno (1977)	
Dihydrocarvyl acetate [09.216]	Rat	NR	Oral	> 5 000	Moreno (1980)	
neo-Dihydrocarvyl acetate [09.355]	Rat	NR	Oral	> 5 000	Moreno (1980)	
Cyclopentanol [02.135]	Rat	NR	Gavage	< 625	Myers et al. (1980)	
3,5,5-Trimethylcyclohexanol [02.209]	Rat	M, F	Oral	3 250	Smyth and Carpenter (1948)	
Cyclohexanone [07.148]	Rat	M, F	Oral	1 705	Kohli et al. (1967)	
	Rat	M, F	Oral	1 840	Deichmann and LeBlanc (1943)	
	Rat	M, F	Oral	1 620	Smyth et al. (1969)	
	Rat	M, F	Oral	1 800	Gupta et al. (1979)	
	Mouse	M, F	Oral	2 070	Gupta et al. (1979)	
	Rabbit	M	Gavage	1 600	Treon et al. (1943)	
	Rabbit	M	IP	1 540	Gupta et al. (1979)	
	Guinea pig	M	IP	930	Price (1951)	

Table 14: Acute toxicity

Chemical Name [FL-no] ^(a)	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
Cyclohexanol [02.070]	Rat	M	Gavage	1 750	Miller and Sherman (1965)	
	Rat	NR	Oral	1 550	Birch (1978)	
	Rat	NR	Oral	2 060 ^(b)	Smyth et al. (1946)	
	Rat	NR	Oral	2 060	Bär and Griepentrog (1967)	
	Rat	M, F	Oral	1 120	Birch et al. (1981)	
	Rabbit	NR	Gavage	2 200–2 600 ^(c)	Treon et al. (1943)	
3-Methylcyclopentadecan-1-one [07.111]	Dog	M, F		> 2 000	You et al. (1997)	
	Rat	M, F		> 5 000	Oh et al. (1997)	
6-Isopropyl-3,9-dimethyl-1,4- dioxyspiro[4.5]decan-2-one [06.136]	Rat	NR	Oral	> 2 000	Flavour Industry (2006a)	
Methyl 3-oxo-2-pentyl-1- cyclopentylacetate [09.520]	Rat	NR	Oral	> 2 000	Flavour Industry (2006a)	
Carveol [02.062]	Rat	NR	Oral	3 000	Keating (1972)	
Carvyl acetate [09.215]	Rat	NR	Oral	> 5 000	Levenstein (1976)	

F, female; M, male; NR, not reported.

(a): Supporting substances are listed in brackets.

(b): Administered as 10 % solution in Tergitol 7.

(c): Minimum lethal dose.

Table 15: Subacute/subchronic/chronic/carcinogenicity studies

Chemical Name [FL-no] ^(a)	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg per day)	Reference	Comments
(Menthol [02.015])	Mouse; M, F 2/50	Diet	2 000, 4 000 ppm	103 weeks	600 ^(b)	National Cancer Institute (1979)	Good quality
	Mouse; F 2/30	Intraperitoneal injection (IP)	500 and 2 000 mg/kg three times week	24 weeks	A NOAEL was not determined	Stoner et al. (1973)	Good quality
	Rat; M, F 3/20	Gavage	0, 200, 400 and 800 mg/kg bw day	28 days	< 200 ^(c)	Thorup et al. (1983)	Relative good quality
	Rat; M, F 2/80	Diet	100 and 200 mg/kg bw	5.5 weeks	200 ^(b)	Herken (1961)	Limited information
	Rat; M, F 2/50	Diet	3 750 and 7 500 ppm	103 weeks	375 ^(b)	National Cancer Institute (1979)	Good quality
trans-Menthone [07.176]	Rat; M, F 3/20	Gavage	200, 400 and 800 mg/kg bw day	28 days	400	Madsen et al. (1986)	Good quality
	Mouse; F 2/30	IP	1 900 and 4 750 mg/kg three times week	24 weeks	A NOAEL was not determined ^(c)	Stoner et al. (1973)	Good quality
Cyclohexanone [07.148]	Mouse; M, F 7/20	Drinking water	400–47 000 ppm	13 weeks	M: approx. 3 300, F: approx. 6 500	Lijinsky and Kovatch (1986)	Good quality
	Rat; M, F 7/10	Drinking water	190–6 500 ppm	25 weeks	Approx. 330	Lijinsky and Kovatch (1986)	Good quality
	Mouse; M, F 3/84–104	Drinking water	6 500, 13 000 and (F) 25 000 ppm	2 years	Approx. 1 600	Lijinsky and Kovatch (1986)	Good quality
	Rat; M, F 2/104	Drinking water	3 300 and 6 500 ppm	2 years	Approx. 330	Lijinsky and Kovatch (1986)	Good quality
	Rat 1/7	IP	200 mg/kg bw (twice a day) five days/week	13 weeks	A NOAEL was not determined ^(b)	Perbellini et al. (1981)	Only neurotoxicity was checked. Limited experimental design

Table 15: Subacute/subchronic/chronic/carcinogenicity studies

Chemical Name [FL-no] ^(a)	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg per day)	Reference	Comments
Cyclohexanol [02.070]	Rats 1/7	IP	200 mg/kg bw (twice a day) five days/week	3 weeks (twice a day) plus 3 weeks (once a day)	A NOAEL was not determined ^(b)	Perbellini et al. (1981)	Limited experimental design
	Rat; M 1/6	Gavage	455 mg/kg day	7 days	455 ^(b)	Lake et al. (1982)	Limited quality
	Rat; M 1/NR	Drinking water	10 ppm	30 days	1 ^(b)	Messiha and Lox (1985)	Limited quality
2-sec- Butylcyclohexanone [07.095]	Rat 3/NR	Diet		91 days	370	Hummler (1969)	Study not available
3- Methylcyclopentadecan- 1-one [07.111]	Rat, M, F 3/20	Gavage		30 days	1 000 ^(d)	Oh et al. (1997)	
	Dog, M, F 3/6	Gavage		28 days	20 ^(d)	You et al. (1997)	
Methyl 3-oxo-2-pentyl- 1-cyclopentylacetate [09.520]	Rat M, F 10/10	Diet	0, 10, 50 or 100 mg/kg bw day	90 days	100	Kelly and Bolte (2000)	

IP, intraperitoneal injection; F, female; M, male; NR, not reported

(a): Supporting substances are listed in brackets.

(b): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(c): The test substance was administered three times per week for eight weeks; animals were observed for an additional 16 weeks.

(d): Study was performed with either a single dose or multiple doses that produced no adverse effect. The value is therefore not a true no observed effect level (NOEL), but is the highest dose tested that produced no adverse effects. The actual NOEL may be higher.

Table 16: Developmental and reproductive toxicity studies

Chemical Name [FL-no] ^(a)	Study type Duration	Species/Sex No/group	Route	Dose levels (mg/kg per day)	NOAEL (mg/kg per day) Including information on possible maternal toxicity	Reference
(Menthol [02.015])	Teratology Gestation days 6–15	Mouse; F 22	Gavage	0, 1.85, 8.59, 39.9, 185	185 ^(b)	Food and Drug Research Laboratories, Inc. (1973)
	Teratology Gestation days 6–15	Rat; F 22–23	Gavage	0, 2.18, 10.15, 47.05, 218	218 ^(b)	Food and Drug Research Laboratories, Inc. (1973)
	Teratology Gestation days 6–15	Hamster; F 20–22	Gavage	0, 4.05, 21.15, 98.2, 405	405 ^(b)	Food and Drug Research Laboratories, Inc. (1973)
	Teratology Gestation days 6–18	Rabbit; F 9–11	Gavage	0, 4.25, 19.75, 91.7, 425	425 ^(b)	Food and Drug Research Laboratories, Inc. (1973)
Cyclohexanol [02.070]	Reproductive NR ^(c)	Mouse; M, F NR	Diet	ca. 1 500 (1 %)	< 1 500 (< 1 %)	Gondry (1972)

F, female; M, male; NR, not reported.

(a): Supporting substances are listed in brackets.

(b): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(c): Animals were exposed during gestation, lactation and weaning over multiple generations. Total length of exposure not reported.

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] ^(a)	Test system	Test object	Concentration	Result	Reference	Comments
(Menthol [02.015])	Ames test	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537	0, and 6 concentrations up to 5 000 µg/plate	Negative ^(b)	Ishidate et al. (1984)	<i>d,l</i> -Menthol was used. The study is considered valid
	Ames test (pre- incubation method)	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535	3–666 µg/plate	Negative ^(b)	Zeiger et al. (1988)	<i>d,l</i> -Menthol was used. The study is considered valid
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA2637	0, 5–500 µg/plate	Negative ^(b)	Nohmi et al. (1985)	<i>d,l</i> -Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA2637	0, 20– 500 µg/plate	Negative ^(b)	Nohmi et al. (1985)	<i>l</i> -Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 6.4, 32, 160 and 800 µg/plate	Negative ^(b)	Andersen and Jensen (1984)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid
	Ames test	<i>E. coli</i> WP2 <i>uvrA</i> (Trp-)	100–800 µg/plate	Negative	Yoo (1986)	<i>l</i> -Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used
	Ames test	<i>S. typhimurium</i> TA97A, TA98, TA100, TA102	0, 5–800 µg/plate	Negative ^(b)	Gomes-Carneiro et al. (1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid
	Rec assay	<i>B. subtilis</i> H17, M45	Up to 10 000 µg/disk	Positive	Yoo (1986)	<i>l</i> -Menthol was used. Inhibition zone for <i>rec</i> - and <i>rec</i> + was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation or a control group was used. The validity of this study cannot be assessed. The method (<i>rec</i> -assay) has poor predictive value
	Rec assay	<i>B. subtilis</i> H17, M45	20 µg/disk	Negative	Oda et al.	<i>l</i> -Menthol was used. The article is not in English.

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] ^(a)	Test system	Test object	Concentration	Result	Reference	Comments
					(1979)	Only one concentration level is mentioned in a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (<i>rec-assay</i>) has poor predictive value
	Alkaline elution assay	Rat hepatocytes	0, 0.1–1.3 mM (203.2 µg/ml) ^(c)	Negative	Storer et al. (1996)	The experiment employed d-Menthol. An increase in DNA breaks was observed only at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid
	Sister chromatid exchange	Chinese hamster ovary cells	5–50 and 0, 2–25 µg/ml ^(d) 0, 16–167 µg/ml ^(e)	Negative ^(b)	Ivett et al. (1989)	<i>d,l</i> -Menthol was used. The compound was tested up to toxic or near-toxic concentration levels. The study is considered valid
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1, 10 mM (1 563 µg/ml) ^(c)	Negative ^(b)	Murthy et al. (1991)	The study is considered valid
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1, 10 µg/ml	Negative	Food and Drug Research Laboratories, Inc. (1975)	The report does not mention exogenous metabolic activation. The study is considered valid
	Chromosome aberration	Chinese hamster fibroblasts	0 and three concentrations up to 200 µg/ml	Negative ^(d)	Ishidate et al. (1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid
	Chromosome aberration	Chinese hamster ovary cells	0, 50–250 µg/ml	Negative ^(b)	Ivett et al. (1989)	<i>d,l</i> -Menthol was used. The compound was tested up to toxic or near-toxic concentration levels. The study is considered valid
	Chromosome aberration	Human lymphocytes	0, 0.1, 1, 10 mM (1 563 µg/ml) ^(c)	Negative ^(b)	Murthy et al. (1991)	The study is considered valid
	Gene mutation assay	Mouse lymphoma L5178Y TK+/- cells	0, 12.5–200 µg/ml	Negative ^(b)	Myhr and Caspary (1991)	<i>d,l</i> -Menthol was used. The maximum concentration was selected by a preliminary test The study is considered valid

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] ^(a)	Test system	Test object	Concentration	Result	Reference	Comments
trans-Menthone [07.176]	Ames test	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537	0, 6.4– 800 µg/plate	Positive ^(b)	Andersen and Jensen (1984)	Concentrations were selected based on preliminary experiments. In the absence of metabolic activation, menthone was mutagenic only to strain TA1537 at 6.4 and 32 µg/ml (slightly less than twofold increase in mutation frequency), but not at higher (toxic) concentrations. In addition, in the absence of metabolic activation, there was a concentration-dependent increase in number of TA97 strain revertants (up to fourfold increase at 600 µg/l). It was stated that metabolic activation did not enhance the mutagenicity of menthone. The study is considered valid
Cyclopentanol [02.135]	Modified Ames test	<i>S. typhimurium</i> G46, TA98, TA100, TA1535, C3076, TA1537, D3052, TA1538 <i>E. coli</i> WP2, WP2 <i>uvrA</i> –	0, 0.1– 1 000 µg/ml	Negative ^(b)	McMahon et al. (1979)	The study was performed with agar plates containing the following concentration gradients: 0.1–1, 1–10, 10–100 and 100–1 000 µg/ml. The study is considered valid, although tabulated data on cyclopentanol were not presented
(Cyclohexanone [07.148])	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	33– 3 333 µg/plate	Negative ^(b)	NTP (2007)	
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 33– 10 000 µg/plate	Negative ^(b)	Haworth et al. (1983)	The highest level tested was the highest of either 10 000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 3 µmol/plate	Negative ^(b)	Florin et al. (1980)	A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03–30 µmol/plate. The validity of the study cannot be evaluated

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] ^(a)	Test system	Test object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	NR	Positive	Massoud et al. (1980)	Only an abstract is available. No reporting with respect to metabolic activation. The substance was also tested with <i>B. subtilis</i> . With this species, toxicity was found, as well as a positive response. The validity of the study cannot be evaluated because of the lack of experimental information
	Cytogenetic assay	Human leucocytes	0.1–10 mM	Inconclusive ^(d)	Collin (1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose–response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate
	Chromosomal aberration	Human lymphocytes	0, 0.005– 0.1 µg/ml	Positive	Dyshlovoi et al. (1981)	Article is not in English. Only an abstract is available in English. The validity of the study cannot be evaluated
	Gene mutation (HPRT)	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative ^(b)	Aaron et al. (1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated
	Chromosomal aberration	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative ^(b)	Aaron et al. (1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated
	Sister chromatic exchange	Chinese hamster ovary cells	0, 7.5 µg/ml	Positive ^(d) Negative ^(e)	Aaron et al. (1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated
	Mutation	Mouse lymphoma <i>L5178Y</i> TK+/- cells	312.5– 5 000 µg/ml	Negative	NTP (2007)	
Cyclohexanol [02.070]	Ames test	<i>S. typhimurium</i> TA98, TA1535, TA1537, TA1538	500– 10 000 µg/plate ^(d) 500– 15 000 µg/plate ^(e)	Negative ^(b)	Barsky (1976)	The highest concentrations showed cytotoxicity. The study is considered valid

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] ^(a)	Test system	Test object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	0, 10– 3 333 µg/plate	Negative ^(b)	Haworth et al. (1983)	The highest level tested was the highest of either 10 000 µg/plate, limit of solubility or maximal non-toxic concentration. Both rat and hamster liver S9 were used. The test was run twice. The study is considered valid
	Chromosomal aberration	Human leucocytes	0.1–10 mM	Inconclusive ^(d)	Collin (1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose–response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate
(Cyclohexyl acetate [09.027])	DNA damage	<i>B. subtilis</i> H17(<i>rec</i> ⁺), M45 (<i>rec</i> [−])	19 mg/disc	Negative ^(b)	Yoo (1986)	
(Cyclohexyl butyrate [09.230])	DNA damage	<i>B. subtilis</i> H17(<i>rec</i> ⁺), M45 (<i>rec</i> [−])	19 mg/plate	Negative ^(b)	Oda et al. (1979)	
(Cyclopentanone [07.149])	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	2.5– 2 500 mg/plate	Negative ^(b)	Florin et al. (1980)	
(2,2,6-Trimethyl cyclo-hexanone [07.045])	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	4.2– 3 600 mg/plate	Negative ^(b)	Florin et al. (1980)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	5 mg/plate	Negative ^(b)	Thompson (2000)	Valid study in compliance with the OECD Guideline 471
	Reverse mutation	<i>E. coli</i> WP2 <i>uvrA</i>	5 mg/plate	Negative ^(b)	Wagner and Klug (2000)	Valid study in compliance with the OECD Guideline 471
	Forward mutation Test	Mouse lymphoma cells <i>L5178y</i>	200 or 300 µg/l 300 µg/l	Positive ^(d) Positive ^(e)	Ross and Harris (1979)	Pre-GLP study—not possible to assess the reliability of these studies.

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] ^(a)	Test system	Test object	Concentration	Result	Reference	Comments
	Forward mutation Test	Mouse lymphoma cells <i>L5178y</i>	100–325 µg/l	Negative ^(b)	Cifone (2001)	Valid study and in compliance with OECD Guideline 476
(Carveol [02.062])	Ames test (pre-incubation)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	560 µg/plate	Negative ^(b)	Mortelmans et al. (1986)	
(Carvyl acetate [09.215])	Ames test (pre-incubation)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	333 µg/plate	Negative ^(b)	Mortelmans et al. (1986)	
(L-Menthyl (R,S)-3-hydroxybutyrate)	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and TA1538	78, 156, 312, 625, 1250, 2 500 or 10 000 µg/plate	Negative ^{(b)(f)}	Morimoto (2005)	The JECFA evaluated the racemate of L-menthyl (R,S)-3-hydroxybutyrate
	Reverse mutation	<i>E. coli</i> WP2 <i>uvrA</i>	78, 156, 312, 625, 1 250, 2 500 or 10 000 µg/plate	Negative ^{(b)(f)}	Morimoto (2005)	

HPRT, hypoxanthine-guanine phosphoribosyltransferase gene; NA, not applicable; NR, not reported.

(a): Supporting substances are listed in brackets.

(b): With and without S9 metabolic activation.

(c): Calculated based on molecular weight of menthol = 156.3 g/mol.

(d): Without S9 activation.

(e): With S9 activation.

(f): Modified pre-incubation method.

(g): Marked differential toxicity was seen at dose levels above 25 µmol/plate. No observations were noted at lower dose levels.

Table 18: Genotoxicity (*in vivo*)

Chemical Name [FL-no] ^(a)	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Menthol [02.015])	Host mediated mutation assay	<i>S. typhimurium</i> TA1530 and G46; <i>S. cerevisiae</i> D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Equivocal	Food and Drug Research Laboratories, Inc., 1975	Negative results, with the exception of the combination <i>S. typhimurium</i> TA1530 – 5000 mg/kg bw and <i>S. cerevisiae</i> D3 – 1150 mg/kg bw per day. This study is considered valid, but the equivocal result might have low relevance since the effect was observed at only very high (lethal) dose levels.
	<i>In vivo</i> cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	Food and Drug Research Laboratories, Inc., 1975)	Oral DL ₅₀ was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, because of lethality.
	<i>In vivo</i> micronucleus assay	B6C3F1 male mouse bone marrow cells	Intra peritoneal	0, 250 - 1000 mg/kg bw per day, for 3 days	Negative	Shelby et al., 1993	<i>d,l</i> -Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	<i>In vivo</i> dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	Food and Drug Research Laboratories, Inc., 1975	This study is considered valid.

Table 18: Genotoxicity (*in vivo*)

Chemical Name [FL-no] ^(a)	Test System	Test Object	Route	Dose	Result	Reference	Comments
(trans-Menthone [07.176])	<i>In vivo</i> SMART assay	<i>D. melanogaster</i> – flr3 × mwh cross	Whole body	0, 1.3 µl/disk	Positive	Franzios et al., 1997	Somatic Mutation and Recombination Test. Only one dose level (1.29 µl/disk; slightly higher than the LD ₅₀) was tested. A two-fold increase in mutation frequency as compared with control was observed. Menthone was not recombinogenic. The validity of this study is unclear.
(Cyclohexanone [07.148])	<i>In vivo</i> sex-linked recessive lethal mutation	<i>D. melanogaster</i>	NR 3 days exposure	0, 1 µl/ml	Negative	Goncharova, 1970	Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.
Cyclohexanol [02.070]	<i>In vivo</i> sex-linked recessive lethal mutation	<i>D. melanogaster</i>	NR 3 days exposure	0, 1 µl/ml	Negative	Goncharova, 1970	The validity of the study cannot be evaluated.
	<i>In vivo</i> micronucleus test	NMRI mouse bone marrow	Oral	500 - 1500 mg/kg bw	Negative	Gelbke, 1991	The study is considered valid. The negative result of this study is of limited relevance, since no bone marrow toxicity could be detected. Testing at higher dose levels might not have been possible because of observed general toxicity at the highest dose.
Methyl 3-oxo-2- pentyl-1- cyclopentylacetate [09.520]	Micronucleus test	ICR mice	Intraperit onal	280, 560 or 1120 mg/kg bw	Negative	Gudi and Krsmanovic, 1998	Valid study in compliance with the OECD Guideline 474.
	Unscheduled DNA Synthesis	Rat hepatocytes	Intraperit onal	333.3 or 1000 mg/kg bw	Negative	Durward, 2001	Valid study in compliance with the OECD Guideline 486.

NR: Not reported.

(a): Supporting substances are listed in brackets.



United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7508C)

September 28, 2004

Report of the Food Quality Protection Act (FQPA) Tolerance Reassessment Progress and Risk Management Decision (TRED) for Menthol



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

CERTIFIED MAIL

Dear Registrant:

This is the Environmental Protection Agency's (hereafter referred to as EPA or the Agency) "Report of the Food Quality Protection Act (FQPA) Tolerance Reassessment Progress and Risk Management Decision for Menthol," which was approved on September 28, 2004. This document is also known as a Tolerance Reassessment Decision, or TRED.

The Federal Food, Drug and Cosmetic Act (FFDCA), as amended by FQPA, requires EPA to reassess all the tolerances for registered chemicals in effect on or before the enactment of the FQPA on August 3, 1996. In reassessing these tolerances, the Agency must consider, among other things, aggregate risks from non-occupational sources of pesticide exposure, whether there is increased susceptibility to infants and children, and the cumulative effects of pesticides with a common mechanism of toxicity. Once a safety finding has been made, the tolerances or exemptions from the requirement of a tolerance are considered reassessed.

Menthol, as an active ingredient, is used in over-wintering beehives to control tracheal mites in honey bees. Menthol has a peppermint flavor and smell and occurs naturally in many plants, especially mint. It is also found in foods derived from those plants, including honey made from pollen that naturally contains menthol. The Agency has evaluated all current registered uses of menthol and has determined that there is a reasonable certainty that no harm to any population subgroup will result from exposure to menthol when considering dietary exposure and all other non-occupational sources of pesticide exposure for which there is reliable information. Therefore, no mitigation measures are needed, and the current exemption from the requirement of a tolerance at 40 CFR 180.1092 is now considered reassessed under section 408(q) of the FFDCA.

FQPA requires that EPA consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity." The Agency considers other substances because low-level exposures to multiple chemical substances that cause a common toxic effect by a common mechanism could lead to the same adverse health effect, as would a higher level of exposure to any of the other substances individually. The Agency has not yet determined whether the chemical class which includes menthol exhibits a common mechanism of toxicity. Therefore, the Agency defers any cumulative risk assessment to a later date. For the purposes of the tolerance exemption reassessment of menthol, EPA is assuming no common mechanism with other compounds. Therefore, a cumulative assessment was not conducted for this TRED.

The current tolerance exemption for menthol at 40 CFR 180.1092 is now considered reassessed under section 408(q) of the FFDCA. This document summarizes the Agency's decision on the tolerance reassessment for menthol. Please contact Mark Perry of my staff with any questions regarding this decision. He may be reached by phone at (703)308-8024 or by e-mail at perry.mark@epa.gov.

Sincerely,

A handwritten signature in black ink, appearing to read "Debra Edwards", with a stylized flourish at the end.

Debra Edwards, Ph.D.

Director

Special Review and Reregistration Division

Enclosures: *FQPA Risk Assessment for Tolerance Reassessment of Menthol* (9/28/2004, OPP
Lower Toxicity Pesticide Chemical Focus Group)

Tolerance Reassessment Eligibility Decision

Menthol

CASE 4063

**Special Review and Reregistration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 South Bell Street
Arlington, VA 22202**

Background:

This document represents the Lower Risk Pesticide Chemical Focus Group's (LRPCFG) Tolerance Reassessment Eligibility Decision (TRED) on menthol. This assessment summarizes available information on the use, physical/chemical properties, toxicological effects, exposure profile, and environmental fate and ecotoxicity for menthol. In performing this assessment, EPA has utilized reviews previously performed by EPA, the World Health Organization (WHO) and the Organisation for Economic Co-operation and Development (OECD). Information from the Hazardous Substance Data Bank (HSDB) has also been used in this assessment.

In January 1993, a Reregistration Eligibility Decision (RED) was made for menthol, although the Agency determined that the need for full documentation was waived (i.e., an actual RED document was not issued). The purpose of this TRED document is to reassess the exemption from the requirement of a tolerance for residues of this chemical when used as an active ingredient (40 CFR 180.1092) and as an inert ingredient in pesticide formulations. Because the menthol RED was prior to the development of the Food Quality Protection Act (FQPA) in August 1996, tolerances need to be reassessed to meet the FQPA standard. The Agency has considered any new data generated after the tolerance exemption was issued, new Agency guidance or other federal regulations, as well as previously available information in this assessment.

I. Executive Summary:

Menthol, as an active ingredient, is used in over-wintering beehives to control tracheal mites in honey bees. As an inert ingredient, menthol is in pesticides products used to control worms and caterpillars on fruits, vegetables, ornamental and shade trees. Toxicity information was collected on menthol and an endpoint for assessing short-term residential dermal exposure to menthol was chosen. The endpoint selected was a No Observed Effect Level (NOEL) of 560 mg/kg/day, based on increased incidence of perivascular lymphoid hyperplasia and interstitial nephritis observed in female mice in the 1100 and 2300 mg/kg-bw dose groups in a 13-week rat oral study. The dermal dose was conservatively converted to an oral equivalent dose using a 100% dermal absorption factor. An uncertainty factor of 100 (10 for interspecies extrapolation and 10 for intraspecies variation) was used in this assessment. EPA had earlier concluded in the RED for menthol that exposure from reasonable pesticide usage is not expected to present an increased dietary risk or residential risk beyond that from ordinary exposure.

Dietary (food) exposures have been assessed for menthol uses as an inert ingredient. Menthol is 7% of the cPAD for the general population and 24% of the cPAD for Children (1-2 years), indicating that the dietary exposures are not of concern to the Agency. Drinking water concentrations were not estimated for menthol due to its limited usage as an active ingredient and an inert ingredient, as well as its various physical and chemical properties (sorption to soil and sediment and volatilization).

Residential exposure to menthol is expected to occur through its use as an inert ingredient in pesticide formulations used in gardens, and through its use in disinfectants. To estimate residential handler and postapplication exposures to the inert uses of menthol, the Pesticide Inert Risk Assessment Tool (PIRAT, test version) was used. It was assumed that exposure would be to soluble and emulsifiable concentrate formulations. For residential handler exposure scenarios for the use of menthol as an inert ingredient, the calculated MOEs ranged from 310,000 for applications of soluble concentrate formulations using a low-pressure handwand to 1,400,000 for applications of emulsifiable concentrate formulations using a hose-

end sprayer in gardens and trees. For residential postapplication exposures, it was assumed that the transfer coefficient for adults would be 1000 cm²/hr based on data from the Agriculture Reentry Task Force (ARTF) for adults performing postapplication tasks on tomatoes. The transfer coefficient for youths is assumed to be 500 cm²/hr. No postapplication calculations were performed for toddlers' incidental oral ingestion, since toddlers are not expected to spend significant time contacting gardens or ornamental trees. For residential postapplication exposures, calculated MOEs ranged from a low of 460,000 for dermal contact with residue in foliage by adults following application of soluble concentrate formulations to a high of 1,400,000 for dermal contact with residue in foliage by youths following application of emulsifiable concentrate formulations. To examine exposure to menthol through the use of products such as general purpose cleaners, the Consumer Exposure Module (CEM) was used and a MOE of 121,000 was estimated. In this assessment, calculated MOEs did not exceed the Agency's level of concern (MOE<100) for any handler or postapplication scenarios. Considering aggregate exposure, it has been qualitatively determined that aggregate exposures due to the pesticidal uses of menthol would not be of concern to the Agency. This conclusion is based on the extremely low residential exposure, low dietary exposure and the absence of concern for drinking water exposures.

II. Use Information:

Menthol has a peppermint flavor and smell and occurs naturally in many plants, especially mint. It is also found in foods derived from those plants, including honey made from pollen that naturally contains menthol. There are various isomers of menthol; the four most commonly used compounds are L-menthol (CAS No. 2216-51-5), D-menthol (CAS No. 15356-60-2), the racemic mixture D/L-menthol (CAS No. 89-78-1) and menthol, unspecified isomers, (CAS No. 1490-04-6). Menthol is used as both an active (PC Code: 051601) and inert ingredient (PC Code: 851601) in various products. There is one product currently registered with menthol as an active ingredient, and it is listed in OPPIN as containing the unspecified isomers of menthol (CAS No. 1490-04-6). This product with menthol as an active ingredient is registered for use as a nearly pure crystalline solid in over-wintering beehives for the treatment of tracheal mites in honey bees.

FDA lists menthol in 21CFR 172.515 as a synthetic flavoring substance that may be safely used as a food additive. It is also considered by FDA to be Generally Recognized as Safe (GRAS) for use in food as an essential oil or natural extractive, with no limitations other than complying with good manufacturing practices (21 CFR 182.20). Menthol is also used extensively in drugs, cigarettes, cosmetics, and liqueurs to produce a mint flavor or odor, as well as in toothpastes. It is also used in medications for humans, as a topical antipruritic, and in inhalers for treatment of upper respiratory disorders or added to water for inhalation in acute bronchitis.

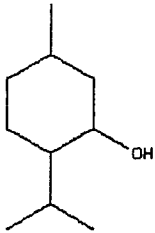
The exemption from the requirement for a tolerance being reassessed in this document, with the respective citation in the Code of Federal Regulations (CFR), and the use patterns as an active and inert ingredient are listed in Table 1. There are no exemptions for the requirement for a tolerance for the use of menthol as an inert ingredient.

Table 1. Tolerance Exemptions Being Reassessed in this Document					
Tolerance Exemption Expression	CAS No.	40 CFR	PC Code	Use Pattern	List Classification
Menthol	Active Ingredient				
	1490-04-6	180.1092	051601	Used in over-wintering bee hives	N/A
	Inert Ingredient				
	89-78-1	N/A	851601	Pesticide	3 ^a

a. Inert ingredients are categorized into four lists as described in the 1987 and 1989 Policy Statements. List 3 inert ingredients are those inerts for which there was no basis for listing the chemical on any of the other lists.

III. Physical/Chemical Properties:

The physical and chemical properties of menthol are provided in Table 2. The information was obtained from the Hazardous Substance Data Bank (HSDB) profile on L-menthol and the (SIDS) Initial Assessment Report for the menthols category. As indicated in the SIDS Initial Assessment Report, L-menthol, D-menthol, D/L-menthol and menthol (unspecified isomers) have similar physicochemical properties.

Table 2. Physical/Chemical Properties of L-Menthol	
Scientific name	5-methyl-2-(1-methylethyl)-cyclohexanol
Structure for menthol (unspecified isomer)	
Molecular formula	C ₁₀ H ₂₀ O
Molecular weight	156.27 g/mole
Physical state	Colorless crystals or granules, peppermint odor and flavor
Boiling point	212 °C
Melting point	41-43 °C
Solubility in water	456 mg/L at 25°C
Density/Specific Gravity	0.890

References:
HSDB, 2003;
OECD, 2003.

Table 2. Physical/Chemical Properties of L-Menthol		
Vapor Pressure	0.0637 mm Hg (25 °C); 0.85 hPa (25 °C)	
Estimated Octanol/Water Coefficient	3.4	
Estimated Henry's Law constant	1.5×10^{-5} atm- m ³ /mole	
Estimated Soil Sorption Coefficient	1500	

IV. Hazard Assessment:

In 1999, the World Health Organization published a document summarizing safety data on select food additives, including menthol, prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In this document, JECFA reviewed various studies conducted with L-menthol and D/L-menthol and concluded that there was no difference in the toxicity of the two isomers. As a result, the JECFA established an acceptable daily intake (ADI) not to exceed 4 mg/kg body weight for the two isomers of menthol, based on a NOEL of 380 mg/kg-bw per day in a long-term study in rats (WHO, 1999).

In 2003, a Screening Information Data Set (SIDS) Initial Assessment Report for the menthols category was published by the Organisation for Economic Cooperation and Development (OECD) under the HPV Chemicals Programme. In the SIDS Initial Assessment Report, the menthols category includes the isomers D-menthol (CAS No. 15356-60-2) and L-menthol (2216-51-5), the racemate D/L-menthol (CAS No. 89-78-1) and menthol, unspecified isomers, (CAS No. 1490-04-9). The SIDS Initial Assessment Report noted that all of these chemicals have similar physicochemical, toxicological, ecotoxicological and environmental fate properties. The SIDS Initial Assessment Report concluded that the chemicals in the menthols category are of low priority for further work due to their low hazard potential to human health. It was noted that although these chemicals may possess a hazard to the environment, no further work was needed, because acute toxicity is only evident at very high dose levels (OECD, 2003).

Key toxicological data for menthol are provided in Table 3. These data were obtained from published studies in peer reviewed journals summarized in the WHO JECFA document (WHO, 1999) and the SIDS Initial Assessment Report (OECD, 2003). Data cited in the initial proposed rules for tolerance exemption were previously reviewed by the Agency in the 1993 Reregistration Eligibility Document.

Menthol is relatively non-toxic in acute testing by the oral and dermal route. It is not a dermal sensitizer. There are no acute inhalation toxicity data for menthol (Table 3). The only long-term toxicity data showed limited toxicity concerns. When rats were exposed to L-menthol vapors (0.087, 0.15, or 0.26 ppm; equivalent to 0.57, 0.96, and 1.68 mg/m³) in whole body exposures for 71-79 days, there were no systemic effects at any dose; however, histological examination revealed irritant effects on lungs and trachea, but only at highest dose, 0.26 ppm = 1.68 mg/m³.

Table 3. Summary of Toxicity Data for Menthol

Acute Toxicity						
Test (Primary Reference)	Route of Administration	Form of Menthol	Dose	Species	Results	Source of Reference
Oral LD ₅₀ (mg/kg-body weight (bw))	Oral	L- menthol	various	Mouse	3400; 4380 mg/kg-bw	WHO, 1999; OECD, 2003
		D/L - menthol		Rat	940; 2615; 3300	
				Mouse	3100	WHO, 1999; OECD, 2003
				Rat	2602; 2900; 3180	
Dermal LD ₅₀	Dermal	D/L- menthol	various	Rabbit	> 5000	OECD, 2003
Inhalation LC ₅₀	Studies on the acute inhalation toxicity of menthol have not been identified. When rats exposed to L-menthol vapors (whole body exposures) for 71-79 days, no systemic effects at any dose, but histological examination revealed irritant effects on lungs and trachea, but only at highest dose.					WHO, 1999; OECD, 2003
Eye Irritation		Not specified	N/A	Rabbit	Severe eye irritation	EPA, 1993
		all			Slightly irritating in testing with all four isomers	OECD, 2003
Dermal Irritation		D/L- menthol	N/A	Rabbit	Mild irritation	EPA, 1993
		all			Moderately irritating in testing with all four isomers	OECD, 2003
Dermal sensitization	Buehler method	L- menthol		Rabbit	negative	OECD, 2003
	Local lymph node assay	L- menthol		Mouse	negative	OECD, 2003
	Modified Draize	L- menthol		Guinea pig	"Ambiguous (positive after rechallenge)"	OECD, 2003

Test (Primary Reference)	Route of Administration	Form of Menthol	Dose	Species	Results	Source of Reference
	skin sensitization and dermal challenge testing	various		humans	“skin sensitization potency is low “	OECD, 2003
Subchronic and Chronic Toxicity						
5-day oral (Litton Bionetics, Inc., 1975)	Oral-gavage	L- menthol	2000, 25000, 32000, 4000, or 5000 mg/kg	Mice	LD ₅₀ = 2600 mg/kg-bw	WHO, 1999; OECD, 2003
13-week oral (NCI, 1979)	Oral-diet	D/L- menthol	0, 140, 280, 560, 1100, or 2300 mg/kg- bw/day	B6C3F ₁ Mice	NOEL: 560 mg/kg-bw/day [d] LOEL: 1100 mg/kg-bw/d, based on increased incidence of perivascular lymphoid hyperplasia and interstitial nephritis in female mice only.	WHO, 1999
			Males: 243, 488, 978, 1956, or 3913 mg/kg-bw/d Females: 290, 595, 1193, 2386, or 4773 mg/kg-bw/d		NOAEL: 1956 mg/kg-bw/d in males; 2386 mg/kg-bw/d in females; LOAEL: 4773 mg/kg-bw/d in males; 3913 mg/kg-bw/d in females, based on reduction in body weight gain only in highest dose group	OECD, 2003
13-week oral (NCI, 1979)	Oral-diet	D/L- menthol	0, 93, 190, 380, 750, or 1500 mg/kg- bw/day	Fischer Rat	NOEL: 750 mg/kg-bw/d LOEL: 1500 mg/kg-bw/d, based on increased incidence of interstitial nephritis in male rats only.	WHO, 1999
			Males: 59, 114, 231, 472, or 937 mg/kg-bw/d Females: 67, 142, 285, 521, or 998 mg/kg-bw/d		NOAEL: 937 mg/kg-bw/d in males; 998 mg/kg-bw/d in females. LOAEL values were identified, since there were no effects on organ weights and no other adverse noted at highest dose tested; however, slight increase in the severity of spontaneous interstitial nephritis in males (which OECD stated was not an adverse effect).	OECD, 2003

Test (Primary Reference)	Route of Administration	Form of Menthhol	Dose	Species	Results	Source of Reference
103-week oral (NCI, 1979)	Oral-diet	D/L- menthol	0, 300, or 600 mg/kg-bw/day	B6C3F ₁ Mice	NOEL: 600 mg/kg-bw No significant carcinogenic nor organ-specific adverse effects at the highest dose tested.	WHO, 1999
			0, 334, or 667 mg/kg-bw/day		NOAEL: 667 mg/kg-bw/d, based on no adverse effects at highest dose tested	OECD, 2003
103-week oral (NCI, 1979)	Oral-diet	D/L- menthol	0, 190, or 380 mg/kg-bw/day	Fischer Rat	NOEL: 380 mg/kg-bw, based on no significant carcinogenic nor any other organ-specific adverse effects at the highest dose tested.	WHO, 1999
			0, 188, or 375 mg/kg-bw/day		NOAEL: 375 mg/kg-bw/d in males (highest dose tested); 188 mg/kg-bw/d in females; LOAEL: 375 mg/kg-bw/d in females, based on slightly reduced body weight in females. There was a slight increase in spontaneous, chronic inflammation of kidney in male rats in both dose groups (which study determined to be not an adverse effect).	OECD, 2003
Pre-natal Developmental (Food and Drug Research Labs, Inc., 1973)	Oral-gavage	L- menthol	0, 1.9, 8.6, 40, or 190 mg/kg-bw/day	CD-1 Mice	no teratogenic effects observed.	WHO, 1999; OECD, 2003
Pre-natal Developmental (Food and Drug Research Labs, Inc., 1973)	Oral-gavage	L- menthol	0, 2.2, 10, 47, or 220 mg/kg-bw/day	Wistar Rat	no teratogenic effects observed.	WHO, 1999; OECD, 2003
Pre-natal Developmental (Food and Drug Research Labs, Inc., 1973)	Oral-gavage	L- menthol	0, 4.1, 21, 98, or 400 mg/kg-bw/day	Golden hamsters	no teratogenic effects observed.	WHO, 1999; OECD, 2003
Pre-natal Developmental (Food and Drug Research Labs, Inc., 1973)	Oral-gavage	L- menthol	0, 4.3, 20, 92, or 430 mg/kg-bw/day	Dutch- belt rabbit	no teratogenic effects observed.	WHO, 1999; OECD, 2003

In rats, most of the orally administered menthol is rapidly eliminated in the urine or feces as the glucuronic acid conjugate or various oxidation products. In rabbits, orally administered menthol is similarly conjugated with glucuronic acid and eliminated in the urine, with about 86% elimination by glucuronidation within 10 hours. Similarly, humans also rapidly metabolize menthol primarily by conjugation with glucuronic acid and elimination in the urine. WHO (1999) postulated that cytochrome P450-mediated oxidation occurs in humans, yielding various alcohol and hydroxy acid derivatives, which would also be eliminated in the urine unchanged or conjugated with glucuronic acid.

In feeding studies with D/L-menthol, no evidence of carcinogenicity was found in mice and rats at the doses tested. According to WHO (1999), the highest doses tested were 380 mg/kg-bw/day in rats, and 600 mg/kg-bw/day in mice, while OECD (2003) reported these maximum doses as 375 and 667 mg/kg-bw/day, respectively for the rats and mice. Note that these data indicate that there was a slight recalculation of the doses between the WHO and OECD documents; however, in addition, the OECD document assessed the data to determine the actual severity of the adverse effects, and reported NOAELs, while the WHO document only reported the levels at which effects were noted, without assessing whether the effects were truly "adverse." Thus, the information listed in Table 3 for the NCI 1979 study is as reported in the respective WHO (1999) and OECD (2003) documents. Note that regardless of whether NOEL or NOAEL values are reported, menthol has a very low degree of subchronic and chronic toxicity to rats and mice.

While no studies of reproductive toxicity were available with menthol, in repeated dose studies with gavage-administered L- menthol to test developmental effects, no effects on reproduction were observed in mice, rats, hamsters, or rabbits. There were no effects on maternal body weights, appearance, behavior, or food consumption. In examinations of the fetuses of each species, there were no effects on nidation (nest building), maternal survival, fetal survival, or fetal abnormalities. The numbers of abnormalities seen in soft or skeletal tissues of treated animals did not differ from those occurring spontaneously in the controls. In addition, OECD (2003) concluded that although no fertility studies were available, histopathological examinations of the reproductive organs of rats and mice showed no changes following repeated dose 90-day and 2-year duration toxicity studies with D/L-menthol and also in carcinogenicity studies with D/L-menthol, and concluded "there is no indication of a potential of D/L-menthol to interfere adversely with reproduction." In addition, OECD (2003) specifically concluded that "L-menthol was not embryo- or fetotoxic and had no teratogenic properties in well performed gavage studies in various species (rat, mouse, rabbit, hamster) at not maternally toxic doses (185-425 mg/kg bw/d). No maternally toxic levels were used in these studies."

Available data indicate that menthol is generally not genotoxic, based on studies conducted *in vitro* with bacterial and mammalian test systems with L-menthol and D/L-menthol (WHO, 1999; OECD, 2003). In 29 studies, the results were negative. When menthol was administered at the maximum tolerated dose to rats and mice, and hepatocytes were removed after 1 to 2 days and replicative DNA synthesis measured, there was a 6% increase in rats and 1.7% in mice; however, WHO (1999) concluded that these results indicate cell replication (i.e., mitogenesis), but not genotoxicity, and OECD (2003) concluded that these results "demonstrated no mutagenic potential."

Special Considerations for Infants and Children

At this time, there is no concern for potential sensitivity to infants and children. Therefore, the additional tenfold FQPA safety factor (SF 10X) is unnecessary, and has been removed (equivalent to 1x). In developmental toxicity studies in which L-menthol was fed to mice, rats, hamsters, and rabbits, no teratogenic effects were observed at doses ranging from maximum doses of 190 to 430 mg/kg-bw,

depending on the species (WHO, 1999; OECD, 2003).

Toxic Endpoint Selection:

The WHO (1999) report stated that the limited data which allow comparisons of metabolism and toxicity between the various forms provide no indication of any differences in the toxicity between L-menthol and D/L-menthol. And OECD (2003) also concluded the available toxicity data indicate very similar toxicity profiles for all of the menthol isomers investigated. Thus, toxicity endpoint selection was made without regard to the specific menthol isomer which had been tested.

For conducting dietary exposures for menthol, there was no single dose (i.e., acute) effect endpoint identified. For chronic dietary exposures, the toxicological endpoint of concern selected was the lowest long-term NOAEL observed, 188 mg/kg/day, for the female rats from the 103-week oral feeding study (Table 3). The selection of this endpoint is very "conservative" (i.e., health-protective), because it represents the maximum dose tested in the study, and there were no effects observed at this dose; thus, if a higher dose had been tested, there may have been an even higher NOAEL.

For the residential exposure assessment of menthol, there are no dermal toxicological studies and no dermal absorption studies available in the existing literature. Therefore, an oral endpoint was used to assess short-term dermal exposure. The dermal dose was conservatively converted to an equivalent oral dose using a 100% dermal absorption factor. The oral toxicological endpoint that was selected was 560 mg/kg-bw/day, which was actually a NOEL. This NOEL was based on increased incidence of perivascular lymphoid hyperplasia and interstitial nephritis in female mice in the 1100 and 2300 mg/kg-bw dose groups in a 13-week oral study in mice (WHO, 1999). This endpoint is similar to the endpoint observed in a 13-week oral study in rats (WHO, 1999) where similar adverse toxicological effects are observed in male rats. Therefore, the adverse effects are not considered sex-specific and a body weight of 70 kg, representing the body weight of an average adult, is used in the assessment. An uncertainty factor of 100 (10 for interspecies extrapolation and 10 for intraspecies variation) was used for this assessment.

There are no acute inhalation toxicity data for menthol, and the only long-term toxicity data showed limited toxicity concerns (Table 3). Therefore, in the absence of adverse toxic effects, and no inhalation toxicity endpoint identified, a residential inhalation exposure assessment of menthol was not completed.

V. Dietary (Food) Assessment

The chronic dietary (food) exposure assessments were conducted for menthol using the Dietary Exposure Evaluation Model software with the Food Commodity Intake Database (DEEM-FCID™, Version 2.03), which incorporates consumption data from US Department of Agriculture (USDA) Continuing Surveys of Food Intakes by Individuals (CSFII), 1994-1996 and 1998. The 1994-96 and 1998 data are based on the reported consumption of more than 20,000 individuals over two non-consecutive survey days. For the chronic exposure assessment, consumption data are averaged for the entire U.S. population and within population subgroups.

For chronic dietary exposure assessment, an estimate of the residue level in each food or food-form (e.g., cucumbers or pickles) on the food commodity residue list is multiplied by the average daily consumption estimate for that food/food form. The resulting residue consumption estimate for each food/food form is summed with the residue consumption estimates for all other food/food forms on the commodity residue

list to arrive at the total average estimated exposure. The exposure is expressed in mg/kg body weight/day, and as a percent of the chronic Population Adjusted Dose (cPAD). The value for the PAD was taken as equal to the Reference Dose (RfD) of 1.88 mg/kg/day, derived from the oral NOAEL of 188 mg/kg/day from the two-year feeding study in the rat, with a Safety Factor of 100 applied to determine the RfD. This exposure estimation procedure for dietary exposures is performed for each population subgroup.

Since there were no available residue data for menthol in the respective food commodities, in conducting this assessment, the Agency has utilized a screening model, the inert screening level assessment, to estimate the residue levels and applied them to the various crops treated with products containing menthol as an inert ingredient. The screening level assessment uses the following generic assumptions and criteria in selecting and assigning residue values:

- Actual crop-specific residue data for various active ingredients can be utilized as surrogate data for menthol residue levels associated with its use as an inert ingredient (including secondary residues in meat, milk, poultry and eggs);
- Menthol is used on all crops and 100% of all crops are "treated";
- No adjustments are made for % of menthol used as an inert ingredient in formulation, the application rate, or multiple applications of the different active ingredient formulations in which menthol is an inert ingredient; and
- The model only considers preharvest applications.

Dietary modeling (Table 4) was performed utilizing the highest established tolerance level residue for each commodity. The results estimated for menthol indicate that chronic dietary exposures are not of concern to the Agency.

Population Subgroup ²	Chronic Dietary	
	Estimated Exposure (mg/kg/day)	% cPAD
General U.S. Population	0.120	7
All Infants (< 1 year old)	0.245	14
Children 1-2 years old	0.422	24
Children 3-5 years old	0.310	18
Children 6-12 years old	0.174	10
Youth 13-19 years old	0.100	6
Adults 20-49 years old	0.087	5
Adults 50+ years old	0.086	5
Females 13-49 years old	0.087	5

¹Exposure estimates are based on highest-tolerance-level residues of high-use active ingredients for all food forms, including meat, milk, poultry, and eggs.

² Only representative population subgroups are shown.

VI. Drinking Water Considerations

According to the Hazardous Substance Data Bank (HSDB) environmental fate profile, volatilization from water surfaces is expected to be an important fate process for L-menthol; estimated volatilization half-lives for a model river are 2 days, and for a model lake 18 days. In soils, L-menthol is expected to have low mobility and will volatilize rapidly under moist conditions.

In addition, there is only one product which contains menthol as an active ingredient, and it has an enclosed usage pattern (the product is used for over-wintering of beehives). The products containing menthol as an inert ingredient, similarly have limited usage. Based on the environmental fate properties (especially sorption to soils/aquatic sediments and the estimated volatilization half-lives) and the limited usage for menthol-containing products, detailed drinking water modeling was not required. Note also, that OECD (2003) had indicted that various forms of menthol exhibited a low (1) Class of danger for water pollution for menthol for all uses of the chemical, with 1 indicating a potential for “weakly water polluting.”

VII. Residential Exposure Assessment

Exposure to menthol can occur through the use of certain pesticide products. In addition, exposure can occur naturally through different foods, or through FDA-approved GRAS uses.

Residential Exposure

Residential exposure to menthol could occur through its use as an inert ingredient in pesticide products used in and around the home. An inhalation exposure assessment was not performed due to absence of toxic endpoints for that route of exposure. In the 1993 RED for menthol, the Agency indicated that potential for inhalation exposure does exist; however, menthol is an ingredient added in liqueurs, candies, as well as inhalers and cough remedies to sooth respiratory congestion, and is of little concern from a toxicity perspective (EPA, 1993).

Outdoor uses

Residential handler dermal exposure to menthol during outdoor applications as an inert ingredient was examined in this risk assessment (Table 5). Postapplication dermal exposure to adults and youths (10-12 years) from contact with residues on foliage were also evaluated (Table 6).

The exposure scenarios chosen for this risk assessment were based on the anticipated use patterns and current labeling for pesticide products containing menthol as an inert ingredient. Exposure estimates for residential handler and postapplication scenarios were calculated using the Pesticide Inert Risk Assessment Tool (PIRAT, test version) (Versar, 2004). This tool is based on weight fractions of inert ingredients in pesticide products. For this risk assessment, it was assumed that exposure would be to pesticide products formulated as soluble and emulsifiable concentrates. Product uses evaluated include applications to trees and garden plants. The 90th percentile default weight fractions were assumed. An application rate of 0.00013 lb/ft² was used. In addition, a 100% dermal absorption factor was used to convert dermal doses to equivalent oral doses. The oral NOEL of 560 mg/kg/day was used to assess risks from short-term exposures. Intermediate-term exposures are not expected in the residential setting. The Agency does not have concern when MOE values are greater than 100.

For postapplication scenarios, the following assumptions are used:

- transfer coefficient of 1000 cm²/hr for adults and 500 cm²/hr for youths (from hand harvesting, hand pruning, staking, thinning, training, or tying tomatoes based on data from the Agriculture Reentry Task Force (ARTF) for adults performing postapplication tasks on tomatoes);
- time in treated area 0.67 hours/day for adults and 0.33 hours/day for youths; and
- body weight is 70 kilograms for adults and 39.1 kilograms for youths.

The scenarios, application rates, areas treated or amounts used and calculated MOEs for residential handler and postapplication exposure are provided in Tables 5 and 6, respectively. For residential handler exposure scenarios, calculated MOEs ranged from 310,000 for applications of soluble concentrate formulations using a low-pressure handwand to 1,400,000 for applications of emulsifiable concentrate formulations using a hose-end sprayer in gardens and trees. For residential postapplication exposures, calculated MOEs ranged from a low of 460,000 for dermal contact with residue in foliage by adults following application of soluble concentrate formulations to a high of 1,400,000 for dermal contact with residue in foliage by youths following application of emulsifiable concentrate formulations. Exposure outputs from the PIRAT model are provided in Appendix A.

Table 5. Residential Handler Risks Due to Exposure to Menthol as an Inert Ingredient					
Formulation Type	Product Use	Application Rate ^a	Application method	Area treated or Amount Used ^a	Dermal MOE ^b
Soluble Concentrate	vegetable garden, ornamental trees	0.00013 lb/ft ²	Low-pressure handwand	1000 ft ²	310,000
			Hose-end sprayer	1000 ft ²	1 x 10 ⁶
Emulsifiable Concentrate	vegetable garden, ornamental trees	0.00013 lb/ft ²	Low-pressure handwand	1000 ft ²	410,000
			Hose-end sprayer	1000 ft ²	1.4 x 10 ⁶

a. Values for application rate and area treated are default values provided by PIRAT.

b. MOE = NOAEL (560 mg/kg)/Potential Dose Rate estimated by PIRAT.

Table 6. Residential Post-Application Risks Due to Exposure to Menthol as an Inert Ingredient							
Formulation Type	Product Use	Application Rate ^a	Population	Exposure Time (hours)	Transfer Coefficient	PDR ^a (mg/kg/day)	Dermal MOE ^b
Soluble Concentrate	Garden/Trees	0.00013 lb/ft ²	Adults	0.67	1000	0.001206	460,000
			Youth (10-12 years)	0.33	500	0.000532	1.1 x 10 ⁶
Emulsifiable Concentrate	Garden/Trees	0.00013 lb/ft ²	Adults	0.67	1000	0.000896	630,000
			Youth (10-12 years)	0.33	500	0.000395	1.4 x 10 ⁶

a. Potential Dose Rate estimated by PIRAT.

Potential Dose Rate = $(AR \cdot WF \cdot F \cdot 4.54e5 \text{ mg/lb} \cdot 1.08E-3 \text{ ft}^2/\text{cm}^2 \cdot T_c \cdot ET) / BW \cdot ABS$; where AR= application rate; WF= weight fraction; F = Fraction Exposed; T_c= Transfer coefficient; ET= Exposure time; BW= Body weight; and ABS= Absorption factor.

b. MOE = NOAEL (560 mg/kg)/Potential Dose Rate estimated by PIRAT.

Indoor uses

In terms of consumer use exposure to products containing menthol, the Consumer Exposure Module (CEM) (Versar, 1999) was used to determine the average daily dose (ADD) from exposure to menthol. The exposure scenario examined was the use of a general purpose cleaner assuming a weight fraction range of 1% to 5%. Table 7 provides the CEM dermal MOE estimates. Exposure output information from the CEM model is provided in Appendix B. Using the oral NOEL of 560 mg/kg-day and assuming 100% dermal absorption, the MOE was calculated to be 121,000.

Postapplication dermal and oral exposure to toddlers resulting from the use of menthol as an inert in general purpose cleaners was not estimated due to lack on information on application rates. However, postapplication exposure is not expected to be significant given the low concentration of menthol in disinfectants.

Table 7. Summary of Consumer Exposure						
Scenario	Weight Fractions ^a	Years of Use	Surface Area/Body Weight Ratio (cm ² /kg)	Frequency of Use (events/yr)	Average Daily Dose Rate (mg/kg-day)	Dermal MOE ^b
General Purpose Cleaner	0.01-0.05	57	15.6	300	4.62e-03	121,000

a. Based on inert ingredient information provided for Comet disinfectant, a weight fraction range of 1% to 5% was assumed for menthol.

b. MOE = NOAEL (560 mg/kg-day)/Average Daily Dose Rate estimated by PIRAT.

VIII. Aggregate Assessment

Based on the extremely low residential exposure, low dietary exposure and the absence of concern for drinking water exposures, it has been qualitatively determined that the aggregate exposures due to the pesticidal uses of menthol would not be of concern to the Agency.

IX. Risk Characterization

Regarding use as an active ingredient, EPA concluded in the 1993 RED for menthol, and is reaffirming in this TRED, that exposure from reasonable pesticide usage is not expected to present an increased dietary risk or occupational risk beyond that from ordinary exposure (EPA, 1993). There were no concerns for dietary (food) exposures for menthol. In addition, none of the handler or postapplication risks were of concern for menthol resulting from its use as an inert ingredient.

X. Cumulative Exposure

Section 408(b)(2)(D)(v) of the FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider “available information” concerning the cumulative effects of a particular pesticide’s residues and “other substances that have a common mechanism of toxicity.” If chemicals are structurally related and all are low toxicity chemicals, then the risks either separately or combined should also be low.

EPA does not have, at this time, available data to determine whether menthol has a common mechanism of toxicity with other substances. Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to menthol and any other substances, and menthol does not appear to produce a toxic metabolite produced by other substances.

For the purposes of this tolerance action, therefore, EPA has not assumed that menthol has a common mechanism of toxicity with other substances. For information regarding the Agency’s efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA’s Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA’s website at <http://www.epa.gov/pesticides/cumulative/>.

XI. Tolerance Reassessment

In 1993, a tolerance exemption was established for residues of menthol in or on beeswax and honey when used in accordance with good agricultural practices in over-wintering hives. The residue data submitted to the Agency in support of the tolerance exemption were adequate, and indicated that menthol residues in honey will not exceed 5 ppm as a result of the registered use. The Agency is concluding that the residue data continue to support the tolerance exemption for the active use of menthol. The technical justification for this finding is based partially on the negligible dietary exposure associated with this pesticide use when compared with confectionary, pharmaceutical and other uses for menthol. At this time the Agency considers the tolerance exemption for menthol to be reassessed.

XII. References

Hazardous Substances Data Bank (HSDB). 2003. L-menthol. <http://toxnet.nlm.nih.gov>.

Organisation for Economic Co-operation and Development (OECD). 2003. Screening Information Data Set (SIDS) Initial Assessment Report.
<http://www.chem.unep.ch/irptc/sids/OECDSIDS/MENTHOLS.pdf>

U.S. EPA. 1993. Reregistration Eligibility Document on Menthol. Office of Prevention, Pesticides, and Toxic Substances (7508W).

Versar, Inc. 1999. Consumer Exposure Module (CEM), Version 1.2. Prepared for the Economics, Exposure and Technology Division of the Office of Pollution Prevention and Toxic Substances.

Versar, Inc. 2004. Pesticide Inert Risk Assessment Tool (PIRAT), Test Version. Prepared for the Office of Pesticide Programs. In review.

World Health Organization (WHO). 1999. Safety Evaluation of Certain Food Additives. International Programme on Chemical Safety. 51st Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). <http://www.inchem.org/documents/jecfa/jecmono/v042je04.htm>

File No: LTD/1409

June 2009

**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
(NICNAS)**

FULL PUBLIC REPORT

**Butanedioic acid, 1-[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl] ester
(INCI: Menthyl succinate)**

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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**Director
NICNAS**

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FULL PUBLIC REPORT**Butanedioic acid, 1-[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl] ester
(INCI: Menthyl succinate)****1. APPLICANT AND NOTIFICATION DETAILS**

APPLICANT(S)

Johnson & Johnson Pacific Pty Ltd (ABN 73 001 121 446)
45 Jones Street
ULTIMO NSW 2007

NOTIFICATION CATEGORY

Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)

No details are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)

Variation to the schedule of data requirements is claimed as follows:

Boiling point, Density, Vapour pressure, Water solubility, Hydrolysis as a function of pH, Partition coefficient, Adsorption/Desorption, Dissociation constant, Particle size, Flash point, Flammability limits, Autoignition temperature.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)

None

NOTIFICATION IN OTHER COUNTRIES

None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)

Listerine Total Care Mouthwash (contains notified chemical at < 0.05%)
Menthyl succinate (INCI name)
Physcool

CAS NUMBER

77341-67-4

CHEMICAL NAME

Butanedioic acid, 1-[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl] ester

OTHER NAME(S)

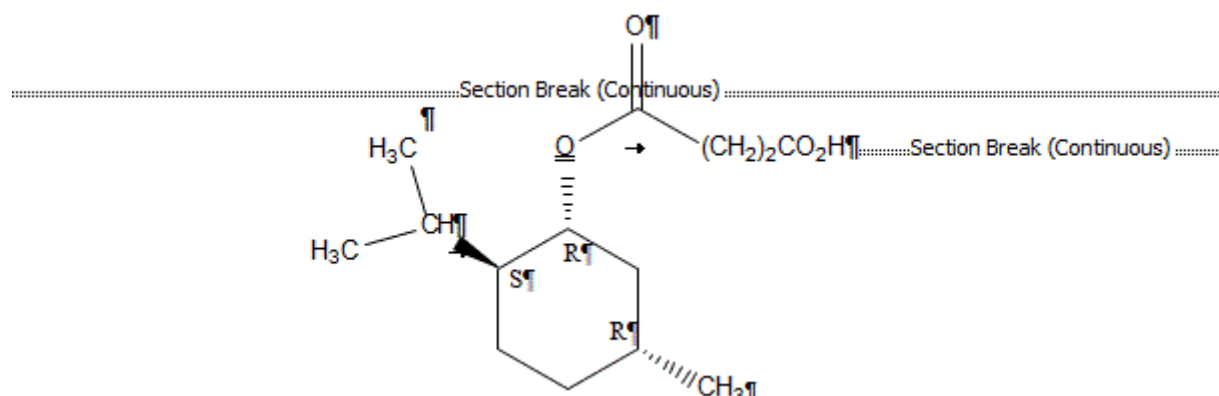
Butanedioic acid, mono[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl] ester (9CI)
Butanedioic acid, mono[5-methyl-2-(1-methylethyl)cyclohexyl] ester, [1R-(1.alpha.,2.beta.,5.alpha.)]-
Mono-menthyl succinate

MOLECULAR FORMULA $C_{14}H_{24}O_4$

MOLECULAR WEIGHT

256.3 Da

STRUCTURAL FORMULA



ANALYTICAL DATA

Reference IR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 99%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight) None

ADDITIVES/ADJUVANTS None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: White powder

Property	Value	Data Source/Justification
Melting Point/Freezing Point	60°C	MSDS
Boiling Point	353°C at 101.3 kPa	Estimated (EPIWEB v 4.0)
Density	Not determined	Not isolated from aqueous solution
Vapour Pressure	2.3×10^{-5} kPa at 25°C	Estimated (EPIWEB v 4.0). Modified Antoine method using measured melting point of 60°C.
Water Solubility	44 mg/L at 20°C	Estimated
Hydrolysis as a Function of pH	Expected to be slow.	Estimated
Partition Coefficient (n-octanol/water)	$\log P_{ow} = 4.1$ at 20°C	Estimated
Adsorption/Desorption	$\log K_{oc} = 2.1-2.4$ at 20°C	Estimated
Dissociation Constant	$pK_a = 4.2$	Analogue data (succinic acid)
Particle Size	Not determined	Not isolated from aqueous solution
Flash Point	Not determined	Not isolated from aqueous solution
Flammability	Not determined	Not isolated from aqueous solution
Autoignition Temperature	Not determined	Not expected to autoignite under normal conditions of use.
Explosive Properties	Not expected to be explosive	The structural formula contains no explosives.

DISCUSSION OF PROPERTIES

The notified chemical is predicted to be lipophilic and to have a low vapour pressure and low water solubility based on modelling (EPIWEB, 2009). While abiotic hydrolysis is expected to be slow, the notified chemical is likely to be rapidly hydrolysed by esterases under environmental conditions.

Reactivity

The notified chemical is expected to be stable under normal storage and handling conditions.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

The notified chemical will be imported as a component of finished personal care products at a concentration of < 0.05%.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

<i>Year</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>
<i>Tonnes</i>	0.4	0.5	0.6	0.7	0.8

PORT OF ENTRY

Sydney

TRANSPORTATION AND PACKAGING

The notified chemical will be imported as a component of a finished cosmetic mouthwash in bottles up to 1L for retail sale and transported by road to the notifiers warehouse and then onto the retail store's central distribution centres.

USE

The notified chemical is a component of a flavour mixture used in a cosmetic mouthwash.

OPERATION DESCRIPTION

There will be no manufacture or reformulation of the notified chemical within Australia.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

EXPOSURE DETAILS

Transport, storage and retail workers are not expected to be exposed to the notified chemical except in the unlikely event of an accident or spill.

6.1.2 Public exposure

The public will be exposed to the notified chemical at a concentration of up to 0.05% when using mouthwash products. Dermal and oral will therefore be the primary routes of exposure. Given the low vapour pressure of the notified chemical inhalation exposure is not expected. Accidental ocular exposure may occur, but is not expected to be significant.

Based on data reported by the Soap and Detergent Association (SDA) for European use, 8.5% of mouthwash is estimated to be ingested, equivalent to 70.8 mg/kg bw/day for a 60 kg adult using 10 g of the mouthwash product per use 5 times a day (SDA, 2005). Therefore exposure to the notified chemical at 0.05% is estimated as 0.04 mg/kg bw/day.

6.2. Human health effects assessment

No toxicity data were submitted.

The notified chemical is generally recognized as safe (GRAS) as a flavor ingredient by the expert panel of the Flavor and Extract Manufacturers Association (FEMA) (Smith et al, 1996).

The Joint FAO/WHO Expert Committee on Food Additives JECFA concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (1999a).

Toxicokinetics

The notified chemical has a low molecular weight and is highly lipophilic; therefore penetration into the strata corneum would be expected. However, given its low water solubility (44 mg/L) the rate of transfer into the epidermis is expected to be low.

The notified chemical would be expected to hydrolyse in the body to (-)-menthol (CAS No. 2216-51-5) and butanedioic acid (CAS No. 110-15-6); the latter being endogenous to humans. Menthol is known to be readily absorbed by the oral route and largely eliminated as glucuronides. It has been reported that 70-80% is eliminated in urine and faeces within 48 hrs (JECFA, 1999a, 1999b).

Acute toxicity

The notified chemical is expected to have a low acute oral toxicity based on a reported LD50 of > 5000 mg/kg on a study conducted on rats (JECFA, 1999a).

Repeated dose toxicity

No repeat dose toxicity data was available for the notified chemical, however in a two year study conducted on groups of 50 rats of each sex fed the equivalent of 190 and 380 mg/kg bw/day of racemic menthol¹ in their diet, the NOEL was determined to be 380 mg/kg bw/day based on the absence of any treatment related effects (JECFA, 1999b).

Irritation and Sensitisation

No irritation/sensitisation data was available for the notified chemical. The notified chemical may possess irritant properties as it contains a carboxylic acid group, a structural alert for skin irritants (Hulzebos, 2005) and eye irritants (Tsakovsha, 2007). Indeed, the notified chemical is listed on Safe Work Australia's hazardous substance information system (HSIS) as a severe eye irritant.

The notified chemical does not contain structural alerts for sensitisation and therefore is not expected to present as a sensitizer.

Genotoxicity

No genotoxicity data was available for the notified chemical, however neither menthol¹ nor its metabolites were found to be genotoxic in a number of in vitro studies, including reverse mutation and chromosomal aberration tests, and in vivo tests (JECFA, 1999b).

Developmental toxicity

No developmental toxicity data was available for the notified chemical, however in a study conducted on the expected metabolite of the notified chemical, (-)-menthol, no teratogenic effects were observed when tested at maximum doses of 190-430 mg/kg bw/day in mice, rats, hamsters and rabbits (JECFA, 1999b).

Carcinogenicity

No carcinogenicity data was available for the notified chemical; however racemic menthol¹ was concluded to be not carcinogenic in studies conducted on rats and mice (JECFA, 1999b).

¹Based on limited data, there is no indication of a difference in the metabolism and toxicity of (-)-menthol, the expected metabolite, and racemic menthol (JECFA, 1999b).

Health hazard classification

The notified chemical is listed on Safe Work Australia's hazardous substance information system (HSIS) as an eye irritant with the following risk phrase:

R41: Risk of serious damage to eyes

Cutoffs:

Conc $\geq$ 10%: Xi; R41

$\geq$ 5%Conc < 10%: Xi; R36

Based on the available data the notified chemical is not classified as hazardous for other endpoints under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation**6.3.1. Occupational health and safety**

Given workers will only be exposed to low levels of the notified chemical in the event of an accident or spill, the risk to workers to the notified chemical is considered to be negligible.

6.3.2. Public health

The greatest risk of exposure to the notified chemical to the public will be by the oral route when using mouthwash products containing the notified chemical. The estimated exposure to the notified chemical in the mouthwash at up to 0.05% is 0.04 mg/kg bw/day. Given the NOEL (380 mg/kg bw/day) of the expected metabolite menthol is three orders of magnitude greater than the estimated exposure, the risk to the public is considered acceptable.

The risk of irritation effects are considered to be low/negligible based on the low concentration.

7. ENVIRONMENTAL IMPLICATIONS**7.1 Environmental Exposure & Fate Assessment****7.1.1 Environmental Exposure****RELEASE OF CHEMICAL AT SITE**

The notified chemical will not be manufactured or reformulated in Australia. It is imported as a component of a finished cosmetic mouthwash in consumer containers.

There is unlikely to be any significant release to the environment, given the proposed relatively low level use for the product containing the notified chemical, the formulation and sealed packaging containers.

RELEASE OF CHEMICAL FROM USE

It is expected that the majority of the imported quantity of the notified chemical (estimated to be 99%) will be washed to the sewer as the chemical is intended for use in personal care products. This release will occur in a diffuse and widespread manner.

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated that 1% of the finished product goes into the garbage collection system as residues in empty containers. These residues may be sent to landfill with the containers, or washed to sewer when consumers rinse the containers before recycling.

7.1.2 Environmental fate

No environmental fate data were submitted. The notified chemical is expected to be easily hydrolysed in sewage treatment plants and the environment to menthol and succinic acid. These natural products can be expected to biodegrade easily. The notified chemical is not expected to bioaccumulate in fish because of the ease of enzymatic (esterase) hydrolysis in living organisms.

7.1.3 Predicted Environmental Concentration (PEC)

A PEC for the aquatic environment can be determined as tabulated below, assuming all the product containing the notified chemical is rinsed to sewer after use.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	800	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	800	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.19	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.374	million
Removal within STP	0%	
Daily effluent production:	4,275	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.51	µg/L
PEC - Ocean:	0.051	µg/L

7.2 Environmental effects assessment

No ecotoxicity data were submitted. The notifier has provided model estimates (EPIWEB, 2009) for the acute aquatic toxicity, as tabulated below.

	Duration	Predicted LC50/EC50
Fish	96 hour	21.06 mg/L
Fish (saltwater)	96 hour	25.43 mg/L
Daphnid	48 hour	32.88 mg/L
Green algae	96 hour	11.14 mg/L
Mysid shrimp	96 hour	9.89 mg/l

7.2.1 Predicted No-Effect Concentration

The PNEC can be determined as tabulated below by application of a thousand-fold assessment factor to the estimated acute toxicity to mysid shrimp.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Mysid 96 hour LC50	9.9	mg/L
Assessment Factor	1000	
PNEC:	9.9	µg/L

7.3 Environmental risk assessment

The risk quotients (Q = PEC/PNEC) are tabulated below.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.51	9.9	0.052
Q - Ocean	0.051	9.9	0.0052

The notified chemical is not considered to pose a risk to the environment as the risk quotients are less than one.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

The notified chemical is listed on Safe Work Australia's hazardous substance information system (HSIS) as an eye irritant with the following risk phrase:

R41: Risk of serious damage to eyes

Cutoffs:

Conc $\geq$ 10%: Xi; R41

$\geq$ 5%Conc < 10%: Xi; R36

Based on the available data the notified chemical is not classified as hazardous for other endpoints under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

and

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

<i>Estimated mysid toxicity</i>	<i>Hazard category</i>	<i>Hazard statement</i>
96 hour LC50 = 9.9 mg/L	Acute 2	Toxic to aquatic life

As eye irritation data has not been cited, GHS classification for this endpoint has not been undertaken.

Human health risk assessment

Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable risk to public health.

Environmental risk assessment

On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical as introduced, however, these should be selected on the basis of all ingredients in the formulation.

Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the MSDS should be easily accessible to employees.
- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, collection and subsequent safe disposal.

Regulatory Obligations

Secondary Notification

This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

- (1) Under Section 64(1) of the Act; if
 - the importation volume exceeds one tonne per annum notified chemical;or
- (2) Under Section 64(2) of the Act; if
 - the function or use of the chemical has changed from as a component of a finished cosmetic mouthwash at a concentration of < 0.05% , or is likely to change significantly;
 - the amount of chemical being introduced has increased from 0.8 tonnes per annum, or is likely to increase, significantly;
 - the chemical has begun to be manufactured in Australia;
 - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Material Safety Data Sheet

The MSDS of the notified chemical and products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

BIBLIOGRAPHY

- Hulzebos E, Walker JD, Gerner I and Schlegel K (2005). Use of structural alerts to develop rules for identifying chemical substances with skin irritation or skin corrosion potential, *QSAR Comb. Sci.*, 24: 332-342.
- JECFA (1999) Safety Evaluation of Certain Food Additives. WHO Food Additives Series: 42, Substances Structurally Related to Menthol. Joint FAO/WHO Expert Committee on Food Additives.
- JECFA (1999b) Safety Evaluation of Certain Food Additives. WHO Food Additives Series: 42, Menthol. Joint FAO/WHO Expert Committee on Food Additives.
- NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.
- NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.
- NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.
- SDA (2005) Exposure and Risk Screening Methods for Consumer Product Ingredients. The Soap and Detergent Association, Washington, DC, USA.
- Smith RL, Newberne P, Adams TB, Ford R, Hallagan JB and the FEMA Expert Panel (1996). GRAS flavouring substances 17. *Food Technol.* 50(10): 72-81.
- Tsakovska I, Saliner Gallegos A, Netzeva T, Pavan M and Worth AP (2007) Evaluation of SARs for the prediction of eye irritation/corrosion potential - structural inclusion rules in the BfR decision support system. *SAR and QSAR in Environmental Research.* 18: 221-235.
- United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.

Evaluation of certain food additives

Eighty-sixth report of the Joint
FAO/WHO Expert Committee on
Food Additives



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Evaluation of certain food additives

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*This report contains the collective views of an international group of experts and
does not necessarily represent the decisions or the stated policy of the World Health Organization*



**Food and Agriculture
Organization of the
United Nations**



**World Health
Organization**

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List of abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AMC	anionic methacrylate copolymer
BMC	basic methacrylate copolymer
bw	body weight
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CCFA49	Forty-ninth Session of the Codex Committee on Contaminants in Food Additives
CIFOCoss	FAO/WHO Chronic Individual Food Consumption Database – Summary statistics
CITREM	citric and fatty acid esters of glycerol
CXG	Codex Guideline
CXS	Codex Standard
EFSA	European Food Safety Authority
EHC 240	<i>Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food</i>
EINECS	European Inventory of Existing Commercial Chemical Substances
F	filial generation (F_0 , F_1 , F_2 , etc.)
FAO	Food and Agriculture Organization of the United Nations
GEWR	Glycerol ester of wood rosin
GLP	good laboratory practice
GRAS	Generally Recognized as Safe
GSFA	General Standard for Food Additives
HPLC	high-performance liquid chromatography
i.p. or IP	intraperitoneal OR intraperitoneal injection
IACM	International Association of Color Manufacturers
IARC	International Agency for Research on Cancer
IC ₅₀	median inhibitory concentration
INS	International Numbering System for Food Additives
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K_m	Michaelis constant (affinity)
LD ₅₀	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
LOQ	limit of quantification
M	male
mADI	microbiological acceptable daily intake

MSDI	maximized survey-derived intake
NCI	National Cancer Institute
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NMC	neutral methacrylate copolymer
no. / No.	number
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NR	not reported
NR	not required
NR	not relevant
NTP	National Toxicology Program
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
OECD	Organisation for Economic Co-operation and Development
<i>P</i>	probability
P90	90th percentile
P95	95th percentile
ppm	parts per million
SCE	sister chromatid exchange
SPET	single-portion exposure technique
TDI	tolerable daily intake
TG	test guideline
TSH	thyroid-stimulating hormone
USA	United States of America
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UV	ultraviolet
UVA	ultraviolet (radiation from about 320–400 nm in wavelength)
v/v	volume per volume
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WHO	World Health Organization

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 12 to 21 June 2018. The meeting was opened by Dr Kazuaki Miyagishima, Director of the Department of Food Safety and Zoonoses of the World Health Organization (WHO), on behalf of WHO Director-General Dr Tedros Adhanom Ghebreyesus. Dr Miyagishima reported that the 2018 World Health Assembly adopted the general programme of work for 2019–2023, and that Codex continues to be a high priority.

Dr Miyagishima welcomed all the delegates. In his opening remarks, he noted the long-standing service of a number of experts, which demonstrates their dedication to JECFA's goals. He also noted the number of new experts, along with the efforts to increase the geographical and gender balance of the Committee, exemplifying JECFA's commitment to knowledge transfer. This was considered particularly welcome as the list of food additives and flavourings awaiting evaluation continues to grow, a sign of the recognition of the quality and importance of JECFA's work.

Dr Miyagishima welcomed JECFA's constant drive to improve its methodology, pointing out that the new methodology for evaluating flavouring agents, agreed on 2 years ago, would be put into practice for the first time by the current Committee. He also informed the Committee that an agreement had been reached between WHO and the International Agency for Research on Cancer (IARC) on new interim standard operating procedures that would streamline the evaluation of compounds and give due recognition of JECFA's primary role in food risk assessments.

Dr Markus Lipp, FAO Joint Secretary, and Dr Angelika Tritscher, WHO Joint Secretary, also welcomed the delegates and thanked them for their hard work before the meeting and their continuing efforts.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the eighty-sixth meeting had completed declaration of interest forms. No conflicts of interest were identified. There were no responses to the public posting of the list of participants for this meeting.

1.2 Modification of the agenda

The agenda (see [Annex 4](#)) was modified to include sorbitol syrup in the list of specific food additives based on request of the CCFA to clarify if sorbitol syrup can be covered by the same acceptable daily intake (ADI) as sorbitol.

The agenda (see [Annex 4](#)) was modified to include Corrigenda for specifications monographs.



2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives (JECFA), held in September 1955 (1), there have been 85 previous meetings of the Committee ([Annex 1](#)). The present meeting was convened on the basis of a recommendation made at the eighty-fourth meeting ([Annex 1](#), reference 234).

The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives including flavouring agents ([section 3](#));
- review and prepare specifications for certain food additives ([sections 3 and 4](#) and [Annex 2](#)); and
- undertake safety evaluations of certain food additives ([sections 3 and 4](#) and [Annex 2](#)).

2.1 Report from the Fiftieth Session of the Codex Committee on Food Additives (CCFA)

Dr Yongxiang Fan, Chair of the CCFA, provided the Committee with an update on the work of CCFA since the eighty-fourth meeting of JECFA in 2017 ([Annex 1](#), reference 234).

The Fiftieth Session of CCFA (CCFA50) noted the conclusions of the eighty-fourth meeting of JECFA on the safety of nine food additives. CCFA50 agreed to include gum ghatti (International Numbering System for Food Additives [INS] 419) and tamarind seed polysaccharide (INS 437) in the Codex *General Standard for Food Additives* (CXS 192-1995) (GSFA) Table 3 (“Additives Permitted for Use in Food in General, Unless Otherwise Specified, in Accordance with Good Manufacturing Practice”). CCFA50 noted the conclusions for β -carotene-rich extract from *Dunaliella salina*, Brilliant Blue FCF (INS 133) and Fast Green FCF (INS 143). CCFA50 called on members to provide more data to JECFA to complete the evaluation for Jagua (Genipin–Glycine) Blue, metatartaric acid (INS 353), tannins (oenological tannins) and yeast extracts containing mannoproteins.

CCFA50 finalized work on over 320 provisions for food additives for inclusion in the General Standard on Food Additives (GSFA) and forwarded specifications for the identity and purity of 10 food additives (two new and eight revised specifications) prepared by the eighty-fourth meeting of JECFA for adoption by the forty-first session of the Codex Alimentarius Commission (CAC).

CCFA50 agreed on a revised Priority List of Substances for Evaluation (or re-evaluation) by JECFA, which currently includes 46 food additives and eight flavourings. CCFA50 agreed to amend the circular letter on the Priority List in order to expedite the process of confirming requests and to provide a mechanism for members to confirm the requests without having to attend the in-session working group meetings. CCFA50 also agreed to change the Priority List table by introducing a summary of information about the request such as its basis and possible trade issues.

CCFA50 established an electronic working group that will develop an inventory of data available on nitrates and nitrites with a view to consulting with JECFA and CCFA regarding next steps on the use of these substances,

The Fifty-first Session of CCFA (CCFA51), to be held on 25–29 March 2019 in China, will continue its work on the food additives provisions for inclusion in the GSFA, including further alignments of food additive provisions in the Codex commodity standards with the corresponding provisions of the GSFA.

2.2 Update on activities relevant to JECFA

2.2.1 Update of Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food (EHC 240)

The Secretariat informed the Committee about ongoing activities on risk assessment methodology and update of certain chapters of EHC 240. In particular, more detailed guidance on the interpretation and evaluation of genotoxicity of compounds in food, including the interpretation of results of genotoxicity tests, is under development. In addition, Chapter 5 on dose-response assessment, including benchmark dose modelling, and the derivation of health-based guidance values is being updated, as is Chapter 6 on dietary exposure assessment. Guidance for the evaluation of enzyme preparations will also be updated. Final drafts are expected in 2019, following wide stakeholder input including public calls for comments.

2.3 Food additive specifications and analytical methods

2.3.1 Corrigenda for specifications monographs

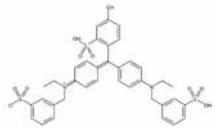
The following requests for corrections, reported to the JECFA Secretariat, were evaluated by the eighty-sixth Committee and found to be necessary (see [Table 1](#)). These corrections, however, will only be made in the online database for specifications.

Table 1

Corrections in JECFA reports and monographs on food additives^a

Food additive	Original text	New text	Additional information
Calcium disodium ethylenediaminetetraacetate (INS 385) Monograph 1 (2006)	CAS No. 662-33-9	CAS No. 62-33-9	Transcription error
Chlorophyllins, copper complexes sodium and potassium salts (INS 141(ii)) Monograph 5 (2008) Test for "Free ionisable copper"	Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil. ...	Accurately weigh about 1 g of the sample and mix in 20 mL of arachid oil. ...	Correction
Curcumin (INS 100(ii)) Monograph 1 (2006)	The criteria for several residual solvents are listed under the heading "Residual solvents" (see Fig. 1).	Acetone: Not more than 30 mg/kg Hexane: Not more than 25 mg/kg Methanol: Not more than 50 mg/kg Ethanol: Not more than 50 mg/kg Isopropanol: Not more than 50 mg/kg Ethyl acetate: Not more than 50 mg/kg	Improves readability It was unclear whether the criterion "Not more than 50 mg/kg" extended to methanol, ethanol, isopropanol and ethyl acetate.
Ethyl acetoacetate ethyleneglycol ketal JECFA No.: 1969 JECFA 73 (2010)	CAS No. 1648615	CAS No. 6413-10-1	Transcription error
Ethyl 2-methyl pentanoate JECFA No.: 214 JECFA 55 (2000)	CAS No. 28959-02-6	CAS No. 39255-32-8	Wrong CAS number
<i>cis</i> -3-Hexen-1-ol JECFA No.: 315 JECFA 51 (1998)	98.0% (sum of (Z) and (E) isomers, =<92.0% (Z))	98.0% (sum of (Z) and (E) isomers, =>92.0% (Z))	Transcription error
Monosodium <i>L</i> -glutamate (INS 621) Monograph 1 (2006)	CAS No. 142-47-2	CAS No. 6106-04-3	Wrong CAS number
Myrcene JECFA No.: 1327 JECFA 63 (2004)	Specific gravity: 0.789–1.793	Specific gravity: 0.789–0.793	Transcription error
Polyoxyethylene (20) sorbitan monostearat (Polysorbate 60) (INS 435) Monograph 16 (2014)	CAS No. 9005-07-6	CAS No. 9005-67-8	Wrong CAS number
Sodium aluminium silicate (INS 554) Monograph 20 (2017)	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on dried basis".	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on ignited basis".	Transcription error
Silicon dioxide, amorphous (INS 551)	CAS No. 112696-00-8 (hydrated silica)	CAS No. 112926-00-8 (hydrated silica)	Transcription error

Table 1 (continued)

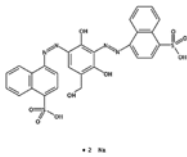
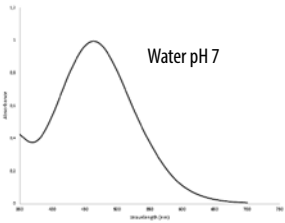
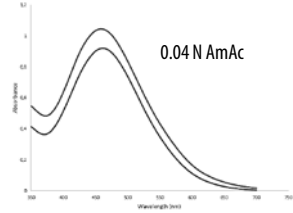
Food additive	Original text	New text	Additional information
Monograph 20 (2017)	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water.	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface adsorbed water.	Transcription error
Sodium thiosulfate (INS 539) Monograph 1 (2006)	CAS No. 7772-98-7	CAS No. 10102-17-7	CAS No. 7772-98-7 refers to the anhydrous form. The specifications in the monograph refer to the pentahydrate form.
Brown HT and its aluminium lake (FAO JECFA Monographs 19, 82nd meeting, 2016)	Text in Table 1: “Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry”	See Table 1, below	
Fast Green FCF (FAO JECFA Monographs 19, 82nd meeting, 2016)	Chemical structure in Table 1: “Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry”		

CAS: Chemical Abstracts Service; INS: International Numbering System for Food Additives; JECFA: Joint FAO/WHO Expert Committee on Food Additives; No.: number
* Bolding and underlining for clarity only. This formatting will not be shown in the online database.

Fig. 1
Residual solvent criteria for curcumin as displayed in Monograph 1, 2006

Residual solvents (Vol. 4)	Acetone:	Not more than 30 mg/kg
	Hexane:	Not more than 25 mg/kg
	Methanol:	}
	Ethanol:	Not more than 50 mg/kg
	Isopropanol:	
	Ethyl acetate:	

Table 2
Replacement of the text for the spectrophotometric data for Brown HT and its aluminium lake originally published in “Table 1. Values for synthetic colours for use in performing tests for Colouring Matters Content by Spectrophotometry” (FAO JECFA Monographs 19, 82nd meeting, 2016)

JECFA name	Sample weight	Structure	Spectral data	Visible absorption spectrum
Brown HT	245.6 mg		Water, pH 7 λ_{max} = 464 $A = 0.9957$ Spec abs = 403 $a = 40.3$ Water λ_{max} = 464 $A = 0.9804$ Spec abs = 397 $a = 39.7$ 0.04 N AmAc λ_{max} = 461 $A = 0.9206$ Spec abs = 373 $a = 37.3$	
Brown HT Aluminium Lake	53.3 mg	—	Straight colour (blue) 0.04 N AmAc λ_{max} = 461 $A = 0.9206$ Lake (red) 0.04 N AmAc λ_{max} = 458 $A = 1.0451$	

3. Specific food additives (other than flavouring agents)

3.1 Toxicological evaluation, exposure assessment and establishment of specifications

3.1.1 Anionic methacrylate copolymer

Explanation

Anionic methacrylate copolymer (AMC; E 1207; International Numbering System Number [INS No.] 1207; Chemical Abstracts Service [CAS] No. 26936-24-3; acrylates copolymers; methyl acrylate, methyl methacrylate, methacrylic acid polymer; methacrylic acid polymer with methyl acrylate and methyl methacrylate) is a copolymer manufactured from the monomers methacrylic acid, methyl methacrylate and methyl acrylate in the molar ratio of 7:3:1.

AMC has been evaluated by the European Food Safety Authority (EFSA) and is approved for use as a food additive in the European Union. Its use in the European Union is restricted to a maximum level of 100 000 mg/kg in solid food supplements, category 17.1 [1].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. AMC is soluble above pH 7 and is used for its taste- and odour-masking properties; as protection from heat, light, moisture and oxidation; and to prevent the fast release of active ingredients once they leave the stomach.

AMC has not previously been evaluated by the Committee. The Committee evaluated the use of AMC as a coating or glazing agent for solid food supplements and foods for special medical purposes when provided in the form of solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 10%, at the request of the Forty-ninth Session of the CCFA (CCFA49 [2]). AMC is also used in pharmaceuticals.

Toxicological data submitted for the evaluation included distribution studies, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the monomers but no additional studies on AMC. The Committee considered the data on the residual monomers, as well as that for AMC itself, because the monomers are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. Due to the low levels of residual monomers present in AMC, the Committee evaluated only absorption, distribution, metabolism and excretion (ADME) data and long-term toxicity and genotoxicity data on the monomers.

The Committee was also aware that AMC contains an oligomer fraction of up to 2%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 1000 Da, with around 75% of the oligomer fraction having a molecular weight between 5000 and 10 000 Da, it is unlikely that the oligomers would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the distribution and toxicity studies was prepared from an aqueous dispersion with 1.5% emulsifier and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of AMC.

The Committee evaluated toxicological and exposure data on sodium lauryl sulfate, polysorbate 80 and simethicone, residual components of AMC that can be present in the final product because they are used in the manufacture of the copolymer. The Committee concluded that these residual components did not pose a safety concern at the maximum estimated exposure levels.

Chemical and technical considerations

AMC is manufactured by emulsion polymerization of the monomers methacrylic acid, methyl methacrylate and methyl acrylate with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low molecular weight substances and coagulum.

AMC has a weight-average molecular weight of 280 000 Da and a number-average molecular weight of 77 000 Da.

Although organic solvents are not used in the manufacture of AMC, methanol may be present at a level not exceeding 1000 mg/kg as a result of hydrolysis of esterified carboxyl groups incorporated in the polymer. The copolymer is standardized as a 30% aqueous dispersion with sodium lauryl sulfate (0.3%) and polysorbate 80 (1.2%). Simethicone emulsion is used as an antifoaming agent during the manufacture and is present in the dispersion at not more than 20 mg/kg. The copolymer dispersion may contain residual monomers: methyl acrylate (not more than 1 mg/kg); methyl methacrylate (not more than 3 mg/kg); and methacrylic acid (not more than 1 mg/kg).

Biochemical aspects

AMC

Radiolabelled AMC administered to rats was excreted in the faeces with mean recovery amounting to 92.38% of the total dose after 72 hours and 94.07% ($\pm 3.42\%$) of the total dose 10 days after dosing. Radioactivity was detected at very low levels in the urine of four of the eight treated animals in this group; this may have been due to contamination with faecal matter [3].

Residual monomers

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration in rats. On the basis of available data, methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [4, 5, 6].

Methyl acrylate is rapidly absorbed. About half the dose is exhaled as carbon dioxide, while the rest is excreted in the urine in the form of cysteine conjugates and other thioethers [7, 8].

Methacrylic acid is rapidly absorbed after oral or inhalational exposure. After a single oral administration of the sodium salt of methacrylic acid to rats, the maximum concentration in blood was found after 10 minutes. After 60 minutes, no more methacrylic acid was detectable [9].

Toxicological studies

AMC

In a good laboratory practice (GLP)-compliant study, a single dose of 2000 mg/kg body weight (bw) of AMC was administered by gavage to rats. Following a 14-day recovery period, there were no abnormal clinical observations, no deaths and no statistically significant differences in body weights between the test animals and historical controls. No abnormalities were observed at necropsy [10].

When gavage doses of 0, 200, 500 or 1500 mg/kg bw per day AMC were administered to rats for 4 weeks, no treatment-related effects were observed. The no-observed-adverse-effect level (NOAEL) was 1500 mg/kg bw per day, the highest dose tested [11]. In a GLP-compliant 26-week oral toxicity study in rats using gavage doses of 0, 200, 500 or 1500 mg/kg bw per day, there were also no treatment-related effects. Some statistically significant findings were observed, but these did not exhibit a dose-response relationship. The NOAEL was 1500 mg/kg bw per day, the highest dose tested [12]. In a 4-week dog study, AMC was administered as copolymer-coated cellulose pellets at doses of the test substance at 0, 100, 200 or 400 mg/kg bw per day. There were no treatment-related effects. The NOAEL was 400 mg/kg bw per day, the highest dose tested [13].

Two reverse mutation assays in bacteria, a mouse lymphoma assay and a chromosomal aberration assay in vitro and an in vivo mouse micronucleus assay all gave negative results. The Committee concluded that AMC was not of concern for genotoxicity.

No long-term toxicity or carcinogenicity studies were available on AMC.

In a GLP-compliant developmental toxicity study, female rats administered AMC at 0 or 1000 mg/kg bw per day by gavage on gestation days 5–19 showed no treatment-related effects. The NOAEL was 1000 mg/kg bw per day, the only dose tested [14].

Studies on cytotoxicity, dermal toxicity, inhalation toxicity, ocular toxicity and phototoxicity produced no effects.

Residual monomers

Methyl methacrylate

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight increased in females at the highest dose but no treatment-related histopathological effects were observed in any organs or tissues [15]. Based on the results of this study, a tolerable daily intake (TDI) of 1.2 mg/kg bw per day was determined [16].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [15].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and sister chromatid exchange (SCE) assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days of exposure. These results were judged by the Committee to be inconclusive.

The Committee concluded that there was some evidence of mutagenicity and clastogenicity in vitro but that there was a lack of adequate in vivo tests following up the equivocal in vitro findings.

Methyl acrylate

In a 2-year inhalation study, groups of rats ($n = 86/\text{sex}$) were exposed to methyl acrylate by inhalation at 0, 15, 45 or 135 parts per million (ppm; 0, 53, 158 and 475 mg/m³) in air for 6 hours per day on 5 days per week for 2 years. No significant difference in mortality was observed between the groups. The incidence of soft-tissue sarcomas varied considerably between the groups but there was no dose-dependence. No increased frequency of any tumour type in any organ could be related to a carcinogenic effect of the test substance [17].

There are no epidemiological data in humans. The IARC [18] concluded that there was inadequate evidence in experimental animals for carcinogenicity and that methyl acrylate was not classifiable as to its carcinogenicity in humans.

The Committee evaluated the genotoxicity studies on methyl acrylate. Reverse mutation studies in bacteria were negative. A well-performed genotoxicity study on L5178Y mouse lymphoma cells, without exogenous metabolism, produced positive outcomes for gene mutation and chromosomal aberrations but only at cytotoxic concentrations. The in vivo study designs have methodological shortcomings. A mouse bone marrow micronucleus assay using intraperitoneal dosing showed an increase in micronuclei induction, but this was limited in magnitude and not dose related. A mouse bone marrow micronucleus assay using oral dosing was negative, but it is not clear if the target tissues were exposed to the test substance. Overall, the Committee concluded that the data were not sufficient to draw conclusions on the genotoxicity of methyl acrylate.

Methacrylic acid

There were no long-term chronic toxicity/carcinogenicity studies on methacrylic acid. The Committee noted that studies on methyl methacrylate, which is metabolized into methacrylic acid, found no carcinogenicity in mice, rats or hamsters [16].

There are several in vitro genotoxicity studies on methacrylic acid, but the most useful data were from negative studies on gene mutations in bacteria. Positive results were obtained in two comet assays and a γ -H2AX assay in human gingival fibroblasts. There were no studies on chromosomal aberrations or gene mutation in mammalian cells and no in vivo studies on methacrylic acid. The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid.

Observations in humans

No human data were available on AMC.

Assessment of dietary exposure

The Committee evaluated exposure to AMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes. As another major use of AMC is in pharmaceuticals, this use was also evaluated in the exposure assessment. The level of use of AMC is a maximum of 10%.

The Committee evaluated exposure to AMC and its residual monomers, methacrylic acid, methyl methacrylate and methyl acrylate. As the Committee evaluated the toxicology of methacrylic acid, a total exposure to this monomer was estimated from the sum of the methacrylic acid monomer exposure and the methacrylic acid product of methyl methacrylate metabolism.

The exposure assessment included estimates provided by the sponsor and an evaluation by EFSA [1] based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. The national consumption data were from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOcOss) and data from Australia and New Zealand submitted to the Committee. A comprehensive literature search was also conducted; no additional studies were identified.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals, given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are not relevant for the general healthy population. This use was not further evaluated by the Committee.

The total monomeric content of AMC is less than 0.01%. This level was used to calculate the exposure to individual monomers based on the ratio of each monomer in the copolymer. The total exposure to methacrylic acid is from the sum of the exposure to methacrylic acid monomers and methacrylic acid from methyl methacrylate with a conversion using molecular weights (Table 3).

The Committee noted that AMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA ranged between 8.0 and 13.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

Evaluation

There were no concerns for the toxicity of AMC itself. However, the presence in AMC of the residual monomer methyl acrylate, for which it is not possible to conclude on genotoxic potential, and the insufficient carcinogenicity data for methyl acrylate preclude establishing an ADI for AMC.

The available toxicology data for AMC itself do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for AMC range from 400 to 1500 mg/kg bw per day, the highest doses tested. Estimated exposures to AMC range from 2.9 to 43 mg/kg bw per day.

Toxicological data on the residual monomers, apart from the genotoxicity data, do not give rise to concerns when taking into account the low

Table 3
Summary of range of estimated exposures to AMC, its monomers and total methacrylic acid from uses in food supplements for average and high exposures

Population group	Range of estimated dietary exposures ^{a,b}				Total methacrylic acid exposure (µg/kg bw per day) ^c
	AMC (mg/kg bw per day)	Methacrylic acid (µg/kg bw per day)	Methyl methacrylate (µg/kg bw per day)	Methyl acrylate (µg/kg bw per day)	
Adults	3.5–37	0.04–0.4	0.1–0.9	0.2–2.4	0.1–1.2
Children	2.9–43	0.03–0.4	0.1–1.1	0.2–2.8	0.1–1.4

AMC: anionic methacrylate copolymer; bw; body weight
^a All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated average exposures, and the upper end of the range is the highest of the estimated high exposures.
^b Includes exposures estimates submitted by the sponsor and the European Food Safety Authority [1], and national estimates calculated by the Committee based on a concentration of 100 mg per 1 g dosage unit.
^c The total methacrylic acid exposure is the sum of the exposure to methacrylic acid monomers and methacrylic acid from methyl methacrylate, with a conversion using molecular weights.

exposures. Genotoxicity data for methyl methacrylate suggest a potential risk for mutagenicity and clastogenicity in vitro, and there is a lack of adequate data on genotoxicity in vivo. However, in carcinogenicity studies in mice, rats and hamsters with methyl methacrylate given by inhalation, there was no evidence of any carcinogenic effects. In a 2-year study in rats given methyl methacrylate in drinking-water, the NOAEL was 121 mg/kg bw per day; from this NOAEL, a TDI of 1.2 mg/kg bw was derived [16]. Estimated exposures to methyl methacrylate range from 0.1 to 1.1 µg/kg bw per day, which are below the TDI.

Data on methyl acrylate are limited. ADME studies suggest that methyl acrylate is rapidly absorbed and excreted. The genotoxicity data are insufficiently adequate to draw conclusions on the genotoxic potential of methyl acrylate. Although a rat carcinogenicity study on methyl acrylate by the inhalation route was negative, no suitable long-term oral toxicity studies were available to support the safety of methyl acrylate. The Committee was unable to conclude on the safety of methyl acrylate as a residual monomer in AMC. Estimated exposures to methyl acrylate range from 0.2 to 2.8 µg/kg bw per day.

There were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid, and no long-term carcinogenicity studies were available. The Committee noted that there was evidence that methyl methacrylate is metabolized to methacrylic acid, and therefore the four negative long-term toxicity oral (via drinking-water) and inhalation carcinogenicity studies on methyl methacrylate could be used to support the safety of methacrylic acid. The Committee concluded that the exposure to methacrylic acid from the sum of the levels present in AMC and as a metabolite of methyl methacrylate, ranging from 0.1 to 1.4 µg/kg bw per day, would be unlikely to be a health concern.

The Committee was unable to complete the evaluation of AMC. While the copolymer itself is not a health concern, genotoxicity concerns remain for the residual monomer methyl acrylate.

A toxicological and dietary exposure monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared. The specifications were made tentative pending the completion of the safety evaluation of AMC.

References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS): Scientific Opinion of the safety of anionic methacrylate copolymer for the proposed uses as a food additive. *EFSA J.* 2010;8(7):1656 [25 pp.] doi:10.2903/j.efsa.2010.1656.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Absorption, distribution and excretion study in the rat following a 13-day administration period. Huntingdon Life Sciences, Eye, Suffolk, England, United Kingdom; 1999. Unpublished study report no. 010/984481 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
4. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer.* 1977;36(1):114–9 [cited in reference 16].
5. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
6. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995 (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
7. Vodicka P, Gut I, Frantík E. Effects of inhaled acrylic acid derivatives in rats. *Toxicology.* 1990;65(1–2):209–21.
8. Sapota A. The disposition and metabolism of methyl acrylate in male Wistar albino rats. *Pol J Occup Med Environ Health.* 1993;6(2):185–93.
9. Bereznowski Z, Mariewski M, Somolenski RT. High performance liquid chromatographic determination of methacrylate in blood serum. *Biomed Chromatogr.* 1994;8(1):42–4 [cited in European Commission, 2002].
10. Acute oral toxicity study in the rat. Research Toxicology Centre, Rome, Italy; 1995. Unpublished study report no. 5090/T/211/95 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Four-week oral toxicity study in rats followed by a 2-week recovery period. Research Toxicology Centre, Rome, Italy; 1998. Unpublished study report no. 861/T/215/98 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
12. Twenty-six week oral toxicity study in rats followed by a 4-week recovery period. Unpublished study by Research Toxicology Centre, Rome, Italy; 2000. Report no. 7250/T/346/2000 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

13. Four-week oral toxicity study in dogs. Unpublished study by Research Toxicology Centre, Rome, Italy; 2004. Report no. 8101 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. Prenatal developmental toxicity study in rats. Unpublished study by Harlan Bioservice for Science, Walsrode, Germany; 2003. Study report no. 20-4-0144-02 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. Borzelleca JF, Larson PS, Hennigar GR, Hluf EG, Crawford EM, Blackwell Smith R. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 16].
16. Methyl methacrylate. Geneva: World Health Organization; 1998 (International Programme on Chemical Safety II. Concise international chemical assessment document 4).
17. Reininghaus W, Koestner A, Klimisch HJ. Chronic toxicity and oncogenicity of inhaled methyl acrylate and *n*-butyl acrylate in Sprague-Dawley rats. *Food Chem Toxicol.* 1991;29(5):329–39.
18. Reevaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 71. Lyon: International Agency for Research on Cancer; 1999.

3.1.2 Basic methacrylate copolymer

Explanation

Basic methacrylate copolymer (BMC; E 1205; INS No. 1205; CAS No. 24938-16-7; basic butylated methacrylate copolymer; amino methacrylate copolymer; aminoalkyl methacrylate copolymer E; butyl methacrylate, dimethylaminoethyl methacrylate, methyl methacrylate polymer; butyl methacrylate, methyl methacrylate, dimethylaminoethyl methacrylate copolymer) is a cationic copolymer manufactured using the monomers dimethylaminoethyl methacrylate [2-(dimethylamino)ethyl methacrylate], butyl methacrylate [*n*-butyl methacrylate] and methyl methacrylate in the molar ratio of 1:2:1.

BMC has been evaluated by EFSA and is an approved food additive within the European Union where its use is restricted to a maximum level of 100 000 mg/kg in solid food supplements, category 17.1 [1].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. BMC is soluble below pH 5 and is used for its taste- and odour-masking properties; as protection from heat, light, moisture and oxidation; and to prevent the fast release of active ingredients in the stomach.

BMC has not previously been evaluated by the Committee. The Committee evaluated the use of BMC as a coating or glazing agent for solid food supplements and foods for special medical purposes in the form of solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 10%, at the request of CCFA49 [2]. Another proposed use of BMC is for microencapsulation, which enhances the stability of micronutrients in food

fortification, specifically for populations with nutrient deficiencies. BMC is also used in pharmaceuticals.

Toxicological data submitted for the evaluation included ADME, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the monomers but no additional studies on BMC. The Committee considered the data on the residual monomers because these are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. As exposure to monomers from BMC is higher than to AMC and neutral methacrylate copolymer (NMC), the Committee evaluated ADME, short- and long-term toxicity studies, and genotoxicity and reproductive and developmental toxicity data on the monomers.

The Committee was also aware that BMC contains an oligomer fraction of up to 10%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 1000 Da, and around two thirds of the oligomer fraction has a molecular weight of between 4000 and 5000 Da, it is unlikely that the oligomers would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the distribution and toxicity studies was prepared from an aqueous dispersion and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of BMC.

The Committee evaluated toxicological and exposure data on 2-propanol, butanol and methanol, the residual components of BMC that can be present in the final products because they are used in the manufacture of the copolymer. The Committee concluded that these residual components do not pose a safety concern at the maximum estimated exposure levels.

Chemical and technical considerations

BMC is manufactured by a controlled polymerization process using a free radical donor initiator system. After completion of polymerization, the viscous copolymer solution is fed into an extruder to remove solvents and volatile substances, by continuous degassing through vacuum and heating. The solid granules of BMC formed in the extruder can be milled to a powder in which not more than 10% of the particles have a diameter less than 3 µm.

BMC has a weight-average molecular weight of 47 000 Da and number-average molecular weight of 22 000 Da.

During the manufacture of BMC, 2-propanol is added as a production aid. Most of the solvent evaporates during the polymerization and extrusion steps in the manufacture of BMC. The methyl methacrylate integrated in the polymer chain may hydrolyse to methacrylic acid and methanol. The methacrylic acid remains in the polymer chain but methanol is released. Similarly, butyl

methacrylate integrated in the polymer chain may hydrolyse to methacrylate and butanol. BMC may contain 2-propanol (not exceeding 0.5%); butanol (not exceeding 0.5%); and methanol (not exceeding 0.1%). The copolymer may also contain residual monomers: dimethylaminoethyl methacrylate (not exceeding 500 mg/kg); butyl methacrylate (not exceeding 200 mg/kg); and methyl methacrylate (not exceeding 50 mg/kg).

Biochemical aspects

BMC

When radiolabelled BMC (purity >98%) was administered to adult rats in two separate studies, the majority was excreted in the faeces within 2–3 days of administration. Levels of radioactivity in the gut returned to normal within 7 days. From the levels of radioactivity in the urine, it can be concluded that less than 0.02% of the radioactivity was absorbed [3].

Residual monomers

There were no toxicokinetic studies on 2-(dimethylamino)ethyl methacrylate. Studies using simulated saliva or intestinal fluids show that this residual monomer is rapidly hydrolysed with 86–90% degradation [4].

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration to rats. Methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [5, 6, 7].

Like methyl methacrylate, *n*-butyl methacrylate, is rapidly metabolized by carboxylesterases. Hydrolysis of *n*-butyl methacrylate yields methacrylic acid and *n*-butanol, which are further metabolized, with methacryl CoA, a physiological substrate of the valine pathway [8].

Toxicological studies

BMC

In acute toxicity studies in mice, no effects were observed after oral, intraperitoneal and subcutaneous administration of BMC (purity >98%) at 3.0, 2.0 and 1.0 g/kg bw, respectively, although deaths occurred at higher doses via the intraperitoneal and oral routes. In rats, the oral median lethal dose (LD₅₀) was greater than 15 g/kg bw. Orally administered lactose granules (125 g) coated in test material (BMC at 5.9 g/kg bw) resulted in deaths in rats, but this mortality was attributed to the amount of the administered dose. When doses of BMC of up to 6 g/kg bw were administered to rats in feed, no adverse effects were observed [9, 10, 11].

Short-term toxicity studies were available in rats and dogs. A 6-month study in rats administered BMC (purity >98%) in feed at 500 or 2000 mg/kg bw per day showed no treatment-related effects [12]. A 28-day study in dogs administered BMC (>98% BMC) in gelatin capsules showed no treatment-related effects at doses of 100–750 mg/kg bw per day [13].

No long-term toxicity or carcinogenicity studies were available on the copolymer itself.

A reverse mutation assay in bacteria, a gene mutation assay in mammalian cells and an in vivo mouse micronucleus assay all produced negative results. The Committee concluded that BMC was not of concern for genotoxicity.

A developmental study in rats showed no treatment-related effects at the only dose tested, 1000 mg/kg bw per day, when administered in feed between gestation days 6 and 16 [14].

Studies of cytotoxicity, dermal toxicity, ocular toxicity and phototoxicity with BMC showed no effects.

Residual monomers

2-(Dimethylamino)ethyl methacrylate

A short-term toxicity study with 2-(dimethylamino)ethyl methacrylate in rats given gavage doses of 0, 100, 200 or 500 mg/kg bw per day for 13 weeks showed no treatment-related adverse effects. The NOAEL was 500 mg/kg bw per day, the highest dose tested [15].

No long-term toxicity studies were available on 2-(dimethylamino)ethyl methacrylate. In genotoxicity studies, 2-(dimethylamino)ethyl methacrylate was negative in two reverse mutation assays in bacteria, apart from one isolated positive response in *Salmonella typhimurium* TA1537 in the absence of metabolic activation (negative in the presence of metabolic activation), and negative in a gene mutation assay in mammalian cells in vitro. The monomer was positive for clastogenicity in two chromosomal aberration tests in mammalian cells in vitro, but negative in a GLP-compliant in vivo mouse erythrocyte micronucleus test conducted according to Organisation for Economic Co-operation and Development (OECD) guideline 474. The Committee concluded that 2-(dimethylamino)ethyl methacrylate does not raise concerns for genotoxicity in vivo.

In a reproductive toxicity study with 2-(dimethylamino)ethyl methacrylate, rats received gavage doses of 0, 40, 200 and 1000 mg/kg per day from 14 days before mating, for 43 days in males and until lactation day 3 in females. There were no effects on reproductive parameters. At the highest dose, maternal toxicity was observed and there were some deaths among dams, with total loss of litters in some dams during the lactation period, reduced pup body weight and reduced pup viability. The NOAEL was 200 mg/kg bw per day [16].

In a developmental toxicity study, 2-(dimethylamino)ethyl methacrylate was administered to female rats by gavage at dose levels of 0, 100, 300 or 600 mg/kg bw per day from gestation days 6 to 19. There were no treatment-related effects on maternal or embryo/fetal parameters. The NOAEL was 600 mg/kg bw per day, the highest dose tested [17]

Methyl methacrylate

In most of the numerous studies on the short-term toxicity of methyl methacrylate in mice, rats, hamsters and dogs, the monomer was administered by inhalation. Most commonly observed effects were decreases in body-weight gain and irritation of the skin, nasal cavity and eye at high concentrations (generally 500 ppm [2050 mg/m³]). At even higher concentrations, renal cortical necrosis and tubular degeneration (in rats and mice) and hepatic necrosis (in mice) were also reported [18].

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight in females increased at the highest dose, but no treatment-related histopathological effects were observed in any organ or tissue [19]. Based on the results of this study, a TDI of 1.2 mg/kg bw per day was determined [18].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [19].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and SCE assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days of exposure. These results were judged by the Committee to be inconclusive. The Committee concluded that there was some evidence of mutagenicity and clastogenicity in vitro. There was a lack of adequate in vivo tests following up the equivocal findings.

There were no conventional reproductive toxicity studies on methyl methacrylate. A dominant lethal study in mice given 100, 1000 or 9000 ppm (410, 4100 and 36 900 mg/m³, respectively) of methyl methacrylate by inhalation for 6 hours/day for 5 days showed no effects on fertility [20].

In older studies on mice and rats, developmental effects including decreases in fetal weight and increases in embryo/fetal death and skeletal abnormalities were observed following inhalation of methyl methacrylate at concentrations that were toxic to the dams [21, 22].

In another older developmental toxicity study, pregnant mice were exposed to 1330 ppm (5450 mg/m³) of methyl methacrylate for 2 hours, twice daily, during gestation days 6–15. There were no adverse developmental effects [23].

In a developmental toxicity study, rats were given methyl methacrylate by inhalation at concentrations from 9 to 2028 ppm (406–8315 mg/m³) for 2 hours daily from gestation days 6 to 15. There were no treatment-related adverse effects. The NOAEL was 8315 mg/m³ [24].

n-Butyl methacrylate

In an inhalation study, rats were exposed to *n*-butyl methacrylate at concentrations of 310, 952 or 1891 ppm for 6 hours/day, 5 days/week for 4 weeks. Microscopic examination of the nasal cavities of the male and female rats exposed to 1891 ppm showed slight and localized bilateral degeneration of the olfactory epithelium lining of the dorsal meati. One male and one female rat exposed to 952 ppm had similar changes in the olfactory epithelium. Rats exposed to 310 ppm had no exposure-related microscopic changes in the nasal cavity [25].

No long-term studies were available on *n*-butyl methacrylate.

n-Butyl methacrylate tested negative in a range of in vitro and in vivo mutagenicity studies covering all the relevant end-points.

In a developmental toxicity study in rats on four methacrylates, including *n*-butyl methacrylate, animals were exposed by inhalation for 6 hours/day on gestation days 6–20. The exposure concentrations for *n*-butyl methacrylate were 0, 100, 300, 600 or 1200 ppm. Fetal toxicity was evident as decreases in fetal body weight at 600 ppm or greater. These exposure levels of *n*-butyl methacrylate were also maternally toxic. No significant increases in embryo/fetal deaths or fetal malformations were observed [26].

Observations in humans

No human data were available on BMC.

Assessment of dietary exposure

The Committee evaluated exposure to BMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes as well as

for micronutrient encapsulation for food fortification. Because another major use of BMC is in pharmaceuticals, this use was also evaluated in the exposure assessment. The level of use of BMC in food supplements, pharmaceuticals and foods for special medical purposes is a maximum of 10%. The level of use of BMC for food fortification differs depending on the nutrient or group of nutrients being microencapsulated.

The Committee evaluated exposure to BMC for the copolymer and its monomers, *n*-butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate.

The exposure assessment included submitted estimates and an evaluation by EFSA based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. National consumption data were from the CIFOCCOSS. Data from Australia and New Zealand were submitted to the Committee. A second sponsor provided estimates of dietary exposure from use in micronutrient encapsulation for food fortification. These estimates included exposures for 12 nutrients at a level that met 100% of their respective recommended dietary allowance (RDA). The exposure was estimated for each nutrient individually and also for the sum of all 12 nutrients.

A comprehensive literature search was also conducted; no additional studies were identified.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals, given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are therefore not relevant for the general healthy population. This use was therefore not further considered by the Committee.

The total monomeric content of BMC is less than 0.3%. This level was therefore used to calculate the exposure to total monomers from the copolymer exposure. Estimates of exposure to the individual monomers were based on exposure to total monomers, taking into account the ratio of each individual monomer in the copolymer.

The estimated exposures to BMC and its monomers for adults and children from uses in food supplements and in micronutrient encapsulation for food fortification are shown in [Table 4](#). All estimates of exposure from uses in food supplements are presented as a range from the lowest of the average exposures to the highest of the high exposures. All estimates of dietary exposure from micronutrient encapsulation for food fortification are presented as a range

Table 4

Summary of the range of estimated exposures to BMC and its monomers for average and high exposures

Population group	Source of exposure	Range of estimated dietary exposures ^{a,b}			
		Copolymer exposure (mg/kg bw per day)	Monomer exposure (µg/kg bw per day)		
			<i>n</i> -Butyl methacrylate	2-(Dimethylamino) ethyl methacrylate	Methyl methacrylate
Adults	Food supplements	3.5–37	2.6–28	5.2–56	2.6–28
	Micronutrient encapsulation for food fortification ^c	0.03–45	0.02–33	0.04–67	0.02–33
	All sources	3.5–82	2.6–61	5.2–120	2.6–61
Children	Food supplements	2.9–43	2.2–32	4.4–64	2.2–32
	Micronutrient encapsulation for food fortification ^c	0.05–92	0.04–69	0.1–140	0.04–69
	All sources	3.0–135	2.2–100	4.4–200	2.2–100

BMC: butyl methacrylate; bw: body weight

^a All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated mean exposures, and the upper end of each range is the highest of the estimated high exposures.^b Includes exposure estimates submitted by the sponsor and the European Food Safety Authority [1] and national estimates calculated by the Committee. Based on a concentration of 100 mg per 1 g dosage unit.^c Based on meeting 100% of the United States/Canadian recommended dietary allowance. The lower end of each range is lowest exposure for a single nutrient and the upper end of each range is the sum of exposure from 12 nutrients.

from the lowest exposure for a single nutrient to the exposure for the sum of the 12 nutrients assessed.

The Committee noted that the exposures estimated by the sponsor were to provide a presumed worst case for the purpose of the safety evaluation. The Committee also noted that national estimates of exposure would be required for the evaluation of safety based on their own nutrient reference values, fortification needs and food consumption patterns.

Estimates of exposure to BMC and its monomers from all sources combined (food supplements and micronutrient encapsulation for food fortification) are also shown in Table 4. The Committee noted that the upper end of the range representing high exposures from all sources is a worst case estimate, and unlikely in terms of actual long-term exposure to BMC.

The Committee noted that BMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA ranged between 8.0 and 13.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

Evaluation

The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not a safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification.

An ADI “not specified” was established for BMC.

The available toxicology data for BMC do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for BMC range from 750 to 2000 mg/kg bw per day, the highest doses tested.

Toxicological data on the residual monomers do not give rise to concerns when taking into account the low exposures. 2-(Dimethylamino) ethyl methacrylate and *n*-butyl methacrylate do not give rise to concerns for genotoxicity. Long-term, reproductive and developmental toxicity studies do not suggest a risk to health at the estimated exposure levels. Genotoxicity data for methyl methacrylate suggest a potential for mutagenicity and clastogenicity in vitro, but there is a lack of adequate in vivo genotoxicity data. However, in carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, there was no evidence of any carcinogenic effects. In a 2-year study in rats given methyl methacrylate in drinking-water, the NOAEL was 121 mg/kg bw per day; from this NOAEL, a TDI of 1.2 mg/kg bw per day was derived [18]. Estimated exposures to methyl methacrylate range from 2.2 to 100 µg/kg bw per day, which are below the TDI.

A toxicological and dietary exposure monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared.

References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the use of basic methacrylate copolymer as a food additive. EFSA J. 2010;8(2):1513 [23 pp.] doi:10.2903/j.efsa.2010.1513.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. [¹⁴C]-Eudragit-E-100: The excretion and tissue distribution of radioactivity after oral administration to rats. Report no. 79/RGC024/278. Life Science Research, Stock, Essex, England, United Kingdom; 1979. Unpublished study report. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

4. 2-Dimethylaminoethyl methacrylate: Basic toxicokinetics. EC no.: 220-688-8. CAS no.: 2867-47-2. Helsinki: European Chemicals Agency; 2015a (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/2/2>, accessed 6 November 2015).
5. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer*. 1977;36(1):114–9 [cited in reference 18].
6. Canadian Environmental Protection Act: Priority Substances List assessment report for methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993.(Cat. no.: En40-215/10E) [cited in reference 18].
7. ECETOC. Methyl methacrylate – CAS no. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995 (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 18].
8. IUCLID [database]. Butyl methacrylate (97-88-1) [2000 CD-ROM edition] [48 pp.] European Commission; 2010 (<http://esis.jrc.ec.europa.eu/>).
9. Determination of acute oral toxicity in rats. Unpublished study report by Battelle-Institute, Frankfurt am Main, Germany; 1966. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
10. The acute toxicity of preparation 2787 on oral administration to rats. Unpublished study report. Hamburg, Germany: Laboratory for Pharmacology and Toxicology; 1970. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Acute toxicity of Eudragit E. Unpublished study by Department of Pharmacology, Nagasaki University, Nagasaki, Japan; 1970. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
12. Six-month toxicity of 2787 B in Sprague-Dawley rats with administration in the feedstuff. Report no. 100692 by Laboratory for Pharmacology and Toxicology, Hamburg, Germany; 1973. Unpublished study report submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
13. Twenty-eight day oral toxicity study of Eudragit E PO in the dog. Unpublished study report no. 20-2-0145-02 by Harlan Bioservice for Science, Walsrode, Germany; 2003. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. Experiments to determine the effect of 2577G and 2697 on pregnant rats and their foetuses. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg, Germany; 1968. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. 2-Dimethylaminoethyl methacrylate. EC no.: 220-688-8. CAS no.: 2867-47-2. Repeated dose toxicity: oral. Helsinki: European Chemicals Agency; 2015b (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/6/2>, accessed 20 June 2018).
16. 2-Dimethylaminoethyl methacrylate: toxicity to reproduction. EC no.: 220-688-8. CAS no. 2867-47-2. Helsinki: European Chemicals Agency; 2015c (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/9/2>, accessed 20 June 2018).
17. 2-Dimethylaminoethyl methacrylate. EC no.: 220-688-8. CAS no.: 2867-47-2. Developmental toxicity / teratogenicity. Helsinki: European Chemicals Agency; 2015d (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/9/3>, accessed 20 June 2018).
18. Methyl methacrylate. Concise international chemical assessment document 4. Geneva: World Health Organization; 1998 (International Programme on Chemical Safety, Concise International Chemical Assessment Document No. 4).

19. Borzelleca JF, Larson PS, Hennigar GR Jr, Huf EG, Crawford EM, Smith RB Jr. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 18].
20. Anderson D, Hodge MCE. Methyl methacrylate monomer: Dominant lethal study in the mouse. ICI, Macclesfield, Cheshire, England, United Kingdom; 1976. Report no. CTL/P/295 [cited in reference 18].
21. Hodge MCE, Palmer S. Methylmethacrylate monomer teratogenicity studies in the rat. Unpublished study by Rohm and Haas Company, Philadelphia, Pennsylvania, USA, to the US Environmental Protection Agency, Washington (DC), USA, under TSCA Section 8(d), July 1979; 1977 [cited in reference 18].
22. Nicholas CA, Lawrence WH, Autian J. Embryotoxicity and fetotoxicity from maternal inhalation of methyl methacrylate monomer in rats. *Toxicol Appl Pharmacol.* 1979;50(3):451–8 [cited in reference 18].
23. McLaughlin RE, Reger SI, Barkalow JA, Allen MS, Diffazio CA. Methyl methacrylate: A study of teratogenicity and fetal toxicity of the vapor in the mouse. *J Bone Joint Surg Am.* 1978;60(3):355–8 [cited in reference 18].
24. Solomon HM, McLaughlin JE, Swenson RE, Hagan JV, Wanner FJ, O'Hara GP et al. Methyl methacrylate: Inhalation developmental toxicity study in rats. *Teratology.* 1993;48(2):115–25.
25. ECHA. Butyl methacrylate. EC no.: 202-615-1. CAS no.: 97-88-1. Repeated dose toxicity: Inhalation. Helsinki: European Chemicals Agency; 2018 (<https://echa.europa.eu/registration-dossier/-/registered-dossier/15151/7/6/3>, accessed 20 June 2018).
26. Saillenfait AM, Bonnet P, Gallissot F, Peltier A, Fabriès JF. Developmental toxicities of methacrylic acid, ethyl methacrylate, *n*-butyl methacrylate, and allyl methacrylate in rats following inhalation exposure. *Toxicol Sci.* 1999;50(1):136–45 [cited in reference 25].

3.1.3 Erythrosine

Explanation

Erythrosine (INS No. 127; CAS No. 16423-68-0) is a xanthene dye permitted as a food colour in China, the European Union, the USA and other regions. It is used for colouring foods including baked goods, breakfast cereals, confectionery products, dairy products, decorations for baking, dressings and sauces, dried fruit, frostings and icings, frozen breakfast foods, frozen treats, hot beverages, juice drinks and processed foods (fish, meat and egg products).

The Committee previously evaluated the safety of erythrosine at its eighth, thirteenth, eighteenth, twenty-eighth, thirtieth, thirty-third and thirty-seventh meetings ([Annex 1](#), references 8, 19, 35, 66, 73, 83 and 94) and for dietary exposure at its fifty-third meeting ([Annex 1](#), reference 143). Toxicological monographs or monograph addenda were published after the thirteenth, eighteenth, twenty-eighth, thirtieth, thirty-third and thirty-seventh meetings ([Annex 1](#), references 20, 36, 67, 74, 84 and 95). At its eighteenth meeting the Committee allocated an ADI of 0–2.5 mg/kg bw. This ADI was reduced at the twenty-eighth meeting to 0–1.25 mg/kg bw and made temporary following observations that erythrosine

produced effects on thyroid function in short-term toxicity studies in rats and that, in long-term toxicity studies, male rats receiving 4% erythrosine in the diet developed benign thyroid tumours.

At the thirtieth meeting the Committee reduced the temporary ADI to 0–0.6 mg/kg bw, based on biochemical effects of erythrosine on thyroid hormone metabolism and regulation in rats. The Committee at the thirtieth meeting requested further data from pharmacokinetic studies relating the amount of erythrosine absorbed to the amount ingested to enable the establishment of a correlation between blood/tissue erythrosine levels and effects on the thyroid. At the thirty-third meeting the Committee further reduced the temporary ADI to 0–0.05 mg/kg bw. This ADI was based on a NOAEL from a study showing slightly increased thyroid-stimulating hormone (TSH) responsiveness in humans ingesting erythrosine at 60 mg per person per day (equivalent to 1 mg/kg bw per day) for 14 days, and applying an uncertainty factor² of 20. The Committee again requested the pharmacokinetic studies required by the previous Committee. At its thirty-seventh meeting, the Committee re-evaluated previously reviewed studies that had since been published and newer studies on thyroid physiology in rats. The Committee concluded that the thyroid tumours in male rats previously reported in long-term toxicity studies were secondary to thyroid hormone changes and species-specific sensitivity.

In view of the differences in thyroid physiology between humans and rats, the Committee based its evaluation on the human data and allocated an ADI of 0–0.1 mg/kg bw on the NOAEL of 60 mg per person per day from the 14-day study in human subjects (equivalent to 1 mg/kg bw per day), with application of an uncertainty factor of 10.

At the present meeting, the Committee re-evaluated erythrosine at the request of CCFA49 [1].

A toxicological dossier that included new studies on genotoxicity, reproductive and developmental toxicity, neurological effects and hypersensitivity was submitted. A comprehensive literature search conducted in PubMed retrieved three additional studies relevant to the present evaluation. The Committee also considered studies evaluated at previous meetings.

Chemical and technical considerations

Erythrosine consists of the disodium salt of 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Erythrosine is manufactured by iodination of fluorescein, the condensation product of resorcinol and phthalic anhydride. Impurities include unreacted starting materials ($\leq 0.4\%$), subsidiary colouring matters except fluorescein

² The previous Committee used the term “safety factor”.

($\leq 4\%$), fluorescein (≤ 20 mg/kg), inorganic iodides ($\leq 0.1\%$), lead (≤ 2 mg/kg) and zinc (≤ 50 mg/kg).

Biochemical aspects

Erythrosine is poorly absorbed and mainly excreted unchanged in rat faeces.

Following oral administration of radiolabelled erythrosine, less than 1% of the dose was excreted in the urine. Blood and plasma radioactivity reached maximum levels by 1 hour, while liver and kidney levels peaked after 4–12 hours; no radioactivity was detectable in the brain or pituitary. No erythrosine was accumulated in the thyroid or other tissues [2].

No additional metabolic or kinetic studies have become available since the previous evaluation by the Committee.

Toxicological studies

Erythrosine has low oral acute toxicity in mice, rats, gerbils and rabbits.

A short-term toxicity study with erythrosine showed pigment deposition in renal tubules in female rats at the 2% dietary level and in males at all but the lowest dietary levels, in a dose-related manner. The NOAEL was 0.25% in the diet (equal to 160 mg/kg bw per day) [3]. No compound-related effects were observed in other short-term toxicity studies in rats, gerbils, dogs and pigs. No additional short-term toxicity studies have become available since the previous evaluation by the Committee.

Several long-term oral toxicity studies showed no compound-related increases in tumour incidences in mice, rats and gerbils. In one long-term study of oral toxicity [4], mice showed decreased body weights at 3.0% erythrosine in the diet. The NOAEL was 1.0% erythrosine in the diet (equal to 1474 mg/kg bw per day). Body-weight decreases were also observed in a rat study [5]. The NOAEL was 1% erythrosine in the diet (equivalent to 500 mg/kg bw per day).

Two long-term feeding studies with erythrosine found an increase in the incidence of thyroid follicular cell adenomas in male rats [6, 7]. The previous Committee considered the occurrence of thyroid follicular tumours in rats secondary to hormonal effects based on results from studies on thyroid function and morphology. Another study indicated that erythrosine promoted the development of thyroid follicular tumours in partially thyroidectomized rats, but not in non-thyroidectomized rats [8]. The present Committee noted that the rat is not considered a suitable model for potential effects on the thyroid in humans [9].

A large number of in vitro and in vivo genotoxicity tests have been conducted on erythrosine. The Committee confirmed that the overall weight of evidence indicates that erythrosine is not genotoxic.

Two short-term oral toxicity studies indicated that erythrosine may affect testicular function in mice. Abdel-Aziz et al. [10] observed decreased epididymal sperm counts and sperm motility at dose levels of 68 and 136 mg/kg bw per day and a significant increase in the incidence of abnormal sperm head morphology at doses of 680 and 1360 mg/kg bw per day. Vivekanandhi et al. [11] observed decreases in sperm motility and sperm counts and increases in sperm abnormalities, with a lowest-observed-adverse-effect level (LOAEL) of 64 mg/kg bw per day. No effects on sperm or on fertility were observed in rats in long-term toxicity studies and a multigeneration study conducted with higher doses administered via the diet [6, 7, 12].

No developmental toxicity was reported in rats.

In a newly available reproductive toxicity study in mice, administration of erythrosine at dietary concentrations of 0, 0.005%, 0.015% or 0.045% (equivalent to 0, 7.5, 22.5 and 67.5 mg/kg bw per day, respectively) resulted in no adverse effects on reproductive parameters or functional developmental parameters in offspring. Significant changes in some measures of exploratory behaviour and motor activity were reported in high-dose dams and their offspring [13]. Motor activity and brain serotonin levels in rat single-dose and repeated-dose studies were inconsistent [14, 15]. In view of the lack of consistency in the data, the poor oral absorption of erythrosine, the evidence that erythrosine does not penetrate the blood–brain barrier and the lack of evidence of behavioural toxicity in a rat study with dose levels equivalent to up to 1000 mg/kg bw per day [16], the Committee concluded that the findings in the Tanaka [13] and Dalal & Poddar [14, 15] studies did not provide robust evidence of behavioural effects and could not be used in the risk assessment.

Observations in humans

Studies in human volunteers showed increased blood iodine levels but no changes in thyroid hormone levels in repeated-dose studies of up to 25 mg/day for 3 weeks or single-dose studies of up to 80 mg. A study with 30 healthy male volunteers found that erythrosine slightly increased thyroid-stimulating hormone (TSH) responsiveness to thyrotropin-releasing hormone (TRH), but there were no effects on other thyroid hormone parameters at the highest dose level of 200 mg per person per day for 14 days [17]. No effect was observed at 60 mg per person per day. At its thirty-third meeting, the Committee concluded that the NOAEL was 60 mg per person per day (equivalent to 1 mg/kg bw per day), and the present Committee concurred.

Assessment of dietary exposure

The Committee previously evaluated dietary exposure to erythrosine at its fifty-third meeting, but significant changes in methodologies for the exposure

Table 5

Summary of the range of estimates of dietary exposure for erythrosine

Source of estimates	Population	Range of estimated dietary exposures (mg/kg bw per day)	
		Means	High percentile (P90 and P95) ^a
Analytical levels ^b	Children	0.00–0.01	0.00–0.09
	Adolescents	0.00–0.02	0.00–0.04
	Adults	0.00–0.03	0.00–0.05
Maximum use levels ^c	Toddlers	0–0.2	0–0.4
	Children	0–0.1	0–0.1
	Adolescents	0–0.1	0–0.1
	Adults	0–0.1	0–0.2
	Elderly adults	0–0.1	0–0.1

bw: body weight; P90: 90th percentile; P95: 95th percentile

^a The upper bound of the range is the maximum of the 90th and 95th percentiles.^b Studies from the Republic of Korea [18] and the USA [19, 20] for consumers only.^c From published national studies [21, 22]) and the Committee's assessment based on consumption data from CIFOCCs for 36 countries.

assessments precluded comparisons between the previous and the current assessments.

A comprehensive literature search retrieved five relevant studies other than those submitted by the sponsor. The Committee reviewed published estimates of dietary exposure to erythrosine conducted in several countries. The Committee also conducted a conservative assessment using consumption data from 36 countries from the CIFOCCs database and Codex maximum use levels. A summary of the dietary exposure estimates is provided in [Table 5](#).

Dietary exposure estimates based on individual consumption data and maximum use levels range from 0 to 0.4 mg/kg bw. Because erythrosine has been in use for many years, national estimates based on analytical data were also available to the Committee. National exposure estimates based on analytical data range from 0.00 to 0.05 mg/kg bw per day for adults and from 0.00 to 0.09 mg/kg bw per day for children, considering consumers only.

The Committee also noted that exposure through pharmaceuticals was previously estimated to occur at up to approximately 0.1 mg/kg bw per day in specific populations, generally over a short period of time. However, the Committee considered that such exposure should not be taken into account in the assessment of exposure to erythrosine as a food additive when looking at long-term exposure in a healthy population.

The Committee considered the approach based on analytical data to be more realistic for preparing long-term dietary exposure estimates than the approach based on maximum use levels. Because the scenarios available from the national studies examined consumers only, the Committee concluded that the

highest estimate of 0.09 mg/kg bw per day for children should be considered in the safety assessment of erythrosine.

Evaluation

The evidence newly available at this meeting indicates that there are no concerns with respect to genotoxicity and reproductive and developmental toxicity of erythrosine. The previously established ADI of 0–0.1 mg/kg bw is based on a NOAEL of 60 mg per person per day (equivalent to 1 mg/kg bw per day for a 60 kg person) identified in a human study, with a default uncertainty factor of 10. In this study [17], minimal effects on thyroid function were observed at 200 mg per person per day (equivalent to 3.3 mg/kg bw per day). Effects in experimental animals were observed at doses at least 60-fold higher than the NOAEL in this human study; these effects supported the use of the human data as the basis for the ADI.

The Committee concluded that the new data that have become available since the previous evaluation do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw.

The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to erythrosine for all age groups do not present a safety concern.

A consolidated toxicological and dietary exposure monograph was prepared.

At the present meeting, the existing specifications for erythrosine were revised. High-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

A Chemical and Technical Assessment was prepared.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
2. Obrist J, LeVan L, Puhl RJ, Duan RJ. Final report: Metabolism of FD&C Red No. 3 in rats, study no. 6145–100. Unpublished report by Hazleton Laboratories America, Inc., 1986. Submitted to WHO by the Certified Color Manufacturers Association, Inc., Washington, DC, USA.

3. Butterworth KR, Gaunt IF, Grasso P, Gangolli SD. Acute and short-term toxicity studies on erythrosine BS in rodents. *Food Cosmet Toxicol.* 1976;14(6):525–31.
4. Borzelleca JF, Hallagen JB. Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in mice. *Food Chem Toxicol.* 1987;25(10):735–7.
5. Hansen WH, Davis KJ, Graham SL, Perry CH, Jacobson KH. Long-term toxicity studies of erythrosine. II. Effects on haematology and thyroxine and protein-bound iodine in rats. *Food Cosmet Toxicol.* 1973;11(4):535–45.
6. Brewer L, Jefferson ND, Blair M, Thorstenson JB, Nair KPC, Richter WR et al. Long-term dietary toxicity/carcinogenicity study in rats. FDC Red No. 3. Unpublished report no. 410–002 from International Research and Development Corporation, Mattawan, MI, USA; 1981. Submitted to WHO by the Certified Color Manufacturers Association Inc., Washington, DC, USA.
7. Borzelleca JF, Capen CC, Hallagen JB. Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in rats. *Food Chem Toxicol.* 1987;25(10):723–33.
8. Hiasa Y, Ohshima M, Kitahori Y, Konishi N, Shimoyama T, Sakaguchi Y et al. The promoting effects of food dyes, erythrosine (Red 3) and Rose Bengal B (Red 105), on thyroid tumors in partially thyroidectomized *N*-bis(2-hydroxypropyl)-nitrosamine-treated rats. *Jpn J Cancer Res.* 1988;79(3):314–9.
9. Part 1: IPCS framework for analysing the relevance of a cancer mode of action for humans and case-studies. Geneva: World Health Organization, International Programme on Chemical Safety; 2007; pp. 1–125. Harmonization Project Document No. 4.
10. Abdel-Aziz AH, Shouman SA, Attia AS, Saad SF. A study on the reproductive toxicity of erythrosine in male mice. *Pharmacol Res.* 1997;35(5):457–62.
11. Vivekanandhi J, Devi CPA, Jayaraman K, Raghavan L. Effect of erythrosine on testicular function of mice. *Toxicol Int.* 2006;13(2):119–25.
12. Aldridge D, Schardein JL, Blair M, Goldenthal EI, Benson BW. FD & C Red No. 3. Three generation reproduction study. Unpublished report no. 410–013 from International Research and Development Corporation, Mattawan, MI, USA; 1981. Submitted to WHO by the Certified Color Manufacturers Association, Inc., Washington, DC, USA.
13. Tanaka T. Reproductive and neurobehavioural toxicity study of erythrosine administered to mice in the diet. *Food Chem Toxicol.* 2001;39(5):447–54.
14. Dalal A, Poddar MK. Short-term erythrosine B-induced inhibition of the brain regional serotonergic activity suppresses motor activity (exploratory behavior) of young adult mammals. *Pharmacol Biochem Behav.* 2009;92(4):574–82.
15. Dalal A, Poddar MK. Involvement of high plasma corticosterone status and activation of brain regional serotonin metabolism in long-term erythrosine-induced rearing motor hyper activity in young adult male rats. *Toxicol Mech Methods.* 2010;20(6):287–97.
16. Vorhees CV, Butcher RE, Brunner RL, Wootten V, Sobotka TJ. A developmental toxicity and psychotoxicity evaluation of FD and C Red Dye No. 3 (erythrosine) in rats. *Arch Toxicol.* 1983;53(4):253–64.
17. Gardner DF, Utiger RD, Schwartz SL, Witorsch P, Meyers B, Braverman LE et al. Effects of oral erythrosine on thyroid function in normal men. *Toxicol Appl Pharmacol.* 1987;91(3):299–304.
18. Ha MS, Ha SD, Choi SH, Bae DH. Exposure assessment of synthetic colours approved in Korea. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2013;30(4):643–53.

19. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry SE. Exposure estimate for FD&C colour additives for the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2016;33(5):782–97.
20. Bastaki M, Farrell T, Bhusari S, Bi X, Scrafford C. Estimated daily intake and safety of FD&C food-colour additives in the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2017;34(6):891–904.
21. Gilsenan MB, Gibney MJ. Assessment of the influence of energy under-reporting on intake estimates of four food additives. *Food Addit Contam.* 2004;21(3):195–203.
22. Final assessment report. Application A603. Red 3 erythrosine in food colouring preparations. Canberra: Food Standards Australia New Zealand; 2010.

3.1.4 Indigotine

Explanation

Indigotine (INS No. 132) consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate (the principal component; CAS No. 860-22-0), disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (an isomer) and subsidiary colouring matters. Indigotine is used in China, the European Union, Japan, the USA and other regions. It is used for colouring foods including blueberry bagels, breakfast cereals, cakes and cupcakes, candies including chocolate, chewing gum, dairy products, decorations for baking, frozen treats, sauces and seasonings.

The Committee evaluated the safety of indigotine at its thirteenth and eighteenth meeting ([Annex 1](#), references 19 and 35). At its eighteenth meeting, the Committee established an ADI of 0–5 mg/kg bw based on a NOAEL of 500 mg/kg bw per day (1% in the diet) in a chronic dietary toxicity study in rats [1], and prepared specifications for indigotine ([Annex 1](#), reference 35). At its seventy-third meeting, the Committee revised the specifications ([Annex 1](#), references 202).

Indigotine was on the agenda for re-evaluation of safety and revision of specifications at the request of CCFA49 [2].

A toxicological dossier was submitted summarizing the available toxicity data, together with relevant study reports and publications. A comprehensive literature search retrieved eight other relevant studies.

Chemical and technical considerations

Indigotine is an indigoid dye. Indigotine consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate, disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (an isomer) and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Indigotine is manufactured by heating indigo in the presence of sulfuric acid. The indigo (or indigo paste) is manufactured by the fusion of

N-phenylglycine (prepared from aniline and formaldehyde) in a molten mixture of sodamide and sodium and potassium hydroxides under ammonia pressure. It is isolated and subjected to purification procedures prior to sulfonation. Impurities include unreacted starting materials ($\leq 0.5\%$), subsidiary colouring matters ($\leq 1\%$), unsulfonated primary aromatic amines ($\leq 0.01\%$ calculated as aniline) and lead (≤ 2 mg/kg).

Biochemical aspects

Although indigotine was poorly absorbed from the gastrointestinal tract in rats, one of its metabolites, 5-sulfoanthranilic acid, formed through microbial fermentation, was absorbed to a greater extent. After administration of a single oral dose of 50 mg/rat, unchanged indigotine, isatin-5-sulfonic acid and 5-sulfoanthranilic acid were identified in urine (0.53%, 0.63% and 0.28% of the dose, respectively). The metabolites isatin-5-sulfonic acid and 5-sulfoanthranilic acid were also identified in bile [3]. After in vitro incubation of indigotine with caecal microflora of rats, four unidentified metabolites were found [4].

In vitro, indigotine significantly inhibited the CYP2A6 monooxygenase activity in a noncompetitive manner, with a median inhibitory concentration (IC_{50}) of 0.05 mmol/L [5, 6].

Toxicological studies

Indigotine has a low acute toxicity. No adverse effects were seen in pigs fed indigotine (purity $>85\%$) at dose levels of 0, 150, 450 and 1350 mg/kg bw per day for 90 days [7].

When mice were fed diets containing 0.2%, 0.4%, 0.8% or 1.6% indigotine (purity $\geq 85\%$) for 80 weeks, the only effect seen was a slight anaemia in animals at 0.8% or 1.6% [8]. The previous Committee concluded that indigotine was not carcinogenic and that the “no-untoward-effect level was 0.4% of the diet equivalent to an intake of approximately 550 mg/kg/day” ([Annex 1](#), references 35 and 36).

In a 2-year combined toxicity and carcinogenicity study, mice were fed indigotine (purity 93%; 7% volatile matter) at dietary concentrations of 0, 0.5%, 1.5% or 5.0% (equal to 0, 825, 2477 and 8259 mg/kg bw per day, respectively). A moderate incidence of blue-green discoloration of the gastrointestinal tract, with occasional discoloration of the liver, gall-bladder and urine, was observed at all doses. There was no evidence of carcinogenicity. The NOAEL was 5% in the diet (equal to 8259 mg/kg bw per day), the highest dose tested [9].

In a long-term combined toxicity and carcinogenicity study, which included an in utero phase, indigotine (purity 93%, 7% volatile matter) was fed to rats at dietary levels of 0, 0.5%, 1.0% or 2.0% (equal to 0, 304, 632 and 1282 mg/kg bw per day, respectively). Treatment began approximately 2 months prior

to mating, and the long-term phase was initiated after random selection of the F₁ animals [10]. No consistent substance-related adverse effects were noted, with the exception of statistically significant increases in incidences of malignant mammary gland tumours and gliomas in males, but not females, at the highest dose. The incidence of mammary gland tumours (carcinomas/adenocarcinomas) in high-dose males was 5.9% (3/51 compared with 0/114 control animals). The incidence of gliomas in high-dose males was 9.9% (7/71) compared with 2.9% in controls (4/140 animals). No increase was seen in the low and mid dose groups (incidence 2.9%, 2/70 animals) or in female rats.

The previous Committee noted the statistically significant increase in incidence of malignant mammary gland tumours and gliomas in male rats at the highest dose level of 1282 mg/kg bw per day. In the absence of any indications for genotoxicity, the Committee concluded that the NOAEL in this study was 632 mg/kg bw per day.

Rats fed a diet containing 1% indigotine (equivalent to 500 mg/kg bw per day) for 2 years showed no treatment-related pathological signs, and fewer malignant and benign tumours than the controls; they also survived for longer than the controls [11]. When indigotine was fed to groups of rats at dietary levels of 0, 0.5%, 1.0%, 2.0% or 5.0% for 2 years, the only effect seen was a reduced growth in males at 2.0% and 5.0% [1]. The NOAEL was 1% in the diet, equivalent to 500 mg/kg bw per day.

Indigotine tested negative in a series of bacterial mutagenicity assays as well as in in vitro mammalian cell chromosomal aberration assays and a number of comet assays. Indigotine was also not genotoxic in in vivo micronucleus tests in mice and rats and a comet assay in mice. The Committee concluded that indigotine does not raise any concerns with respect to genotoxicity.

No reproductive or developmental toxicity was observed in one 3-generation rat study (doses up to 250 mg/kg bw); two 2-generation rat studies (doses up to 250 or 500 mg/kg bw); one rat teratogenicity study (doses up to 250 mg/kg bw); and two rabbit teratogenicity studies (doses up to 250 mg/kg bw).

Assessment of dietary exposure

Dietary exposure to indigotine has not been previously reviewed by the Committee.

A comprehensive literature search retrieved five relevant studies other than those submitted by the sponsor. The Committee reviewed published estimates of dietary exposure to indigotine conducted in several countries and regions. A summary of these estimates, showing the results from mean and high percentile calculations, is provided in [Table 6](#).

As indigotine has provisions in 51 food categories in the General Standard on Food Additives (GSFA), an exposure assessment based on maximum use

Table 6
Summary of the range of estimates of dietary exposure for indigotine

Source of estimates	Population	Range of estimated dietary exposures (mg/kg bw per day)	
		Mean	High percentile (P90 and P95) ^a
National ^b	Children	0.00–0.01	0.00–0.09
	Children	0.00–0.03	0.0–0.3
	Adolescents	0.00–0.01	0.00–0.03
	Adults	0.00–0.01	0.00–0.02
Europe ^c	Toddlers	0.0–0.2	0.3–0.8
	Children	0.0–0.1	0.2–0.8
	Adolescents	0.0–0.1	0.1–0.5
	Adults	0.0–0.4	0.1–0.3
	Elderly adults	0.0–0.03	0.1–0.2

bw: body weight; P90: 90th percentile; P95: 95th percentile

^a The upper bound of the range is the maximum of the 90th and 95th percentiles.

^b Data from Australia [12]; Kuwait [13]; the Netherlands [14]; the Republic of Korea [15]; and the USA [16, 17].

^c Based on consumption data from 17 European countries [18].

levels was considered by the Committee to be unrealistic. Moreover, analytical and reported use levels for the main contributing food categories were far below the maximum use levels, reinforcing the Committee's decision not to consider estimates based on maximum use levels for the exposure assessment.

Because indigotine has been authorized for use for many years, estimates based on analytical data were available and were considered by the Committee to be more appropriate for determining long-term dietary exposure estimates. Exposure estimates based on analytical data range from 0.0 to 0.8 mg/kg bw per day for adults, adolescents and children at the 95th percentile. Because of the conservative assumptions in high percentile exposure estimates, the Committee concluded that an estimate of 0.8 mg/kg bw per day for children and toddlers should be considered in the safety assessment for indigotine.

Evaluation

The Committee noted that indigotine is poorly absorbed from the gastrointestinal tract, has a low acute toxicity, is not genotoxic and does not show any potential for reproductive or developmental toxicity. The previous Committee identified a NOAEL of 500 mg/kg bw per day from a 2-year rat study of 1% indigotine in the diet and established an ADI of 0–5 mg/kg bw. The current Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies. In one long-term toxicity study, slight anaemia was observed in mice fed diets with 0.8% or 1.6% indigotine. In another long-term toxicity study, body-weight gain was reduced in male rats at 2.0% and 5.0%

indigotine in the diet. In a third long-term toxicity study, increased incidences of malignant mammary gland tumours and gliomas were observed in male rats at 1282 mg/kg bw per day but not at 304 and 632 mg/kg bw per day.

The Committee concluded that there are no reasons to revise the ADI and confirmed the ADI of 0–5 mg/kg bw.

The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper limit of the ADI of 0–5 mg/kg bw established for indigotine. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.

A consolidated toxicological and dietary exposure monograph was prepared.

The existing specifications for indigotine were revised. HPLC methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

The specifications monograph was revised, and a Chemical and Technical Assessment was prepared.

References

1. Hansen WH, Fitzhugh OG, Nelson AA, Davis KJ. Chronic toxicity of two food colors, brilliant blue FCF and indigotine. *Toxicol Appl Pharmacol*. 1966;8(1):29–36.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Lethco EJ, Webb JM. The fate of FD&C Blue No. 2 in rats. *J Pharmacol Exp Ther*. 1966;154(2):384–9.
4. Singh S, Das M, Khanna SK. Bio-metabolism of green S and indigo carmine through caecal microflora of rats. *Biochem Biophys Res Commun*. 1993;195(1):490–96.
5. Kuno N, Mizutani T. Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. *J Toxicol Environ Health A*. 2005;68(16):1431–44.
6. Mizutani T. Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner. *J Environ Public Health*. 2009;953952:1–9. doi:10.1155/2009/953952.
7. Gaunt IF, Kiss IS, Grasso P, Gangolli SD. Short-term toxicity study on indigo carmine in the pig. *Food Cosmet Toxicol*. 1969;7(1):17–24.
8. Hooson J, Gaunt IF, Kiss IS, Grasso P, Butterworth KR. Long-term toxicity of indigo carmine in mice. *Food Cosmet Toxicol*. 1975;13(2):167–76.
9. Borzelleca JF, Hogan GK. Chronic toxicity/carcinogenicity study of FD & C Blue No. 2 in mice. *Food Chem Toxicol*. 1985;23(8):719–22.

10. Borzelleca JF, Hogan GK, Koestner A. Chronic toxicity/carcinogenicity study of FD & C Blue No. 2 in rats. *Food Chem Toxicol.* 1985;23(6):551–8.
11. Oettel H, Froberg H, Nothdurft H, Wilhelm G. [Testing of some synthetic dyes for their suitability as food coloring agents]. *Arch Toxikol.* 1965;21(1):9–29 (in German).
12. Survey of added colours in foods available in Australia. Study of concentration in foods including dietary exposure assessment and risk characterisation. Canberra: Food Standards Australia New Zealand; 2008.
13. Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N et al. Estimates of dietary exposure of children to artificial food colours in Kuwait. *Food Addit Contam.* 2006;23(3):245–51.
14. Kist-van Holthe JK, Altenburg TM, Bolakhrif S, El Hamdi L, Man MW, Tu J et al. Consumption of artificial food colourings by school children in the Netherlands. *Adv Pediatr Res.* 2015;2(5). doi:10.12715/apr.2015.2.5.
15. Ha MS, Ha SD, Choi SH, Bae DH. Exposure assessment of synthetic colours approved in Korea. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2013;30(4):643–53.
16. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry KM. Exposure estimate for FD&C colour additives for the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2016;33(5):782–97.
17. Bastaki M, Farrell T, Bhusari S, Bi X, Scrafford C. Estimated daily intake and safety of FD&C food-colour additives in the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2017;34(6):891–904.
18. Scientific Opinion on the re-evaluation of Indigo Carmine (E 132) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *EFSA J.* 2014;12(7):3768. doi:10.2903/j.efsa.2014.3768.

3.1.5 Lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic)

Explanation

Lutein esters from *Tagetes erecta* (INS No. 161b(iii)) and lutein from *Tagetes erecta* (INS No. 161b(i)) are used as food colouring agents and nutrient supplements in a wide range of baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogues, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 2 to 330 mg/kg.

Zeaxanthin (INS No. 161h(i)) is used as a nutritional supplement and colour in a wide range of foods such as baked goods, beverages, chewing gum, egg products, fats and oils, gravies and sauces, hard and soft candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 0.5 to 70 mg/kg.

Lutein esters contain lutein (all-*E*,3*R*,3'*R*,6'*R*)- β , ϵ -carotene-3,3'-diol, which is a naturally occurring oxygenated xanthophyll pigment and a macular carotenoid. Lutein occurs with its isomeric xanthophyll, zeaxanthin, in many

foods but particularly in vegetables and fruit. Lutein occurs either esterified to fatty acids or in a non-esterified “free” form. Studies show that *meso*-zeaxanthin, a structurally related xanthophyll of lutein, and zeaxanthin may originate from foodstuffs that are sources of these xanthophylls, rather than being derived from the bioconversion of retinal lutein [1]. Extracts containing xanthophylls (free and/or esterified) are used as colours and as nutritional supplements in a wide range of applications.

At the thirty-first meeting, the Committee prepared tentative specifications for xanthophylls obtained from *Tagetes erecta* petals, but no toxicological evaluation was performed ([Annex 1](#), reference 77). Xanthophyll preparations (*Tagetes* extract) containing lutein esters at low concentrations were evaluated at the fifty-fifth and fifty-seventh meetings ([Annex 1](#), references 149 and 154). The tentative specifications ([Annex 1](#), reference 151) were subsequently superseded by full specifications ([Annex 1](#), reference 156). At the sixty-third meeting, the Committee evaluated biochemical data and the results of toxicological and human studies on *Tagetes* preparations with a high content of non-esterified lutein (>80%) and established a group ADI of 0–2 mg/kg bw per day for lutein from *Tagetes erecta* and synthetic zeaxanthin ([Annex 1](#), reference 173). At the seventy-ninth meeting, the Committee evaluated the results of new toxicity studies on preparations with a higher content of xanthophyll esters (>60%), identified as “lutein esters from *Tagetes erecta*” ([Annex 1](#), reference 220). A temporary ADI “not specified” for lutein esters from *Tagetes erecta* was established; the ADI was made temporary because the specifications for lutein esters from *Tagetes erecta* were tentative.

At the eighty-second meeting, the Committee evaluated additional information on manufacturing and composition of lutein esters from *Tagetes erecta* that permitted the adoption of final specifications and the removal of the temporary designation ([Annex 1](#), reference 230). An ADI “not specified” for lutein esters from *Tagetes erecta* was established. The Committee was unable to consider establishing a group ADI “not specified” for lutein esters from *Tagetes erecta*, lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls, and recommended that this be taken up at a future meeting.

At the present meeting, newly available data for lutein, zeaxanthin and *meso*-zeaxanthin were submitted by the sponsor. A comprehensive literature search retrieved two other studies. The Committee assessed available toxicity studies for re-evaluation of safety, dietary exposure and specifications to consider establishing a group ADI “not specified” for lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic).

Chemical and technical considerations

Lutein, lutein esters (including lutein dipalmitate) and zeaxanthin are members of a group of pigments known as xanthophylls; they have no provitamin A activity.

Tagetes extract (INS No. 161b(ii)) is obtained by hexane extraction of dried petals of marigold (*Tagetes erecta* L.), with subsequent removal of the solvent. The major colouring principles are lutein (all-*E*,3*R*,3'*R*,6'*R*- β , ϵ -carotene-3,3'-diol) and lutein dipalmitate (helenien; β , ϵ -carotene-3,3'-diol dipalmitate). Hydroxy derivatives of carotenes together with other oxy derivatives, such as epoxides, may also be present in *Tagetes* extract. The product may contain fats, oils and waxes that occur naturally in the plant material. The articles of commerce are usually further formulated to standardize the colour content or to obtain water-soluble or dispersible products.

Lutein esters from *Tagetes erecta* (INS No. 161b(iii)) is a purified extract obtained from marigold (*Tagetes erecta* L.) oleoresin, which is extracted using organic solvents. The preparation contains lutein esters, of which lutein dipalmitate accounts for the major part; a smaller proportion of zeaxanthin esters is also present. The balance of the extract is made up of naturally occurring waxes.

Lutein from *Tagetes erecta* (INS No. 161b(i)) is a purified extract obtained from marigold (*Tagetes erecta* L.) oleoresin, which is extracted using organic solvents. The final product, after saponification and crystallization, contains lutein as the major component and a smaller proportion of zeaxanthin.

Zeaxanthin (synthetic) (INS No. 161h(i)) is the synthetic all-*trans* isomer of zeaxanthin (3*R*,3'*R*- β , β -carotene-3,3'-diol) produced by the Wittig reaction from raw materials that are commonly used in the production of other carotenoids with application in foods. Minor quantities of *cis*-zeaxanthins and by-products 12-apozeaxanthinal, parasiloxanthin, diatoxanthin and triphenyl phosphine oxide may be present in the final product.

Biochemical aspects

The ADME of lutein and lutein esters from *Tagetes erecta* was extensively described in the monographs at the sixty-third and seventy-ninth meetings ([Annex 1](#), references 174 and 221). The absorption of lutein involves emulsification by bile, followed by lipolysis by pancreatic lipases into the micellar fraction for absorption by intestinal cells. Absorption of xanthophylls from esters requires hydrolysis. Hydrolysis is an efficient process as esterified lutein is not normally found in human serum. These processes have been shown to be influenced by food matrices, for example, dietary fibres reduce absorption whereas dietary fats promote absorption due to the hydrophobic nature of these xanthophylls. The bioavailability of lutein following administration of lutein esters has been shown to be equivalent to the administration of free lutein.

Sheshappa et al. [2] investigated the influence of food matrices in lutein-deficient rats. Lutein levels in plasma, liver and eyes were higher than in the controls when animals were administered lutein-mixed micelles containing either 3% fat, phosphatidyl choline (PC) or lyso-phosphatidyl choline (lysoPC). In contrast, the administration of lutein-mixed micelles containing pectin and mixed xanthophylls resulted in lutein levels that were lower than in the controls. Evans et al. [3] found that in humans given a single dose of 20 mg of lutein in a starch-based matrix or cross-linked to an alginate matrix, lutein from the starch-based product was better absorbed than lutein from the alginate one.

In humans, dietary supplements of lutein (containing 5% zeaxanthin) at 10 mg/day for 6 months increased the mean serum level of lutein from a baseline value of 210 to 1000 nmol/L and of zeaxanthin from 56 to 95 nmol/L [4].

Albert et al. [5] observed that monkeys fed a xanthophyll-free diet supplemented with either pure lutein or pure zeaxanthin for 12–92 weeks formed 3'-dehydrolutein in plasma. In addition, two 3'-dehydrolutein diastereomers, (3*R*,6'*S*)- and (3*R*,6'*R*)-3'-dehydrolutein, were present in nearly equimolar concentrations. The authors considered these findings to be comparable to those in human plasma after dietary supplementation with either lutein or zeaxanthin at doses from 1 to 20.5 mg/day for 42 days [6, 7].

Toxicological studies

The Committee previously concluded that the NOAEL for lutein was 200 mg/kg bw per day, the highest dose tested in a 13-week rat toxicity study, and 1000 mg/kg bw per day, the highest dose tested in a rat developmental toxicity study. For lutein esters from *Tagetes erecta*, the NOAEL was 1000 mg/kg bw per day (equivalent to 540 mg/kg bw per day of lutein), the highest dose tested in both a 13-week and a developmental toxicity study in rats.

The studies reviewed by the Committee confirm that lutein, zeaxanthin and *meso*-zeaxanthin, tested in their free form, are of very low toxicity. The only substance-related finding was discoloration of faeces and fur or skin in animals at higher doses. The NOAELs in short-term and long-term toxicity studies were approximately 210–400 mg/kg bw per day for lutein, 87.5–260 mg/kg bw per day for zeaxanthin and 200–300 mg/kg bw per day for *meso*-zeaxanthin in rats and 10 mg/kg bw per day for lutein and zeaxanthin in monkeys. These NOAELs were generally the highest dose levels tested [8, 9, 10].

A 2-generation reproductive toxicity study in rats found that administration of zeaxanthin at up to 500 mg/kg bw per day, the highest dose tested, did not cause any adverse effects [11].

Based on previously and newly available data, the Committee considered that lutein, zeaxanthin and *meso*-zeaxanthin did not raise concerns with respect to genotoxicity.

Observations in humans

The Committee previously reviewed the results of clinical studies in which lutein and lutein esters from *Tagetes erecta* were given as nutritional supplements or therapeutic agents for age-related macular degeneration ([Annex 1](#), references 173 and 220). Although these studies were not designed as safety assessments, lutein and lutein esters were found to be well tolerated. This was confirmed based on evaluations of newly available clinical studies. These clinical studies included healthy and preterm infants given lutein in infant formula at concentrations equal to 0.5 mg/kg bw per day for 6 weeks. Doses for adults were up to 10 mg/person per day [4, 12].

Assessment of dietary exposure

At the sixty-third meeting, the Committee estimated mean and 90th percentile dietary exposure to lutein from *Tagetes erecta* as approximately 7 and 13 mg/day, respectively (equivalent to 0.12 and 0.22 mg/kg bw per day, assuming a 60 kg body weight) ([Annex 1](#), reference 173).

At the seventy-ninth meeting, the use of lutein esters from *Tagetes erecta* was considered to be substitutional for the use of lutein from *Tagetes erecta*.

The present Committee estimated exposure to lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic). When used as a food colour, these substances are substitutional on a molar basis, and exposures are expressed as lutein throughout the report. The estimates included exposure from the use in food supplements. Exposure to *meso*-zeaxanthin was not explicitly assessed by the Committee although it is present in some commercial food supplements.

A comprehensive literature search retrieved 20 additional references relevant to the dietary exposure assessment.

Dietary exposure from the use of lutein, zeaxanthin and their esters and zeaxanthin (synthetic) as a food colour was estimated as 0.3 mg/kg bw per day for adults and 1 mg/kg bw per day for children by EFSA [13]. These estimates, based on maximum reported use levels and national consumption data from 11 European countries, were higher than those estimated by the Committee at the sixty-third meeting.

Dietary exposure from the use of lutein, zeaxanthin and their esters and zeaxanthin (synthetic) in food supplements was estimated by the Committee from dosage information on product labels and from intervention studies to be 0.67 mg/kg bw per day for both adults and children.

Dietary exposure from natural occurrence in food was estimated as 0.13 mg/kg bw per day for adults and 0.69 mg/kg bw per day for children. These estimates were based on national food consumption data from China for adults

Table 7

Estimates of high dietary exposure to lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic)

Source of exposure	Estimated dietary exposure, mg/kg bw per day (%) ^a	
	Adults	Children
Food colour	0.4 (33)	1 (42)
Food supplements	0.67 (56)	0.67 (28)
Natural occurrence	0.13 (11)	0.69 (29)
Aggregated exposure	1.2	2.4

bw: body weight

^a Estimated dietary exposure in mg/kg bw per day and, in parentheses, the estimated exposure as a percentage of the total (aggregated) exposure.

[14] and from the Republic of Korea for children [15]. These estimates were also higher than the previous Committee's estimates.

The present Committee estimated a conservative aggregated high dietary exposure from the use of lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic) as food colour and from food supplements in combination with the natural occurrence of these xanthophylls to be 1.2 mg/kg bw per day for adults and 2.4 mg/kg bw per day for children (Table 7).

Evaluation

Free lutein, lutein esters and free zeaxanthin including *meso*-zeaxanthin are biochemically and toxicologically equivalent. The esters of lutein and zeaxanthin are hydrolysed in the gastrointestinal tract, and systemic exposure is to free lutein and zeaxanthin, compounds that differ only in the position of a single double bond. Lutein and zeaxanthin are naturally present in food. In addition, both xanthophylls are essential constituents of the primate retina; biologically controlled mechanisms and pathways exist for absorption of both xanthophylls from the diet and their distribution within the body and deposition in the retina.

The Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from *Tagetes erecta*, synthetic zeaxanthin and *meso*-zeaxanthin. The Committee considered the available toxicological data together with the dietary exposure of the general population.

No adverse effects were observed in a broad range of toxicological studies of free lutein, lutein esters and free zeaxanthin and *meso*-zeaxanthin in laboratory animals and in clinical studies in humans. Results from a new 2-generation reproductive toxicity study of zeaxanthin in rats indicated no adverse effects at up to 500 mg/kg bw per day, the highest dose tested [11].

The estimated dietary exposure from the use of free lutein, lutein esters and free zeaxanthin as colours or food supplements are in the same order of magnitude as the intakes from foods where these xanthophylls are naturally present.

Based on the absence of toxicity in a wide range of studies with NOAELs of approximately 210–400 mg/kg bw per day for lutein, 87.5–500 mg/kg bw per day for zeaxanthin and 200–300 mg/kg bw per day for *meso*-zeaxanthin and estimated dietary exposure of up to 2.4 mg/kg bw per day, the Committee established a group ADI “not specified” for lutein from *Tagetes erecta*, lutein esters from *Tagetes erecta* and zeaxanthin (synthetic).

Meso-zeaxanthin was not included in this group ADI as specifications are not currently available.

The group ADI of 0–2 mg/kg bw for lutein from *Tagetes erecta* and zeaxanthin (synthetic) was withdrawn.

A toxicological and dietary exposure monograph addendum was prepared.

The specifications for lutein from *Tagetes erecta* were revised, and the Chemical and Technical Assessment was updated. The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

References

1. Nolan JM, Meagher K, Kashani S, Beatty S. What is *meso*-zeaxanthin, and where does it come from? *Eye* (Lond). 2013;27(8):899–905.
2. Sheshappa MB, Ranganathan A, Bhatiwada N, Talahalli RR, Vallikannan B. Dietary components affect the plasma and tissue levels of lutein in aged rats with lutein deficiency—a repeated gavage and dietary study. *J Food Sci*. 2015;80(10):2322–30.
3. Evans M, Beck M, Elliott J, Etheve S, Roberts R, Schalch W. Effects of formulation on the bioavailability of lutein and zeaxanthin. A randomized, double-blind, cross-over, comparative, single-dose study in healthy subjects. *Eur J Nutr*. 2013;52(4):1381–91.
4. Khachik F, de Moura FF, Chew EY, Douglass LW, Ferris FL, Kim J et al. The effect of lutein and zeaxanthin supplementation on metabolites of these carotenoids in the serum of persons aged 60 or older. *Invest Ophthalmol Vis Sci*. 2006;47(12):5234–42.
5. Albert GL, Hoeller U, Schierle J, Neuringer M, Johnson EJ, Schalch W. Metabolism of lutein and zeaxanthin in rhesus monkeys. Identification of (3R,6'R)- and (3R,6'S)-3'-dehydro-lutein as common metabolites and comparison to humans. *Comp Biochem Physiol B Biochem Mol Biol*. 2008;151(1):70–8.
6. Hartmann D, Thürmann PA, Spitzer V, Schalch W, Manner B, Cohn W. Plasma kinetics of zeaxanthin and 3'-dehydro-lutein after multiple oral doses of synthetic zeaxanthin. *Am J Clin Nutr*. 2004;79(3):410–7.
7. Thürmann PA, Schalch W, Aebischer JC, Tenter U, Cohn W. Plasma kinetics of lutein, zeaxanthin and 3'-dehydro-lutein after multiple oral doses of a lutein supplement. *Am J Clin Nutr*. 2005;82(1):88–97.
8. Ravikrishnan R, Rusia S, Ilamurugan G, Salunkhe U, Deshpande J, Shankaranarayanan ML et al. Safety assessment of lutein and zeaxanthin (Lutemax 2020): subchronic toxicity and mutagenicity studies. *Food Chem Toxicol*. 2011;49(11):2841–8.

9. Thurnham DI, Howard AN. Studies on *meso*-zeaxanthin for potential toxicity and mutagenicity. *Food Chem Toxicol.* 2013;59:455–63.
10. Ravi KB, Raghunatha Reddy KR, Shankaranarayanan J, Deshpande JV, Juturu V, Soni MG. Safety evaluation of zeaxanthin concentrate (OmniXan™): acute, subchronic toxicity and mutagenicity studies. *Food Chem Toxicol.* 2014;72:30–9.
11. Clode S. Zeaxanthin 10% WS beadlets (Ro 01-9509): Two generation oral (dietary administration) reproduction toxicity study in the rat. Amendment to final report study no.: 2416/001. Unpublished study by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom; 2018. Submitted to WHO by DSM Nutritional Products Ltd, Kaiseraugst, Switzerland.
12. Costa S, Giannantonio C, Romagnoli C, Vento G, Gervasoni J, Persichilli S et al. Effects of lutein supplementation on biological antioxidant status in preterm infants: a randomized clinical trial. *J Matern Fetal Neonatal Med.* 2013;26(13):1311–5.
13. Statement on the safety assessment of the exposure to lutein preparations based on new data on the use levels of lutein. *EFSA J.* 2012;10(3):2589. doi:10.2903/j.efsa.2012.2589.
14. Wang L, Li B, Pan MX, Mo XF, Chen YM, Zhang CX. Specific carotenoid intake is inversely associated with the risk of breast cancer among Chinese women. *Br J Nutr.* 2014;111(9):1686–95.
15. Lee HS, Cho YH, Park J, Shin HR, Sung MK. Dietary intake of phytonutrients in relation to fruit and vegetable consumption in Korea. *J Acad Nutr Diet.* 2013;113(9):1194–99.

3.1.6 Neutral methacrylate copolymer

Explanation

Neutral methacrylate copolymer (NMC; E 1206; INS No. 1206; CAS No. 9010-88-2; ethyl acrylate methyl methacrylate polymer; ethyl acrylate methyl methacrylate polymer; ethyl acrylate polymer with methyl methacrylate; methyl methacrylate ethyl acrylate polymer; methyl methacrylate polymer with ethyl acrylate) is a copolymer manufactured from the monomers ethyl acrylate and methyl methacrylate in the molar ratio of 2:1. NMC is described in the European Pharmacopeia [1] and United States Pharmacopeia and National Formulary [2].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. NMC is insoluble in aqueous media and its release is not pH dependent. NMC is used in sustained release formulations that enable continuous dissolution of an active ingredient over a defined time. Release periods can be controlled by changing the amount of copolymer used.

NMC has not previously been considered by the Committee. The Committee evaluated the use of NMC as a coating or glazing agent for solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 20%, at the request of CCFA49 [3]. NMC is also used in pharmaceuticals.

Toxicological data submitted for evaluation included absorption, distribution and excretion studies, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the residual monomers but no other studies on NMC. The Committee also considered the data on the residual monomers because the monomers are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. Due to the low levels of residual monomers present in NMC, the Committee considered only the ADME, long-term toxicity and genotoxicity data on the monomers.

The Committee was also aware that NMC contains an oligomer fraction of between 0.06% and 0.13%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 5000 Da, it is unlikely that they would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the absorption, distribution and excretion and toxicity studies was prepared from an aqueous dispersion with 0.7% emulsifier and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of NMC.

The Committee evaluated toxicological and exposure data on polyethylene glycol monostearyl ether, methanol and ethanol, residual components of NMC that can be present in the final product because they are used in the manufacture of the copolymer. The Committee concluded that these residual components do not pose a safety concern at the maximum estimated exposure levels.

Chemical and technical considerations

NMC is manufactured by emulsion polymerization of the monomers ethyl acrylate and methyl methacrylate with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low molecular weight substances and coagulum.

NMC has a weight-average molecular weight of 600 000 Da and a number-average molecular weight of 220 000 Da.

Although organic solvents are not used in the manufacture of NMC, methanol may be present at a level not exceeding 100 mg/kg and ethanol may be present at a level not exceeding 1000 mg/kg. The copolymer is standardized as a 30% aqueous dispersion with polyethylene glycol monostearyl ether (0.7%). The copolymer dispersion may contain the residual monomers methyl methacrylate (not more than 50 mg/kg) and ethyl acrylate (not more than 20 mg/kg).

Biochemical aspects

NMC

In a pre-GLP study, NMC was found to be poorly absorbed and quickly eliminated from the body when single doses of ^{14}C -labelled NMC at 600 mg/kg bw per day were administered to rats by gavage. An average of 97.6% of the radioactivity was excreted in the faeces within 48 hours. Seven days after dosing, levels of radioactivity in tissues of treated animals did not differ significantly from that of control animals [4].

Residual monomers

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration to rats. Methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [5, 6, 7].

Ethyl acrylate is rapidly absorbed and metabolized following inhalation or oral administration in rats. Metabolism occurs via hydrolysis of the ester linkage by carboxylesterases, forming ethanol and acrylic acid, both of which are ultimately metabolized to carbon dioxide, or via conjugation of ethyl acrylate with glutathione. Following conjugation with glutathione, ethyl acrylate is rapidly eliminated via urinary excretion [8].

Toxicological studies

NMC

Two acute toxicity studies were available, in rats and in dogs. Rats received doses of 25.2–28.2 g/kg bw in their feed; dogs received doses of 7950 or 9100 mg/kg bw in their feed. No treatment-related effects were seen during the observation period or at macroscopic examination of the organs. Urine analysis also found no treatment-related effects [9, 10].

Two short-term toxicity studies were available in rats. In the first study, doses of 0, 500, 1000 or 2000 mg/kg bw per day were given by gavage for 35 days. It is not clear if the doses were expressed in dry weight of polymer or of the preparation. No treatment-related effects were observed [11]. In the second study, doses of 0, 500 or 2000 mg/kg bw per day were given in the feed for 6 months. No treatment-related effects were observed [12]. In both studies, the NOAELs were 2000 mg/kg bw per day, the highest doses tested. In a 26-week study, dogs were given NMC at doses of 0, 50, 125 or 250 mg/kg bw per day administered as NMC-coated cellulose pellets. Apart from decreases in body-weight gain and feed consumption at the highest dose, and the presence of white granular material in the gut, attributed to the physical characteristics of the test material, no treatment-related effects were observed. The NOAEL was 250 mg/kg

bw per day, the highest doses tested [13]. A 28-day study in mini pigs, in which NMC was given by gavage as NMC-coated cellulose pellets, the calculated doses were 0, 113, 227 and 454 mg/kg bw per day. Other than the presence of white granular material in the intestines, attributed to the physical characteristics of the test substance, no treatment-related effects were found. The NOAEL was 454 mg/kg bw per day, the highest dose tested [14].

Two reverse mutation studies in bacteria, one in vitro mouse lymphoma assay and an in vivo mouse micronucleus assay gave negative results. The Committee concluded that the NMC does not give rise to concern for genotoxicity.

No long-term toxicity or carcinogenicity studies with NMC were available.

Developmental toxicity studies were available in rats and rabbits, both using doses of 0, 500 or 2000 mg/kg bw per day in the feed during the period of organogenesis. No treatment-related effects were observed. The NOAEL for both studies was 2000 mg/kg bw per day, the highest doses tested.

Studies on cytotoxicity and dermal, inhalation and ocular toxicity found no effects.

Residual monomers

Methyl methacrylate

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight increased in females at the highest dose but no treatment-related histopathological effects were observed in any organs or tissues [15]. Based on the results of this study, a TDI of 1.2 mg/kg bw per day was determined [16].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term toxicity studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [15].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and SCE assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days

of exposure. These results were judged by the Committee to be inconclusive. The Committee concluded that there was some evidence of mutagenicity and clastogenicity *in vitro*. There was a lack of adequate *in vivo* tests following up the equivocal findings.

Ethyl acrylate

Studies in mice and rats administered ethyl acrylate at 0, 100 or 200 mg/kg bw per day for 103 weeks by gavage showed an increased incidence of squamous cell hyperplasia and papillomas in the forestomach of both species, and, in male rats, squamous cell carcinomas in the forestomach. In contrast, studies using other routes of administration including via inhalation (with concentrations up to 310 mg/m³) and in the drinking-water (with doses up to 280 mg/kg bw per day) showed no such effects. An IARC working group concluded that the mechanism of formation of the forestomach tumours in rodents is not relevant to humans; rather it can be attributed to the irritating effect of high bolus doses of ethyl acrylate delivered to the contact site (forestomach) by gavage [17]. This conclusion was recently confirmed by Health Canada [8]. The Committee concluded that ethyl acrylate is not a carcinogenic risk to humans.

Genotoxicity results for ethyl acrylate are mixed, with some positive results *in vitro*, some negative results in reverse mutation assays in bacteria and positive results in a mouse lymphoma assay. There were both positive and negative results in *in vitro* SCE assays and in chromosomal aberration assays with metabolic activation, but no evidence of clastogenicity in the absence of metabolic activation.

In *in vivo* studies on ethyl acrylate, two early mouse micronucleus studies using intraperitoneal dosing gave positive results, although one of these gave positive results in only one of the five experiments carried out [18, 19]. However, three other micronucleus studies in mice (one dermal exposure, two by intraperitoneal injection) all gave negative results ([Annex 1](#), reference 174). A chromosomal aberration assay and an *in vivo* SCE assay and a recent point mutation assay in mice were also negative. The Committee concluded that the genotoxic potential observed in some *in vitro* studies was not expressed *in vivo*. The Committee noted that Health Canada [8] reached a similar conclusion.

Observations in humans

No human data were available on NMC.

Assessment of dietary exposure

The Committee evaluated exposure to NMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes. As another

major use of NMC is in pharmaceuticals, this use was also considered in the exposure assessment. The level of use of NMC is a maximum of 20%.

The Committee evaluated exposure to NMC for the copolymer and its monomers, methyl methacrylate and ethyl acrylate. The exposure assessment included estimates submitted by the sponsor and an evaluation by EFSA [20] based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. The national consumption data were from CIFOCoSS and data submitted to the Committee from Australia and New Zealand. A comprehensive literature search was also conducted; no additional studies relevant to the exposure assessment were found.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are therefore not relevant for the general healthy population. This use was therefore not further considered by the Committee.

The total monomeric content of NMC is less than 0.01%. This level was used to calculate the exposure to total monomers from the copolymer exposure. Estimates of exposure to the individual monomers were based on the exposure to total monomers, taking into account the ratio of each individual monomer in the copolymer.

All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures.

The estimated exposures to NMC and its monomers from uses in food supplements are shown in [Table 8](#).

The Committee noted that NMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA [20] ranged between 10.0 and 23.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

Evaluation

New specifications for NMC were prepared and made tentative, requiring a suitable validated method for its assay. A Chemical and Technical Assessment was prepared.

The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the

Table 8

Summary of range of estimated exposures to NMC and its monomers from uses in food supplements for average and high exposures

Population group	Range of estimated dietary exposures ^{a,b}		
	Copolymer exposure (mg/kg bw per day)	Monomer exposure (µg/kg bw per day)	
		Methyl methacrylate	Ethyl acrylate
Adults	6.9–74	0.2–2.2	0.5–5.2
Children	5.8–86	0.2–2.5	0.4–6.0

bw: body weight; NMC: neutral methacrylate copolymer

^a All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated mean exposures, and the upper end of each range is the highest of the estimated high exposures.

^b Includes exposure estimates submitted by the sponsor and EFSA (2010) and national estimates calculated by the Committee, based on a concentration of 200 mg per 1 g dosage unit.

food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes.

The Committee therefore established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative.

The available toxicology data for NMC do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for NMC range from 454 to 2000 mg/kg bw per day, and these were the highest doses tested. Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day.

Toxicological data on the residual monomers do not give rise to concerns when taking into account the low exposures. Genotoxicity data for methyl methacrylate suggest a potential risk for mutagenicity and clastogenicity in vitro, and there is a lack of adequate data on genotoxicity in vivo. However, in carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, there was no evidence of any carcinogenic effects. In a 2-year drinking-water study on methyl methacrylate in rats, the NOAEL was 121 mg/kg bw per day, from which a TDI of 1.2 mg/kg bw per day was derived [16]. Estimated exposures to methyl methacrylate range from 0.2 to 2.5 µg/kg bw per day, which are below the TDI.

Although there were some positive genotoxicity findings for ethyl acrylate, the Committee concluded that the genotoxic potential observed in some in vitro studies was not expressed in vivo. Long-term toxicity studies on ethyl acrylate in mice and rats produced forestomach tumours, but the Committee concurred with the conclusions of IARC [17] and, more recently, Health Canada [8], that the mechanism of forestomach tumour formation in rats and mice is not relevant to humans. The Committee was also reassured by the long-term carcinogenicity studies on ethyl acrylate that did not use gavage as the route of

administration; these suggest that ethyl acrylate is not carcinogenic at the doses tested. In the 2-year drinking-water study on ethyl acrylate, the NOAEL was 280 mg/kg bw per day. Estimated dietary exposures to ethyl acrylate range from 0.4 to 6 µg/kg bw per day. The margin of exposure (MOE) based on the highest estimated exposure was calculated to be 46 000.

Assessments of dietary exposure to methyl methacrylate and ethyl acrylate due to their residual occurrence in NMC suggest that exposure to these monomers from the uses of NMC is not a safety concern.

A toxicological and dietary exposure monograph was prepared.

References

1. Polyacrylate Dispersion 30 Per Cent. 9 01/2017:0733. European Pharmacopeia; 2017.
2. United States Pharmacopeia and National Formulary (USP 41-NF 36); Ethyl acrylate and methyl methacrylate copolymer dispersion. Vol. 1. Rockville, Maryland: United States Pharmacopeial Convention; 2017.
3. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
4. The excretion and tissue distribution of radioactivity after oral administration to rats. Huntingdon Life Sciences, Suffolk, England, United Kingdom; 1986. Report no. RTS 010/984481(2nd amendment). Unpublished study report submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
5. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer*. 1977;36(1):114–9 [cited in reference 16].
6. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
7. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995. (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
8. Screening assessment for the challenge 2-propenoic acid, ethyl ester (ethyl acrylate). Chemical Abstracts Service Registry Number 140-88-5. Ottawa (ON): Environment Canada, Health Canada; 2011.
9. The acute toxicity of preparations 2807B and 2837B on oral administration in rats. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1971. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
10. The acute toxicity of preparations 2807B and 2837B on oral administration in dogs when administered in the feedstuff. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1972a. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Subacute toxicity study with rats by oral administration for one month and the recovery study. Unpublished study by BoZo Research Centre, Co Ltd, Japan; 1981. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

12. Six-month toxicity of 2837D in Sprague Dawley rats by administration in the diet. Unpublished study report by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1972b. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
13. 26-Week oral toxicity study in dogs followed by a 3-week recovery period. Unpublished study report by Research Toxicology Centre, Rome, Italy; 2007a. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. 4-Week oral toxicity study in minipigs. Unpublished study report no. 31030 by Research Toxicology Centre, Rome, Italy; 2006. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. Borzelleca JF, Larson PS, Hennigar GR Jr, Huf EG, Crawford EM et al. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 16].
16. Methyl methacrylate. Geneva: World Health Organization; 1998. (International Programme on Chemical Safety II. Concise International Chemical Assessment Document No. 4).
17. Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluation carcinogenic risks to humans. Views and Expert Opinions of an IARC Working Group. Lyon: International Agency for Research on Cancer, World Health Organization; 2003 (IARC Technical Publication No. 39) [cited in [Annex 1](#), reference 174].
18. Przybojewska B, Dziubałtowska E, Kowalski Z. Genotoxic effects of ethyl acrylate and methyl acrylate in the mouse evaluated by the micronucleus test. *Mutat Res.* 1984;135(3):189–91.
19. Ashby J, Richardson CR, Tinwell H. Inactivity of ethyl acrylate in the mouse bone marrow micronucleus assay. *Mutagenesis.* 1989;4(4):283–5.
20. Scientific Opinion on the safety of anionic methacrylate copolymer for the proposed uses as a food additive. *EFSA J.* 2010;8(7):1655. doi: 10.2903/j.efsa.2010.1656.

3.1.7 Sorbitol syrup

Explanation

Sorbitol syrup (INS No. 420(ii)) is currently included in the Codex GSFA ([Annex 3](#)) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. At its Fiftieth Session, the Codex Committee on Contaminants in Food Additives [1] requested the Committee to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances and advise on the need for a separate evaluation of sorbitol syrup or if the ADI “not specified” for sorbitol is also applicable for sorbitol syrup.

Evaluation

The Committee acknowledges that sorbitol and sorbitol syrup are chemically similar but distinct substances that should be assigned separate ADIs.

Sorbitol syrup consists of not less than 99.0% of hydrogenated saccharides and not less than 50.0% of D-sorbitol on an anhydrous basis. The D-sorbitol portion of sorbitol syrup is not of toxicological concern, and the Committee

established an ADI “not specified” for sorbitol at its twenty-sixth meeting ([Annex 1](#), reference 61).

The remaining portion of sorbitol syrup consists of other hydrogenated saccharides. These hydrogenated saccharides were evaluated previously by the Committee. Evaluations of maltitol syrup ([Annex 1](#), reference 83) and polyglycitol syrup ([Annex 1](#), reference 137) concluded that the presence of maltitol and higher-order polyols in these syrups was not of toxicological concern, and each of the syrups was assigned an ADI “not specified”. The evaluations of these syrups were based on toxicological findings and biochemical aspects.

The hydrogenated saccharides are poorly absorbed. They are partially hydrolysed by enzymes in the gastrointestinal tract and also metabolized by colonic microflora. The previous Committee concluded that these substances were fully metabolized into natural body constituents ([Annex 1](#), reference 70) and not of toxicological concern.

Based on the similarity of the chemical constituents of sorbitol syrup to sorbitol, maltitol syrup and polyglycitol syrup, the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI “not specified” for sorbitol syrup.

No toxicological monograph was prepared.

Reference

1. Report of the Fiftieth Session of the Codex Committee on Food Additives, Xiamen, China, 26–30 March 2018. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2018 (REP18/FA).

3.1.8 *Spirulina* extract

Explanation

Spirulina extract (INS No. 134) is used as a food additive, as a blue colouring agent. *Spirulina* extract is used in food in China, the European Union, Japan, Mexico and the USA, among others. It is used as a colour in a wide range of foods and beverages including flavoured dairy products, cheese, dairy-based desserts, processed fruits and vegetables, baked goods and baking mixes, alcoholic and non-alcoholic beverages and beverage bases, breakfast cereals, cocoa products, confectionery products (including soft and hard candy and chewing gum), egg products, gravies and sauces, herbs and spices, condiments and soup and soup mixes. It is also used as a colouring agent in nutritional supplements and in pharmaceuticals. Intended use levels range from 400 to 40 000 mg/kg, depending on the food item or category and the colour strength of the formulated *spirulina* extract used.

Spirulina extract has not been evaluated previously by the Committee. It was placed on the agenda for its use as a food colour at the request of CCFA49 [1].

A toxicological dossier submitted by the sponsor summarized the available toxicity data on spirulina, together with relevant study reports and publications. A few of the studies were conducted using spirulina extract, but most toxicity studies used dried spirulina as the test material. A comprehensive literature search retrieved an additional 17 relevant toxicological study reports.

Chemical and technical considerations

Spirulina extract is produced from *Arthrospira platensis* (commonly called *Spirulina platensis*), an edible cyanobacterium cultivated in open or covered ponds or in bioreactors. Commercial cultivation occurs in alkaline aqueous medium containing sodium bicarbonate, nitrates, phosphates, sulfates and other nutrients including trace minerals. No herbicides or additional solvents are used during the cultivation of *A. platensis* or in the manufacture of spirulina extract. Cultivation conditions are optimized to control for contaminating organisms.

Following cultivation, the culture is harvested, concentrated, washed and prepared as a paste or dried to a powder. The dried or fresh biomass undergoes aqueous extraction and pH adjustment, as needed. Following extraction, the mixture undergoes centrifugation and filtration to remove cell debris and water-insoluble components. The resulting aqueous phase contains proteins, carbohydrates, minerals and two phycobiliproteins (also referred to as phycocyanins) that impart the blue colour. The mixture is concentrated to the desired pigment concentration and then pasteurized and/or sterilized before packaging. Spirulina extract products may undergo additional standardization and/or drying to achieve the desired formulation and pigment concentration. Commercially available products occur in either liquid (aqueous) or powder form with a wide range of pigment concentrations.

The primary colouring principles in spirulina extract are C-phycocyanin (CAS No. 11016-15-2; EINECS No. 234-248-8; ~30 kDa) and allophycocyanin (no CAS number assigned; ~105 kDa), in various ratios, with C-phycocyanin occurring in higher proportions. Phycocyanins are complexes of proteins with the pigment molecule phycocyanobilin [2]. The total content of C-phycocyanin and allophycocyanin in spirulina extract varies depending on the desired colour effect and degree of dilution of the extract. Phycocyanin concentrations in spirulina extract range from 1.5–15% in liquid products to 1.5–65% in powder products, as the sum of C-phycocyanin and allophycocyanin.

Commercial spirulina extracts typically contain peptides and proteins (10–90%, dry weight, including the proteins complexed with phycocyanobilin), carbohydrates and polysaccharides (≤65%, dry weight), fat (<1%, dry weight), fibre (<6%, dry weight), minerals/ash (<6%, dry weight) and water (<6% for

powder products and $\leq 95\%$ for liquid formulations). Spirulina extract may contain trace amounts ($<1\%$) of carotenoids and chlorophylls, which are largely removed during production.

Biochemical aspects

ADME data on spirulina extract are not available.

Spirulina extract is described as consisting of various proportions of proteins and carbohydrates as well as much smaller amounts of fibre and fats. These extract components are digested through normal biochemical pathways like other common dietary constituents.

An in vitro simulated gastric fluid digestion assay demonstrated that the protein portion of the C-phycocyanin is rapidly digested by pepsin into small chromopeptides, consisting of two to 13 amino acids [3]. Because the chemical structure of the chromophore phycocyanobilin is very similar to biliverdin, a non-reduced form of bilirubin [4], phycocyanobilin is expected to be metabolized and excreted like bilirubin, through the bile and into the faeces [5, 6].

No evidence of bioaccumulation of coloured matter was observed in repeated-dose toxicity studies in laboratory animals fed spirulina extract or dried spirulina.

Toxicological studies

As noted, most of the tests conducted used dried spirulina and not spirulina extract. However, based on the similarity of the constituents and the high concentrations of the dried spirulina test materials used in the toxicity studies, the Committee considered this acceptable in the evaluation of spirulina extract. In some studies conducted with spirulina extract, the phycocyanin content was reported; no study conducted with dried spirulina reported its phycocyanin content. The Committee also noted that the source of the test material in some studies was *Spirulina maxima*, as opposed to *S. platensis*. Given the chemical, genetic and nutritional similarity of these two species of edible cyanobacteria [7, 8, 9], this was considered acceptable in the evaluation of spirulina extract. Most reports did not state if the studies were GLP or guideline compliant. However, the Committee concluded that the studies were of acceptable quality and the findings valid.

In acute gavage toxicity studies, no clinical signs of toxicity were observed in mice administered spirulina extract (phycocyanin content not reported) at a dose of up to 3000 mg/kg bw [10]; in rats administered spirulina extract (24–26% phycocyanin content) at a dose of up to 5000 mg/kg bw [11, 12]; or in rats administered dried spirulina at a dose of up to 10 000 mg/kg bw [13].

No toxicity was seen in rats administered spirulina extract (24% phycocyanin content) by gavage at a dose of 3000 mg/kg bw per day for 14 days

[12]; in rats administered spirulina extract (phycocyanin content not reported) by gavage at doses of up to 4000 mg/kg bw per day for 12 weeks [14]; in rats fed diets with spirulina extract (26% phycocyanin content) at a concentration of 0.4% (equivalent to 400 mg/kg bw per day) for 14 weeks [11]; or in rats in a long-term toxicity study where the dietary concentration of spirulina extract (8–9% phycocyanin content) was 1.0% (equivalent to 500 mg/kg bw per day) for 12 months [15].

In a short-term mouse toxicity study conducted with dietary concentrations of dried spirulina of up to 5% (equivalent to 7500 mg/kg bw per day) for 6 months, the NOAEL was the highest concentration tested [16]. In a set of well-conducted toxicity studies conducted with dietary concentrations of dried spirulina of up to 30% for 13 weeks, the NOAELs were the highest concentrations tested (equivalent to 45 000 and 30 000 mg/kg bw per day in mice and rats, respectively) [17, 18]. Several additional short-term feeding studies conducted with dried spirulina in mice [19] and rats [13, 20, 21] showed no toxicity under the conditions of the studies. Similarly, no evidence of systemic toxicity or carcinogenicity was observed in long-term feeding studies in rats where the dietary concentration of dried spirulina was 30% (equivalent to 15 000 mg/kg bw per day) for 84 weeks [22].

With dried spirulina, a dose-related increase in relative seminal vesicle weight was the only treatment-related effect observed in 13-week-long toxicity studies in mice [18] and rats [17]. This finding was not reproduced in other short-term or long-term toxicity studies where seminal vesicle weight was investigated. As there were no histopathological changes in the seminal vesicles and no effects on reproduction in the test animals, the Committee considered this finding of no toxicological relevance.

A bacterial reverse mutation assay and in vitro and in vivo chromosomal aberration assays with spirulina extract (26% phycocyanin content) showed no evidence of genotoxicity [12]. Dominant lethal assays conducted in mice and rats with dried spirulina also showed no evidence of genotoxicity under the conditions of the assays [23, 24].

There was no evidence of reproductive toxicity when mice and rats were fed dietary concentrations of dried spirulina of up to 30% (equivalent to 45 000 and 18 000 mg/kg bw per day, respectively) prior to mating (male mice and rats for 9 weeks, female mice for 8 weeks, female rats for 2 weeks) and during mating and gestation. No toxicity was observed in dams or pups when mice, rats and hamsters were fed dietary concentrations of dried spirulina up to 30% (equivalent to 45 000, 30 000 and 27 000 mg/kg bw per day, respectively) over the entire gestation period [25, 26, 27].

In a 2-generation toxicity study in which dosing was limited to F₀ parental females from gestation day 15 through to lactation day 21, mice were fed

diets containing dried spirulina at 0, 10%, 20% or 30% (equivalent to 0, 15 000, 30 000 and 45 000 mg/kg bw per day, respectively). F₁ and F₂ offspring were not directly exposed to the test material. At 30% dried spirulina in the diet, reduced F₁ pup weight at birth and reduced F₁ survival rate on postnatal days 0–4 were reported; no effects were observed in the F₂ animals [26]. The Committee noted that there was no effect on pup survival rate in a 2-generation toxicity study in which F₀ female rats were exposed from gestation day 17 to lactation day 21, in a dosing schedule similar to that used in the Chamorro et al. mouse study [26, 28]. There were also no effects on fetal weight or pup weight at birth in several other reproductive and developmental toxicity studies in the mouse, rat and hamster.

Nutritional studies of dried spirulina fed to rats, rabbits, pigs, sheep and cows showed that the animals maintained good health [29] even when the dietary concentrations were very high (e.g. up to 40% of the diets in rats, equivalent to 40 000 mg/kg bw per day). From a toxicological perspective, these nutritional studies were limited in terms of their observations.

Observations in humans

Observations in humans included case reports, clinical studies and nutritional studies.

The case reports of adverse effects were relatively few considering the long history and widespread use of dried spirulina as a food ingredient and dietary supplement [30]. A few reports cited adverse immunological reactions, such as allergy, associated with the ingestion of dried spirulina dietary supplements. The Committee noted that dried spirulina was well tolerated in clinical and nutritional studies conducted with gram quantities consumed daily for months.

Assessment of dietary exposure

Dietary exposure to spirulina extract from its use as a food colour was assessed by the present Committee.

A comprehensive literature search retrieved nine studies relevant for the assessment of dietary exposure. Consumption of spirulina or components of spirulina occurs from uses other than food colour, that is, dried spirulina in dietary supplements, dried spirulina and spirulina extract as food ingredients, and spirulina extract in coatings for dietary supplements and pharmaceuticals. In order to assess the aggregated dietary exposure from these uses, exposures have been normalized based on phycocyanin content. This was considered appropriate since the colour component of spirulina extract is due to its content of phycocyanins (C-phycocyanin and allophycocyanin). This approach allowed for a comparison of the two test substances used in the toxicological assessments, dried spirulina and spirulina extract.

Dietary exposure expressed as phycocyanins from the use of spirulina extract as a food colour was estimated using the budget method [31]. The theoretical maximum daily exposure was estimated to be 123 and 385 mg/kg bw per day for adults and children respectively. The conversion to phycocyanin content was based on the content of phycocyanins (28%) in the spirulina extracts proposed for use by the sponsor.

Dietary exposure expressed as phycocyanins from the use of dried spirulina in dietary supplements was estimated to be 33 and 133 mg/kg bw per day for adults and children, respectively. This estimate was based on dosage information on product labels and from intervention studies. The conversion to phycocyanin content was based on phycocyanin content (20%) in products from the high end of the range reported in Generally Recognized as Safe (GRAS) notices for commercial dried spirulina.

Dietary exposure expressed as phycocyanins from the use of dried spirulina or spirulina extract as food ingredients was estimated for high consumers to be 33 and 133 mg/kg bw per day for adults and children, respectively. This estimate was based on proposed uses in four GRAS notices, with the conversion to phycocyanin content based on information in the GRAS documentation.

Dietary exposure expressed as phycocyanins from use of spirulina extract in coatings of dietary supplements was estimated for high consumers to be 0.1 and 0.2 mg/kg per day for adults and children, respectively. The conversion to phycocyanin content was based on the assumption that the concentration was the same as for food colour, that is, 28% in spirulina extract.

The Committee noted that spirulina extract could be used in coatings of pharmaceuticals comparable to the use in coatings for dietary supplements, resulting in a similar dietary exposure (0.1 mg/kg bw per day for adults and 0.2 mg/kg bw per day for children). However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure in a healthy population.

The Committee estimated a conservative aggregated exposure to dried spirulina and spirulina extract from all the assessed uses to be 190 and 650 mg/kg bw per day for adults and children, expressed as phycocyanins (Table 9). Based on this assessment, the estimated exposure to phycocyanins from the use of dried spirulina and spirulina extract as a food colour contributes approximately 60% to this total exposure and as dietary supplements and food ingredients contributes approximately 20% each, while the contribution from the use in coatings of dietary supplements is negligible.

Evaluation

The Committee established a temporary ADI “not specified” for spirulina extract. The ADI was based on the absence of toxicity in repeated-dose animal studies

Table 9

Estimates of dietary exposure to phycocyanins from the use of spirulina products

Use (source of phycocyanins)	Exposure to phycocyanins, mg/kg bw per day (%) ^a	
	Adults	Children
Food colour (spirulina extract)	123 (65)	385 (59)
Dietary supplements (dried spirulina)	33 (17)	133 (20)
Food ingredients (dried spirulina and spirulina extract)	33 (17)	133 (20)
Dietary supplement coating (spirulina extract)	0.1 (0.06)	0.2 (0.03)
Aggregated exposure	190	650

bw: body weight

^a Estimated dietary exposure in mg/kg bw per day and, in parentheses, the estimated exposure as a percentage of the total (aggregated) exposure.

conducted with spirulina extract and dried spirulina. These included well-conducted short-term toxicity studies in mice and rats fed dried spirulina at doses of up to 45 000 and 30 000 mg/kg bw per day, respectively. Assuming a phycocyanin content of 10% based on commercial dried spirulina, the doses of phycocyanin were estimated to be 4500 and 3000 mg/kg bw per day, respectively. No evidence of carcinogenicity or systemic toxicity was observed in long-term toxicity studies in rats fed spirulina extract or dried spirulina. There were no concerns regarding genotoxicity. Reproductive and developmental toxicity were not of concern based on the absence of toxicity in feeding studies conducted with dried spirulina in mice, rats and hamsters.

Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour, based on the budget method, and exposure to spirulina extract and dried spirulina from other dietary sources including food ingredients, dietary supplements and coatings of dietary supplements was 190 mg/kg bw for a 60 kg adult and 650 mg/kg bw for a 15 kg child. The Committee concluded that this dietary exposure does not present a health concern.

The ADI “not specified” was made temporary due to the tentative nature of the specifications.

A toxicological and dietary exposure monograph was prepared.

A new tentative specifications monograph and a Chemical and Technical Assessment were prepared.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

2. Glazer AN, Fang S. Chromophore content of blue-green algal phycobiliproteins. *J Biol Chem.* 1973;248(2):659–62.
3. Minic SL, Stanic-Vucinic D, Mihailovic J, Krstic M, Nikolic MR, Cirkovic Velickovic T. Digestion by pepsin releases biologically active chromopeptides from C-phycocyanin, a blue-colored biliprotein of microalga *Spirulina*. *J Proteomics.* 2016;147:132–9.
4. Schram BL, Kroes HH. Structure of phycocyanobilin. *Eur J Biochem.* 1971;19(4):581–94.
5. McCarty MF. Clinical potential of spirulina as a source of phycocyanobilin. *J Med Food.* 2007;10(4):566–70.
6. Eriksen NT. Production of phycocyanin – a pigment with applications in biology, biotechnology, foods and medicine. *Appl Microbiol Biotechnol.* 2008;80(1):1–14.
7. Ciferri O. *Spirulina*, the edible microorganism. *Microbiol Rev.* 1983;47(4):551–78.
8. Scheldeman P, Baurain D, Bouhy R, Scott M, Mühling M, Whitton BA et al. *Arthrospira* ('*Spirulina*') strains from four continents are resolved into only two clusters, based on amplified ribosomal DNA restriction analysis of the internally transcribed spacer. *FEMS Microbiol Lett.* 1999;172(2):213–22.
9. Kwei CK, Lewis D, King K, Donohue W, Nielan BA. Molecular classification of commercial *Spirulina* strains and identification of their sulfolipid biosynthesis genes. *J Microbiol Biotechnol.* 2011;21(4):359–65.
10. Romay C, Ledón N, González R. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm Res.* 1998;47(8):334–8.
11. Naidu AK, Sarada R, Manoj G, Khan MY, Swamy MM, Viswanatha S et al. Toxicity assessment of phycocyanin – a blue colorant from blue green alga *Spirulina platensis*. *Food Biotechnol.* 1999;13(1):51–66.
12. Chen YH, Chang GK, Kuo SM, Huang SY, Hu IC, Lo YL et al. Well-tolerated *Spirulina* extract inhibits influenza virus replication and reduces virus-induced mortality. *Sci Rep.* 2016;6(24253):1–11.
13. Hutadilok-Towatana N, Reanmongkol W, Satitit S, Panichayupakaranant P, Ritthisunthorn P. A subchronic toxicity study of *Spirulina platensis*. *Food Sci Technol Res.* 2008;14(4):351–58.
14. Song L, Liu B, Zhao Y, Tian J, Qin S. Chronic toxicity study of phycocyanin on Sprague Dawley rats. *Chin Med Herald.* 2012;9(33):15–21.
15. Takemoto K. Chronic toxicity study of phycocyanin (Linablue A). Unpublished study by Saitama Medical School, Moroyama, Saitama Prefecture, Japan; 1980. Submitted to WHO by the International Association of Color Manufacturers (IACM), Washington, DC, USA.
16. Yang Y, Park Y, Cassada DA, Snow DD, Rogers DG, Lee J. In vitro and in vivo safety assessment of edible blue-green algae, *Nostoc commune* var. *sphaeroides* Kützinger and *Spirulina plantensis*. *Food Chem Toxicol.* 2011;49(7):1560–4.
17. Chamorro GA, Herrera G, Salazar M, Salazar S, Ulloa V. Subchronic toxicity study in rats fed spirulina. *J Pharm Belg.* 1988;43(1):29–36.
18. Salazar M, Martinez E, Madrigal E, Ruiz LE, Chamorro GA. Subchronic toxicity study in mice fed *Spirulina maxima*. *J Ethnopharmacol.* 1998;62(3):235–41.
19. Yang Y, Kim B, Park YK, Lee JY. Effects of long-term supplementation of blue-green algae on lipid metabolism in C57BL/6J mice. *J Nutrit Health Food Sci.* 2014;1(1):1–6.

20. Vidé J, Romain C, Feillet-Coudray C, Bonafos B, Cristol JP, Fouret G et al. Assessment of potential toxicological aspects of dietary exposure to silicon-rich spirulina in rats. *Food Chem Toxicol.* 2015;80:108–13.
21. Bigagli E, Cinic L, Niccolai A, Tredici MR, Biondi N, Rodolfi L et al. Safety evaluations and lipid-lowering activity of an *Arthrospira platensis* enriched diet: a 1-month study in rats. *Food Res Int.* 2017;102:380–6.
22. Chamorro-Cevallos G. Toxicologic study of the spirulina algae pilot plant for the production of proteins (Spirulina de Soso, Texcoco LLP). Vienna: United Nations Industrial Development Organization (UNIDO); 1980 (UNIDO/IO 387).
23. Chamorro G, Salazar M. Dominant lethal assay of *Spirulina maxima* in male CD-1 mice after short-term and prolonged-term feeding. *J Food Prot.* 1989;52(2):125–7.
24. Chamorro G, Salazar M, Pages N. Dominant lethal study of *Spirulina maxima* in male and female rats after short-term feeding. *Phytother Res.* 1996;10:28–32.
25. Chamorro G, Salazar S, Salazar M, Pages N. [Evaluation of the teratological effect of Spirulina in hamster.] *Belg J Food Chem Biotech.* 1987;42:188–91 (in French).
26. Chamorro G, Salazar S, Favila-Castilo L, Steele C, Salazar M. Reproductive and peri- and postnatal evaluation of *Spirulina maxima* in mice. *J Appl Phycol.* 1997;9(2):107–12.
27. Chamorro G, Salazar M. Teratogenic study of Spirulina in mice. *Arch Latinoam Nutr.* 1990;40(1):86–94.
28. Salazar M, Chamorro GA, Salazar S, Steele CE. Effect of *Spirulina maxima* consumption on reproduction and peri- and postnatal development in rats. *Food Chem Toxicol.* 1996;34(4):353–9.
29. Madeira M, Cardoso C, Lopes P, Coelho D, Afonso C, Bandrra N et al. Microalgae as feed ingredients for livestock production and meat quality: A review. *Livest Sci.* 2017;205:111–21.
30. Marles RJ, Barrett ML, Barnes J, Chavez ML, Gardiner P, Ko R et al. United States pharmacopeia safety evaluation of spirulina. *Crit Rev Food Sci Nutr.* 2011;51(7):593–604.
31. Hansen SC. Conditions for use of food additives based on a budget for an acceptable daily intake. *J Food Prot.* 1979;42:429–32.

3.2 Revision of specifications and analytical methods

3.2.1 Cassia gum

Cassia gum was on the agenda at the request of the Committee at its eighty-second meeting. The specifications for cassia gum were made tentative by the eighty-second JECFA ([Annex 1](#), reference 230) pending submission of a validated method for the determination of anthraquinones. The Committee, at the current meeting, considered the analytical methods provided and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with *n*-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified.

The tentative specifications were revised and the tentative status was removed. The Chemical and Technical Assessment was revised.

3.2.2 Citric and fatty acid esters of glycerol

Citric and fatty acid esters of glycerol (CITREM) was on the agenda at the request of the Committee at its seventy-ninth and eighty-second meetings ([Annex 1](#), references 174 and 230), requesting a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in the specifications monographs. The Committee did not receive any suitable replacement method. The specifications for CITREM were made tentative, pending a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.

The Committee noted that the method for total glycerol still uses chloroform. The Committee encourages the submission of a method for total glycerol that eliminates the use of chloroform.

The Committee noted that neutralizing agents, other than sodium and potassium hydroxides, are in use for the partial or full neutralization of CITREM products intended for some food applications. The Committee noted that a request was made to extend the definition of CITREM to include the use of alternative neutralizing agents. However, the Committee emphasizes that such requests shall be made through the corresponding Codex committees.

Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by December 2019.

A Chemical and Technical Assessment was prepared.

3.2.3 Glycerol ester of wood rosin

Glycerol ester of wood rosin (GEWR) was on the agenda of the current meeting at the request of Codex Committee on Food Additives [1] to allow the use of additional species of pine as source materials in the manufacture of GEWR. The current specifications refer to two pine species: *Pinus palustris* and *Pinus elliottii*. The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species, namely *Pinus halepensis* and *Pinus brutia*, as source materials. The total esterified abietic acids in GEWR (abietic acid, dehydroabietic acid and neoabietic acid) prepared from the four pine species range from approximately 72% to 85%. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. The Committee further noted that this harmonizes the JECFA specifications with those of other regulatory authorities.

Identification of GEWR is based on the presence of characteristic acids. Since the specifications monograph for GEWR does not contain an assay, the Committee recommends that the JECFA Secretariat be notified upon the development and validation of an appropriate assay.

The existing specifications were revised. The Chemical and Technical Assessment was revised.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

3.2.4 Modified starches

General considerations

The Committee at its seventy-ninth meeting ([Annex 1](#), reference 220) recommended the separation of the combined specifications for the modified starches into 16 individual monographs (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451). The recommendation was based on the difficulty of revising individual specifications for any given modified starch within the existing combined specifications monograph.

The Committee at its eighty-second meeting ([Annex 1](#), reference 230) reviewed individual specifications monographs for each of the 16 modified starches and, based on the limited information received, prepared full specifications for three (INS Nos 1404, 1420, 1451) and tentative specifications for the remaining 13 (INS Nos 1400, 1401, 1402, 1403, 1405, 1410, 1412, 1413, 1414, 1422, 1440, 1442, 1450). The Committee noted that all the modified starches may also be subjected to bleaching and therefore included the appropriate purity tests in all the revised specifications. Data and information necessary to complete and revise the 13 individual tentative specifications monographs, as well as information on the method of manufacture for each of the 16 modified starches, were requested through a further call for data.

At the current meeting, the Committee reviewed data on the method of manufacture, identity and purity of all 16 modified starches.

The Committee noted the following:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered a read-across approach to be appropriate for the toxicological evaluation of these substances.

- All 16 modified starches had been assigned an ADI “not specified”.
- All modified starches can be additionally bleached or fragmented; therefore, revision of the specifications of bleached or fragmented starches would require the revision of all 16 monographs.
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals content and microbiological considerations). Revision of those common specifications would affect all 16 monographs.
- Because of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, unavailable and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.

Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture and the corresponding diversity of impurities.

The Committee recommended that all modified starches be included in a modular monograph titled “Modified Starches” that contains common requirements (“General specifications for modified starches”) consisting of specifications that apply to all 16 modified starches (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451) and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The annexes are as follows:

- Annex 1 – Fragmentation
- Annex 2 – Bleaching
- Annex 3 – Esterification and/or crosslinking with phosphorus-containing compounds
- Annex 4 – Acetylation
- Annex 5 – Oxidation
- Annex 6 – Esterification with octenyl succinic anhydride
- Annex 7 – Etherification with propylene epoxide
- Annex 8 – Esterification and crosslinking with adipic anhydride

Table 10

List of modified starches considered and applicable annexes

Modified starch	INS No.	Annex
Dextrin roasted starch	1400	1
Acid treated starch	1401	1
Alkaline treated starch	1402	1
Bleached starch	1403	2
Oxidized starch	1404	5
Enzyme-treated starch	1405	1
Monostarch phosphate	1410	3
Distarch phosphate	1412	3
Phosphated distarch phosphate	1413	3
Acetylated distarch phosphate	1414	3, 4
Starch acetate	1420	4
Acetylated distarch adipate	1422	4, 8
Hydroxypropyl starch	1440	7
Hydroxypropyl distarch phosphate	1442	3, 7
Starch sodium octenyl succinate	1450	6
Acetylated oxidized starch	1451	4, 5

INS: International Numbering System for Food Additives

Each modified starch should fulfil the specification requirements of the General specifications as well as the specification requirements of all the annexes applicable to it.

The Committee determined that, using this approach, revisions made to the section “General specifications for modified starches” would affect all the modified starches. However, annexes can be revised individually and the revisions would only affect the modified starches mentioned in that annex. A tentative status prompted by information missing from the General specifications would affect all 16 modified starches. A tentative status of an annex would only affect the status of modified starches covered by that annex.

In response to the considerations noted above, the Committee drafted a new modular specifications monograph titled “Modified starches” consisting of an explanatory introduction, “General specifications for modified starches”, and eight annexes. The new modular specifications monograph for modified starches is to be included in FAO Monographs 22, and will replace the 16 existing individual specifications for modified starches (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

Technical considerations

In line with previous decisions, the Committee noted that the processes of starch modification should not result in any additional contamination from heavy metals and therefore recommended that the limit for lead included in the General specifications be decreased from 2 to 0.2 mg/kg. This change is in line with the limit for lead in grains and cereals currently contained in the Codex General Standard for Contaminants in Food [1].

Starch sodium octenyl succinate (INS No. 1450) was on the agenda of the eighty-second JECFA meeting at the request of CCFA at its Forty-seventh Session [2] to assess the data on the levels of lead when the additive is used in infant formula and formula for special medical purposes for infants and to consider a specific limit in the specifications.

At the current meeting, the Committee received data for the lead content from 12 batches of starch sodium octenyl succinate for use in infant formula and formula for special medical purposes intended for infants. The limit of lead for starch sodium octenyl succinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications. This limit is in line with the recommendation from the seventy-ninth JECFA that the introduction of lead limits of 0.1 mg/kg for starch sodium octenyl succinate would result in this additive not exceeding the maximum limit for lead in the final infant formula (i.e. 0.01 mg/kg) if the additive were included in infant formula at the maximum use level reviewed by JECFA.

The Committee further noted that during the manufacture of hydroxypropyl starch (INS No. 1440) and hydroxypropyl distarch phosphate (INS No. 1442), propylene chlorohydrins can occur as impurities. The current specification for propylene chlorohydrins is "Not more than 1 mg/kg". Based on information available in the literature [3] and the limited data received, the Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in these modified starches in order to consider lowering this limit.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added. Should more suitable methods become available, the Committee recommends that these methods be communicated to the JECFA secretariat.

The Committee requests suitable microbiological acceptance criteria and supporting data for all modified starches. Information required in the annexes is summarized in [Table 11](#).

Table 11
Information required in the annexes

Annex	Modification	Starches	Information required
1	Minor fragmentation	INS No. 1400: Dextrin roasted starch INS No. 1401: Acid treated starch INS No. 1402: Alkaline treated starch INS No. 1405: Enzyme-treated starch All modified starches that are additionally fragmented.	A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes
2	Bleaching	INS No. 1403: Bleached starch All modified starches if additionally bleached.	Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s)
3	Esterification and/or crosslinking with phosphorus-containing compounds	INS No. 1410: Monostarch phosphate INS No. 1412: Distarch phosphate INS No. 1413: Phosphated distarch phosphate INS No. 1414: Acetylated distarch phosphate INS No. 1442: Hydroxypropyl distarch phosphate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches
4	Acetylation	INS No. 1420: Starch acetate INS No. 1414: Acetylated distarch phosphate INS No. 1422: Acetylated distarch adipate INS No. 1451: Acetylated oxidized starch	Currently no additional information required
5	Oxidation	INS No. 1404: Oxidized starch INS No. 1451: Acetylated oxidized starch	A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method
6	Esterification with octenyl succinic anhydride	INS No. 1450: Starch sodium octenyl succinate	Currently no additional information required
7	Etherification with propylene epoxide	INS No. 1440: Hydroxypropyl starch INS No. 1442: Hydroxypropyl distarch phosphate	A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of hydroxypropyl starch using the method
8	Crosslinking with adipic anhydride	INS No. 1422: Acetylated distarch adipate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches Levels of free adipic acid in at least 5 representative batches

INS: International Numbering System for Food Additives; No.: number

References

1. General standard for contaminants and toxins in food and feed. Rome: Food and Agriculture Organization of the United Nations, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 1995 (Codex Stan 193-1995).
2. Report of the Forty-seventh Session of the Codex Committee on Food Additives, Xi'an, China, 23–27 March 2015. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2015 (REP15/FA).
3. Scientific Opinion on the re-evaluation of oxidised starch (E 1404), monostarch phosphate (E 1410), distarch phosphate (E 1412), phosphated distarch phosphate (E 1413), acetylated distarch phosphate

(E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422), hydroxypropyl starch (E 1440), hydroxypropyl distarch phosphate (E 1442), starch sodium octenyl succinate (E 1450), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452) as food additives. EFSA J. 2017;15(10):4911 [96 pp.] doi:10.2903/j.efsa.2017.4911.

4. Flavouring agents

4.1 Toxicological evaluation and exposure assessment

4.1.1 Alicyclic primary alcohols, aldehydes, acids and related esters

Introduction

The Committee evaluated an additional three flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters. These three flavouring agents have not previously been evaluated by the Committee. The Committee also re-evaluated six previously evaluated flavouring agents in this group.

The Committee previously evaluated 26 members of this group of flavouring agents at its fifty-ninth meeting ([Annex 1](#), reference 160) and 11 members of this group at its seventy-third meeting ([Annex 1](#), reference 202). The Committee concluded that all 37 flavouring agents were of no safety concern at estimated dietary exposures.

The three additional flavouring agents in this group are the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253); (1-methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl) methanol (No. 2254); and ($\pm$)-bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester (No. 2255). These three flavouring agents have not been reported to occur as natural components of foods.

The six previously evaluated flavouring agents in this group that were re-evaluated at the present meeting are *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), *p*-mentha-1,8-dien-7-ol (No. 974), *p*-mentha-1,8-dien-7-yl acetate (No. 975), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), myrtenol (No. 981) and myrtenyl acetate (No. 982). These six flavouring agents are all reported to occur as natural components of foods, including bergamot oil, blackberry, black tea, cumin, ginger, grapefruit oil, kabosu oil, kumquat oil, lamb's lettuce, lemon peel oil, lime oil, mandarin oil, orange juice and oil, pepper, peppermint, pistachio, spearmint, thyme, yuzu oil and other foods [1].

Two of the six previously evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), contain an α,β -unsaturated aldehyde group that is considered to be a structural alert for genotoxicity [2]. The remaining four re-evaluated flavouring agents in this group are not α,β -unsaturated aldehydes, but are structurally related to Nos 973 and 980. Additional in vitro and in vivo genotoxicity data were available for all six of these previously evaluated flavouring agents. Also, one of the two major components of an additional flavouring agent evaluated by the Committee at the present meeting (No. 2253, which is a mixture of 1-vinyl-3-

cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde) contains an α,β -unsaturated aldehyde group. In vitro and in vivo genotoxicity data on No. 2253 were also available for evaluation.

The evaluations of the three additional members of this group and the re-evaluation of six previously considered flavouring agents in this group were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

Assessment of dietary exposure

The total annual volumes of production of the nine flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters are 36 kg in Europe, 34 kg in the USA, 3030 kg in Japan and 28 kg in Latin America [3, 4]. More than 97% of the annual production volume in Japan is accounted for by *p*-mentha-1,8-dien-7-al (No. 973). More than 97% of the annual production volume in Europe and the USA are accounted for by three flavouring agents: *p*-mentha-1,8-dien-7-al (No. 973); *p*-mentha-1,8-dien-7-yl acetate (No. 975); and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980). More than 96% of the annual production volume in Latin America is accounted for by three flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980) and myrtenol (No. 981).

Annual volumes of production in the USA for the three additional flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters are 0.4 kg each for Nos 2253 and 2254 and 0.1 kg for No. 2255. Annual production volume for these three flavouring agents in Europe, Japan or Latin America is reported as 0 kg [3, 4].

Dietary exposures were estimated using both the single-portion exposure technique (SPET) and the maximized survey-derived intake (MSDI) method, and the higher of the two values for each flavouring agent is reported in [Table 12](#). The SPET and MSDI method values are in the range of 30–1500 and 0.01–780 $\mu\text{g}/\text{day}$, respectively, with the SPET yielding the highest estimate for each flavouring agent. The estimated daily dietary exposure was highest for the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253) (1500 $\mu\text{g}/\text{day}$, the SPET value obtained for non-alcoholic beverages).

Absorption, distribution, metabolism and excretion

Information on the ADME of this group of flavouring agents was described in the reports of the fifty-ninth and seventy-third meetings ([Annex 1](#), references 160 and 202). No additional information was available for this meeting.

Consideration of genotoxicity data

The Committee considered new genotoxicity data on six members of this group. The six members were evaluated at the fifty-ninth meeting ([Annex 1](#), reference 160) and re-evaluated at the present meeting: *p*-mentha-1,8-dien-7-al (No. 973), *p*-mentha-1,8-dien-7-ol (No. 974), *p*-mentha-1,8-dien-7-yl acetate (No. 975), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), myrtenol (No. 981) and myrtenyl acetate (No. 982). Two of these six flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), contain an α,β -unsaturated aldehyde group considered to be a structural alert for genotoxicity [2].

The Committee considered the new genotoxicity data for No. 973 and noted that there were reproducible positive results in a reverse mutation assay in the TA98 strain of *Salmonella typhimurium* [5]. In addition, a study in rats investigated micronucleus induction in bone marrow and DNA damage in liver and duodenum (comet assay) [6, 7]. In this GLP-compliant study, the micronucleus assay was conducted according to OECD guideline 474 while the comet assay was conducted in accordance with published guidelines developed by an expert working group [8]. The results of the micronucleus assay indicated that *p*-mentha-1,8-dien-7-al (No. 973) did not induce an increase in micronucleated polychromatic erythrocytes. Although the comet assay did not indicate DNA damage in duodenum compared to the negative control, a 3-fold increase in DNA strand breaks (statistically significant, $P < 0.001$) was observed in liver at the highest dose tested (700 mg/kg bw per day), and there was a dose-dependent trend in the response ($P < 0.001$). Based on these new data, the Committee concluded that there are concerns for potential genotoxicity for *p*-mentha-1,8-dien-7-al (No. 973).

For No. 980, new in vitro reverse mutation [9, 10] and micronucleus induction [11, 12] studies were negative. Also available were new in vivo assays on micronucleus induction in bone marrow and DNA damage in liver and duodenum [13] conducted in the same laboratory and using the same protocols as used for the study on No. 973. The results of the micronucleus assay indicated no increase in micronucleated polychromatic erythrocytes and the results of the comet assay indicated no DNA damage in duodenum or liver. The Committee concluded that there are no concerns for potential genotoxicity for formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980).

The remaining four re-evaluated flavouring agents in this group are not α,β -unsaturated aldehydes but are structurally related to Nos 973 and 980. Additional in vitro and in vivo genotoxicity data were available on these flavouring agents for evaluation at the present meeting. None of these studies indicated potential for genotoxicity.

One of the two major components of an additional flavouring agent evaluated by the Committee at the present meeting (No. 2253, which is a mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde) also contains an α,β -unsaturated aldehyde group, and in vitro and in vivo genotoxicity data on No. 2253 were available for evaluation. In a bacterial reverse mutation assay, No. 2253 was negative in all five strains tested in both the presence and absence of metabolic activation [14]. In an in vitro mammalian chromosomal aberration assay, No. 2253 induced an increase in the percentage of aberrant cells [15]. However, a GLP-compliant in vivo micronucleus induction assay in mice, conducted in accordance with OECD guideline 474, was negative [16]. The Committee concluded that there are no concerns for potential genotoxicity for the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253).

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Genotoxicity data on the three additional flavouring agents in this group (Nos 2253–2255) and five of the six re-evaluated flavouring agents in this group do not indicate that these flavouring agents have the potential for genotoxicity. For one of the re-evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) the Committee concluded that there were concerns for genotoxicity. Therefore, No. 973 was not further considered using the Procedure for the Safety Evaluation of Flavouring Agents.

Step 2. In applying the Procedure, the Committee assigned six flavouring agents (Nos 974, 975, 980–982 and 2253) to structural class I, one flavouring agent (No. 2254) to structural class II and one flavouring agent (No. 2255) to structural class III [17].

Step 3. Dietary exposures were estimated using both the MSDI method and the SPET.

Step 4. The highest estimated dietary exposures for all eight flavouring agents were below the threshold of toxicological concern applicable to each flavouring agent. The Committee therefore concluded that these eight flavouring agents would not pose a safety concern at current estimated dietary exposures.

Table 12 summarizes the evaluations of the eight flavouring agents belonging to this group of alicyclic primary alcohols, aldehydes, acids and related esters that were considered at the present meeting (Nos 974, 975, 980–982 and 2253–2255).

Consideration of combined intakes from use as flavouring agents

The Committee previously considered the potential combined intakes for this group of alicyclic primary alcohols, aldehydes, acids and related esters and did not identify any safety concerns. The three additional flavouring agents in this

Table 12

Summary of the results of the safety evaluations of alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents^{a,b,c,d}

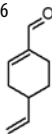
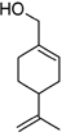
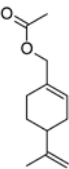
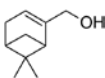
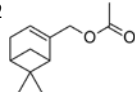
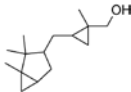
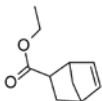
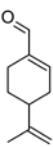
Flavouring agent	No.	CAS no. and structure	Step 4 ^e Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Structural class I				
Mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde	2253	1049017-63-1 	1049017-68-6 	No, SPET: 1 500 No safety concern
<i>p</i> -Mentha-1,8-dien-7-ol	974	536-59-4 	No, SPET: 1 000	No safety concern
<i>p</i> -Mentha-1,8-dien-7-yl acetate	975	15111-96-3 	No, SPET: 1 000	No safety concern
Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	564-94-3 	No, SPET: 1 000	No safety concern
Myrtenol	981	515-00-4 	No, SPET: 1 000	No safety concern
Myrtenyl acetate	982	1079-01-2 	No, SPET: 1 000	No safety concern
Structural class II				
(1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	198404-98-7 	No, SPET: 30	No safety concern
Structural class III				
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	10138-32-6 	No, SPET: 60	No safety concern

Table 12 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 ^e Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Flavouring agent excluded at Step 1 of the Procedure				
<i>p</i> -Mentha-1,8-dien-7-al (perillaldehyde)	973	2111-75-3 	NA	Genotoxicity data for <i>p</i> -mentha-1,8-dien-7-al raise concerns for potential genotoxicity

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; NA: not applicable; No.: number; NOAEL: no-observed-adverse-effect level; SPET: single-portion exposure technique

^a Thirty-seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 160 and 202). Six of those flavouring agents (Nos 973–975 and 980–982) and three additional flavouring agents in this group (Nos 2253–2255) were also evaluated at the present meeting.

^b Step 1: Genotoxicity data for one of the nine flavouring agents in this group, *p*-mentha-1,8-dien-7-al (No. 973), raise concerns for potential genotoxicity. Genotoxicity data for the remaining eight flavouring agents in this group do not indicate potential genotoxicity.

^c Step 2: The additional flavouring agents in this group are in structural class I (No. 2253), class II (No. 2254) and class III (No. 2255). The five re-evaluated flavouring agents in this group are in structural class I.

^d Step 3: Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in Table 12. The SPET gave the highest estimate for each flavouring agent. Dietary exposure values are expressed in µg/day.

^e Step 4: The thresholds for human dietary exposure for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively.

group (Nos 2253–2255) have low MSDI values (0.01–0.04 µg/day) and therefore would make a negligible contribution to the combined intake of this group.

Consideration of additional data on previously evaluated flavouring agents

In addition to new genotoxicity data on the six flavouring agents that were re-evaluated at the present meeting, the Committee considered additional data on several other previously evaluated flavouring agents in this group. Acute toxicity data were evaluated for Nos 961, 967, 977 and 981, and studies of genotoxicity were evaluated for Nos 967, 977–979 and 984. These new toxicological data support the conclusions of previous Committee evaluations that these flavouring agents are not safety concerns.

Conclusions

In previous evaluations of flavouring agents in this group of alicyclic primary alcohols, aldehydes, acids and related esters, studies of hydrolysis; ADME; acute, short-term and long-term toxicity; and genotoxicity were available. None of the 37 previously evaluated flavouring agents raised safety concerns.

At the present meeting, the Committee concluded that the three flavouring agents (Nos 2253–2255) that are additions to the group of alicyclic primary alcohols, aldehydes, acids and related esters, would not give rise to safety concerns at current estimated dietary exposures.

The Committee also concluded that five of the six previously evaluated flavouring agents in this group (Nos 974, 975 and 980–982) that were re-evaluated at the present meeting do not give rise to safety concerns. For one of the re-evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), the Committee concluded that there were concerns for potential genotoxicity. Therefore No. 973 was not further considered using the Procedure.

An addendum to the monograph was prepared.

References

1. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 5 January 2018).
2. Eder E, Scheckenbach S, Deininger C, Hoffman C. The possible role of alpha, beta-unsaturated carbonyl compounds in mutagenesis and carcinogenesis. *Toxicol Lett.* 1993;67(1–3):87–103.
3. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Bowen R. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2011. Study no. 8221207. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Beevers C. *p*-Mentha-1,8-dien-7-al: Combined bone marrow micronucleus test and comet assay in the liver of treated rats. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2014a. Study no. 8288455. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Beevers C. *p*-Mentha-1,8-dien-7-al: Analysis of comet slides from Covance study 8288455. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2014b. Study no. 8307194. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
8. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR et al. Fourth International Workgroup on Genotoxicity testing: results of the in vivo comet assay workgroup. *Mutat Res.* 2007;627(1):31–5.
9. McGarry S. Myrtenal: Bacterial reverse mutation assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2016b. Study no. 8332788. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

10. Dakoulas E. 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS No. 564-94-3): Bacterial reverse mutation assay. BioReliance Corporation, Maryland, USA; 2017. Study no. AE67ZC.503.BTL. Unpublished report to the Research Institute for Fragrance. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
11. McGarry S. Myrtenal: In vitro human lymphocyte micronucleus assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2016a. Study no. 8332792. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
12. Lloyd M. Myrtenal: In vitro human lymphocyte micronucleus assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2017. Study no. 8351223. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
13. Pant K. In vivo mammalian erythrocyte micronucleus assay and mammalian alkaline comet assay in rats. BioReliance Corporation, Maryland, USA; 2018. Study no. AF08WZ.433M.BTL. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
14. Sokolowski A. *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay with GR-50-0091. Harlan Laboratories Ltd, Rossdorf, Germany; 2011. Study no. 1406304. Unpublished report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
15. Hall C. In vitro chromosome aberration test in Chinese hamster V79 cells with G-50-0091. Harlan Laboratories Ltd, Rossdorf, Germany; 2011a. Study no. 1406305. Unpublished report to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
16. Hall C. Micronucleus assay in bone marrow cells of the mouse with [redacted]. Harlan Laboratories Ltd, Rossdorf, Germany; 2011b. Study no. 1439400. Unpublished report to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
17. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 1978;16(3):255–76.

4.1.2 Carvone and structurally related substances

Introduction

The Committee evaluated five flavouring agents belonging to the previously evaluated group of carvone and structurally related substances. The Committee re-evaluated two flavouring agents, (+)-carvone (No. 380.1; *d*-carvone) and (–)-carvone (No. 380.2; *l*-carvone), and evaluated three additional flavouring agents. These three additional flavouring agents included two esters, pinocarvyl isobutyrate (No. 2242) and carvyl palmitate (No. 2243), and one alicyclic secondary alcohol, 6-hydroxycarvone (No. 2244). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference

230). (+)-Carvone (No. 380.1) and (–)-carvone (No. 380.2) were re-evaluated because of new data had become available.

The Committee previously evaluated nine members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), reference 137). The Committee concluded that all nine flavouring agents were of no safety concern at estimated dietary exposures.

Carvone (Nos 380.1 and 380.2) was evaluated at the eleventh meeting ([Annex 1](#), reference 14) at which a conditional³ ADI of 0–1.25 mg/kg bw for the (+)- and (–)-enantiomers was established. At the twenty-third meeting ([Annex 1](#), reference 50), a temporary ADI of 0–1 mg/kg bw was established for (+)- and (–)-carvone. This temporary ADI was extended at the twenty-fifth, twenty-seventh, thirtieth and thirty-third meetings ([Annex 1](#), references 56, 62, 73 and 83). At its thirty-seventh meeting, the Committee determined that the (+)- and (–)-enantiomers of carvone should be evaluated separately ([Annex 1](#), reference 94). The Committee established an ADI for (+)-carvone of 0–1 mg/kg bw per day based on a no-observed-effect level (NOEL) of 93 mg/kg bw per day from a 3-month toxicity study in rats. The temporary ADI for (–)-carvone was not extended because insufficient data were available for the toxicological evaluation of this enantiomer. The Committee at its fifty-first meeting maintained the ADI of 0–1 mg/kg bw for (+)-carvone (No. 380.1) ([Annex 1](#), reference 137).

Two of the five flavouring agents, (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), have been reported to occur naturally in foods, mainly botanicals. (+)-Carvone (No. 380.1) has been reported in *Carum* (caraway) and *Anethum* (dill). (–)-Carvone (No. 380.2) has been reported in the oils of *Mentha* (spearmint) [2].

A comprehensive literature search for toxicological data was performed in Scopus; no additional relevant references were identified.

Assessment of dietary exposure

The total annual volume of production of the three new flavouring agents belonging to the group of carvone and structurally related substances is 2 kg in Japan [3, 4]. The total production volume for the flavouring agents presented for re-evaluation, (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), is 48 300 kg in Europe, 53 700 kg in the USA, 1780 kg in Japan and 6340 kg in Latin America [3, 4]. Separate production volumes for the two enantiomers are not available.

Dietary exposure was estimated using both the SPET and the MSDI method, with the highest values reported in [Table 13](#). The estimated daily dietary exposure is highest for carvone (Nos 380.1 and 380.2) at 37 800 µg/day, the SPET

³ "Conditional ADI is a term no longer used by JECFA to signify a range above the 'unconditional ADI', which may signify an acceptable intake when special problems, different patterns of dietary intake, and special groups of the population that may require consideration are taken into account." [1]

value obtained from beer and malt beverages. The MSDI values for carvone range from 350 to 5573 µg/day for the four regions. For the other flavouring agents, the estimated daily dietary exposures range from 0.05 to 0.3 µg/day (MSDI values) and from 3 to 450 µg/day (SPET values).

Absorption, distribution, metabolism and excretion

Relevant information apart from that described in the monographs of the eleventh, twenty-third, thirty-seventh and fifty-first meetings ([Annex 1](#), references 15, 51, 95, 138) was available on the ADME of the flavouring agents belonging to the group of carvone and structurally related substances.

In general, carboxylesterases or esterases catalyse the hydrolysis of esters to their corresponding alcohol and carboxylic acid. The carvyl esters in this group (Nos 2242 and 2243) can be expected to readily hydrolyse to pinocarveol (No. 1403) and carveol (No. 974), respectively, and their respective carboxylic acids. Carvone (Nos 380.1 and 380.2) is metabolized to carveol, dihydrocarveol, carvonic acid, dihydrocarvonic acid and uroterponolone in humans [5]. Oxidation of (+)- or (–)-carvone by human or rat liver microsomes is stereospecific; (+)-carvone is oxidized exclusively to (+)-carveol and (–)-carvone to (–)-carveol, with (–)-carvone having a significantly higher affinity for microsomal enzymes (lower apparent K_m). Only (–)-carveol is converted to a glucuronide conjugate by human or rat liver microsomes [6]. This is expected to impact the toxicity profiles of the enantiomers and their metabolites. 6-Hydroxycarvone is expected to undergo conjugation with glucuronic acid or glutathione followed by excretion.

Flavouring agents not evaluated according to the Procedure for the Safety Evaluation of Flavouring Agents

Exposure to (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) occurs from different food and non-food sources as well as from their use as flavouring agents. Both enantiomers of carvone occur naturally in food and are used in one or more of the following applications: pesticides, feed additives, veterinary products, personal care products, natural insect repellents, food supplements and herbal medicinal products [2, 7].

For the current re-evaluation of carvone (Nos 380.1 and 380.2), additional biochemical data and studies of acute toxicity and genotoxicity were submitted by the flavour industry. The Scientific Committee of the EFSA published an evaluation of carvone based on data from the flavour and pesticide industries. An ADI of 0.6 mg/kg bw per day for (+)-carvone was established, but an ADI for (–)-carvone could not be established due to lack of data. The highest level of aggregated exposure to (+)-carvone was at the level of the ADI for (+)-carvone. The highest level of aggregated exposure to (–)-carvone was 3-fold higher than that of (+)-carvone [7].

The Committee previously established an ADI for (+)-carvone of 0–1 mg/kg bw per day based on a NOEL of 93 mg/kg bw per day from a 3-month toxicity study in rats [8]. At the current meeting, the Committee noted that when identifying the NOEL, no correction was made for the 5-day (rather than 7-day) dosing scheme. Shortly before the meeting, data from the pesticide industry (studies of acute toxicity, short-term toxicity and a 2-generation study) were made available to the Committee by the sponsor. The Committee considered these data and concluded that a review of the ADI for (+)-carvone is recommended based on the evaluation of all biochemical and toxicological data. Also, additional data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources to complete the re-evaluation of (+)-carvone (No. 380.1).

The Committee previously concluded that the ADI for (+)-carvone could not be extended to (–)-carvone because insufficient data were available for the toxicological evaluation of this enantiomer ([Annex 1](#), reference 94). Data are also needed in an oral exposure assessment to (–)-carvone from all sources to complete the re-evaluation of (–)-carvone (No. 380.2).

The Committee therefore did not re-evaluate (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) according to the Procedure for the Safety Evaluation of Flavouring Agents at the current meeting.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for the additional flavouring agents (Nos 2242–2244) in this group. Chemical-specific genotoxicity data on flavouring agents previously evaluated within this group do not indicate that the flavouring agents in this group have the potential to be genotoxic.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents to Nos 2242–2244, the Committee assigned Nos 2242 and 2243 to structural class I and No. 2244 to structural class III [9].

Steps 3 and 4. The highest estimated dietary exposures of Nos 2242–2244 are below their respective thresholds of toxicological concern (i.e. 1800 µg/day for structural class I and 90 µg/day for structural class III). The Committee therefore concluded that these three flavouring agents would not pose a safety concern at current estimated dietary exposures.

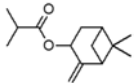
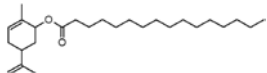
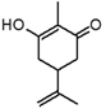
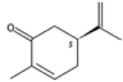
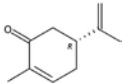
[Table 13](#) summarizes the evaluations of the three flavouring agents (Nos 2242–2244) in the group of carvone and structurally related substances.

Consideration of combined intakes from use as flavouring agents

The three additional flavouring agents in the group of carvone and structurally related substances have low MSDIs (0.05–0.3 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional

Table 13

Summary of the results of the safety evaluations of carvone and structurally related substances used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Structural class I				
Pinocaryl isobutyrate	2242	929116-08-5 	No, SPET: 450	No safety concern
Carvyl palmitate	2243	929222-96-8 	No, SPET: 3	No safety concern
Structural class III				
6-Hydroxycarvone	2244	51200-86-3 	No, SPET: 4	No safety concern
Flavouring agents not evaluated according to the Procedure				
(+)-Carvone	380.1	2244-16-8 	A review of the ADI is recommended based on an evaluation of all biochemical and toxicological data. Also, data are needed for an assessment of oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.	
(-)-Carvone	380.2	6485-40-1 	Additional toxicological data on (-)-carvone are necessary. Also, data are needed for an assessment of oral exposure to (-)-carvone from all sources to complete the evaluation for (-)-carvone.	

ADI: acceptable daily intake; CAS: Chemical Abstracts Service; no.: number; SPET: single-portion exposure technique

^a Nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 137).

^b Step 2: Two additional flavouring agents in this group are in structural class I and one additional flavouring agent in this group is in structural class III.

^c The threshold of toxicological concern for human dietary exposure for structural class I is 1800 µg/day and for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest estimated daily dietary exposures calculated using the SPET.

flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of additional data on previously evaluated flavouring agents

In the previous evaluations of substances in this group of carvone and structurally related substances, studies of biochemistry, acute toxicity, short-term and long-term toxicity and genotoxicity were available ([Annex 1](#), references 15, 51, 95 and 138). None of the nine flavouring agents of this group raised safety concerns.

Besides data on carvone (see the section “Flavouring agents not evaluated according to the Procedure for the Safety Evaluation of Flavouring Agents”), additional biochemical data on carveol (No. 381), a study of acute toxicity with *p*-menthan-2-one (No. 375) and studies of genotoxicity with dihydrocarveol (No. 378) and carveol (No. 381) were available. These data support the conclusions of the previous evaluation.

Conclusions

The Committee concluded that the three flavouring agents pinocarvyl isobutyrate (No. 2242), carvyl palmitate (No. 2243) and 6-hydroxycarvone (No. 2244), which are additions to the group of carvone and structurally related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures.

The Committee did not re-evaluate (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) according to the Procedure for the Safety Evaluation of Flavouring Agents given the lack of information on the oral exposure from all sources, the need to review the ADI of (+)-carvone and the lack of toxicological data on (–)-carvone.

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. In addition, data are needed for an assessment of oral exposure to (+)-carvone from all sources to complete the re-evaluation. This could not be completed during the current meeting.

For (–)-carvone (No. 380.2), the Committee concluded that additional toxicological data are necessary. Also, data are needed for an assessment of oral exposure to (–)-carvone from all sources to complete the re-evaluation.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within 3 years.

An addendum to the monograph was prepared.

References

1. FAO/WHO. Principles and methods for the risk assessment of chemicals in food. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. Geneva: World Health Organization, International Programme on Chemical Safety; 2009 (Environmental Health Criteria 240).

2. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
3. 2015 IOFI Global Poundage Survey. Brussels: International Organization of the Flavor Industry; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Engel W. Detection of a "nonaromatic" NIH shift during in vivo metabolism of the monoterpene carvone in humans. *J Agric Food Chem*. 2002;50(6):1686–94.
6. Jäger W, Mayer M, Platzer P, Reznicek G, Dietrich H, Buchbauer G. Stereoselective metabolism of the monoterpene carvone by rat and human liver microsomes. *J Pharm Pharmacol*. 2000;52(2):191–7.
7. EFSA. Scientific Opinion on the safety assessment of carvone, considering all sources of exposure. *EFSA J*. 2014;12(7):3806 [74 pp]. doi:10.2903/j.efsa.2014.3806.
8. National Toxicology Program. Toxicology and carcinogenesis studies of *d*-carvone (CAS No. 2244-16-8) in B6C3F1 mice and toxicology studies in F344/N rats (gavage studies). (NTP/TR No. 381, draft; NIH No. 90-2836). Washington, DC, USA: US Department of Health and Human Services; 1989. Draft report submitted to WHO by Flavor and Extract Manufacturer's Association of the United States, Washington, DC, USA [cited in [Annex 1](#), reference 94].
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol*. 1978;16(3):255–76.

4.1.3 Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

Introduction

The Committee re-evaluated 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (Nos 1491–1526 and 2103–2105). At three previous meetings, the Committee noted that there were positive in vitro genotoxicity data for several members of this group and that there was a paucity of in vivo genotoxicity data to allay concerns ([Annex 1](#), references 178, 190 and 211). Based on these considerations, the Committee concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group.

The concerns with this group arose primarily from the carcinogenicity of furan itself, which is believed to involve a reactive genotoxic metabolite formed by epoxidation and opening of the furan ring. Furan is not a member of this group of flavouring agents, but all the members of the group contain a furan ring with substituents of varying complexity. Four members of this group, namely 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), 2-ethylfuran (No. 1489) and 2-butylfuran (No. 1490), have short-chain alkyl substituents on the

furan ring; although considered at previous meetings, these four members are no longer supported by industry and were not considered in this re-evaluation.

At the present meeting, the Committee considered additional studies of *in vitro* genotoxicity (for seven flavouring agents: Nos 1495, 1497, 1503, 1504, 1511, 1514 and 1520) and *in vivo* genotoxicity (for four flavouring agents: Nos 1491, 1497, 1503 and 1511). Additional short-term studies of toxicity for two flavouring agents in this group (Nos 1491 and 1500) were also available.

Twenty of these 39 flavouring agents (Nos 1491–1494, 1497, 1499, 1503–1505, 1508–1513, 1520–1522, 2104 and 2105) have been reported to occur as natural components in foods including cheese, chicken, cocoa, coffee, honey, rye bread, spirituous beverages, tomatoes, wheaten bread, wine and other foods [1].

The re-evaluations of the 39 flavouring agents in this group were conducted using the Procedure for the Safety Evaluation of Flavouring Agents. The first step in the Procedure is consideration of genotoxicity ([Annex 1](#), reference 230).

Assessment of dietary exposure

The total annual volumes of production of the 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers is 585 kg in Europe, 798 kg in the USA, 246 kg in Japan and 490 kg in Latin America [2, 3]. More than 83%, 70%, 93% and 85% of the annual production volume in Europe, the USA, Japan and Latin America, respectively, is accounted for by 2-furyl methyl ketone (No. 1503).

Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in [Table 14](#). Estimated daily dietary exposures range from 0.3 to 1200 µg/person (SPET values) and from 0.01 to 61 µg/person (MSDI method values). The estimated daily dietary exposure is highest for 2,5-dimethyl-3-oxo-(2*H*)-furan-4-yl butyrate (No. 1519) at 1200 µg/person, the SPET value for non-alcoholic beverages. The SPET yielded the highest estimated daily dietary exposure in each case with the exception of phenethyl 2-furoate (No. 1517; MSDI = 2 µg/person).

Absorption, distribution, metabolism and excretion

Detailed information on the ADME of this group of flavouring agents was described in the monograph of the sixty-ninth meeting of the Committee ([Annex 1](#), reference 191). At that meeting, the Committee noted that the biotransformation processes applicable to members of this group of furan-substituted flavouring agents are, in large part, dependent on the presence or absence of specific functional groups attached to the furan ring. The Committee also noted that at higher dose levels, low relative molecular mass alkyl furans (e.g. 2-methylfuran)

can undergo ring oxidation to yield reactive 2-ene-1,4-dicarbonyl intermediates that can react with protein and DNA. For example, furan has limited metabolic options and is biotransformed via ring oxidation to an enedialdehyde species that is a potent hepatotoxin. The Committee further noted that the presence of an extended side-chain attached to the furan ring would reduce the potential for epoxidation of the double bond and provide a site for detoxication via metabolism and elimination.

No new data on the ADME of specific members of this group of flavouring agents were available for the present meeting.

Consideration of genotoxicity data

Additional studies of genotoxicity on furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers were evaluated for the present meeting. A total of 16 in vitro genotoxicity studies were available for seven flavouring agents (Nos 1495, 1497, 1503, 1504, 1511, 1514 and 1520), and a total of eight in vivo genotoxicity studies were available for four flavouring agents (Nos 1491, 1497, 1503 and 1511).

A positive result was observed for 2-furyl methyl ketone (No. 1503) in an in vitro SCE assay. However, an in vitro chromosomal aberration assay was negative and in vivo studies of DNA damage (comet assays) and micronucleus induction were also negative. All other in vitro and in vivo genotoxicity assays considered at the present meeting were negative.

The Committee concluded that the newly available in vitro and in vivo genotoxicity data evaluated at the present meeting allay the previous concerns of the Committee. Those concerns arose primarily from the carcinogenicity of furan itself and from some positive genotoxicity findings for four flavouring agents with short-chain alkyl substituents on the furan ring. Those four flavouring agents, namely 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), 2-ethylfuran (No. 1489) and 2-butylfuran (No. 1490), are no longer supported by industry, and were not considered in this re-evaluation.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Genotoxicity data on members of this group of 39 flavouring agents do not raise concerns for genotoxicity.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned all 39 flavouring agents to structural class III [4].

Step 3. Dietary exposures were estimated using both the MSDI method and the SPET.

Step 4. The highest estimated dietary exposures for 14 flavouring agents (Nos 1492, 1500, 1502, 1512, 1517, 1518, 1522–1526, 2103–2105) are below the class III threshold of toxicological concern (i.e. 90 µg/day). The Committee

therefore concluded that these 14 flavouring agents would not pose a safety concern at current estimated dietary exposures.

The highest estimated dietary exposures for 25 flavouring agents (Nos 1491, 1493–1499, 1501, 1503–1511, 1513–1516, 1519–1521) are above the class III threshold of toxicological concern. Therefore, these 25 flavouring agents proceeded to Step 5 of the Procedure.

Step 5. For 2-pentylfuran (No. 1491) the NOAEL of 30 mg/kg bw per day from a 90-day study in rats [5] provides an adequate MOE of 10 000 in relation to the estimated dietary exposure to No. 1491 when used as a flavouring agent. This NOAEL is also appropriate for assessment of the structurally related flavouring agents 2-decylfuran (No. 1493) and 2,4-difurfurylfuran (No. 1496). This NOAEL provides adequate MOEs of 6000 and 4500 in relation to the estimated dietary exposure to No. 1493 and No. 1496, respectively, when used as flavouring agents.

For 3-(2-furyl)acrolein (No. 1497), the NOAEL of 45 mg/kg bw per day from a 90-day study in rats [6] provides an adequate MOE of 5400 in relation to the estimated dietary exposure to No. 1497 when used as a flavouring agent. This NOAEL is also appropriate for assessment of the structurally related flavouring agents 3-methyl-2-(3-methylbut-2-enyl)-furan (No. 1494), 2-methyl-3(2-furyl)acrolein (No. 1498), 3-(5-methyl-2-furyl)prop-2-enal (No. 1499) and 2-furfurylidene-butyraldehyde (No. 1501) and provides adequate MOEs of 9000, 10 800, 2700 and 10 800 in relation to the estimated dietary exposures to Nos 1494, 1498, 1499 and 1501, respectively, when used as flavouring agents.

For 2,3-dimethylbenzofuran (No. 1495), the NOAEL of 0.6 mg/kg bw per day from a 90-day study in rats [7] provides an adequate MOE of 360 in relation to the estimated dietary exposure to No. 1495 when used as a flavouring agent.

For 3-acetyl-2,5-dimethylfuran (No. 1506), the NOAEL of 10 mg/kg bw per day from a 14-day study in rats [8] provides an adequate MOE of 3000 in relation to the estimated dietary exposure to No. 1506 when used as a flavouring agent. The NOAEL of 10 mg/kg bw per day for No. 1506 is also appropriate for assessment of the structurally related flavouring agents 2-acetyl-5-methylfuran (No. 1504) and 2-acetyl-3,5-dimethylfuran (No. 1505) and provides adequate MOEs of 6000 and 600 in relation to the estimated dietary exposures to 1504 and 1505, respectively, when used as flavouring agents.

For 2-furyl methyl ketone (No. 1503), the NOAEL of 25 mg/kg bw per day obtained from a 90-day study in rats [6] provides an adequate MOE of 4300 in relation to the estimated dietary exposure to No. 1503 when used as a flavouring agent. The NOAEL of 25 mg/kg bw per day for No. 1503 is also appropriate for assessment of the structurally related flavouring agents 2-butyrylfuran (No. 1507), (2-furyl)-2-propanone (No. 1508), 2-pentanoylfuran (No. 1509), furfuryl methyl ether (No. 1520) and ethyl furfuryl ether (No. 1521) and provides adequate MOEs of 2400, 10 000, 1500, 7500 and 12 000, respectively, in relation to the estimated

dietary exposures to Nos 1507, 1508, 1509, 1520 and 1521, respectively, when used as flavouring agents.

For 4-(2-furyl)-3-buten-2-one (No. 1511), the NOAEL of 30 mg/kg bw per day from a 14-day study in rats [9] provides an adequate MOE of 2600 in relation to the estimated dietary exposure to No. 1511 when used as a flavouring agent. The NOAEL of 30 mg/kg bw per day for No. 1511 is also appropriate for assessment of the structurally related flavouring agents 1-(2-furyl)butan-3-one (No. 1510), ethyl 3-(2-furyl)propanoate (No. 1513), isobutyl 3-(2-furan)propionate (No. 1514), isoamyl 3-(2-furan)propionate (No. 1515), isoamyl 3-(2-furan)butyrate (No. 1516) provides adequate MOEs of 4500, 3900, 4500, 4500 and 12 000 in relation to the estimated dietary exposures to Nos 1510, 1513, 1514, 1515 and 1516, respectively, when used as flavouring agents.

For 2,5-dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate (No. 1519), the NOAEL of 200 mg/kg bw per day for the structurally related substance 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (No. 1446) obtained from a 2-year study in rats [10] provides an adequate MOE of 10 000 in relation to the estimated dietary exposure to No. 1519 when used as a flavouring agent.

Based on the adequate margins of exposure for each of the 25 flavouring agents considered at Step 5 of the Procedure, the Committee concluded that these 25 flavouring agents (Nos 1491, 1493–1499, 1501, 1503–1511, 1513–1516, 1519–1521) would not pose a safety concern at current estimated dietary exposures.

Consideration of combined intakes from use as flavouring agents

Twenty-five of the flavouring agents in this group have MSDI values of less than 0.1 µg/day, and the four highest MSDI values are 3, 6, 13 and 61 µg/day. The Committee considered that combined intakes of members of this group of flavouring agents do not raise safety concerns.

Consideration of secondary components

Two flavouring agents in this group (Nos 1519 and 1524) have a minimum assay value of less than 95% (see [Annex 3](#)). For No. 1519, the major secondary components are 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (present at 1–3%) and butyric acid (present at 1–3%). The SPET value for No. 1519 is 1200 µg/day and 3% of this value is 36 µg/day, which is below the class III threshold of toxicological concern (90 µg/day). Butyric acid (JECFA flavour No. 87) was evaluated at the forty-ninth meeting, and it was concluded that there were no safety concerns from its use as a flavouring agent ([Annex 1](#), reference 131). The major secondary components of No. 1519 are therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1519 as a flavouring agent.

For No. 1524, the major secondary component is di-(2-methyl-3-furyl) disulfide (present at 6–7%). The SPET value for No. 1524 is 10 µg/day and 7%

of this value is 0.7 µg/day, which is below the class III threshold of toxicological concern (90 µg/day). The major secondary component of No. 1524 is therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1524 as a flavouring agent.

Conclusions

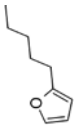
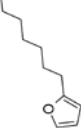
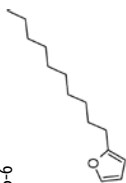
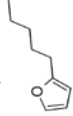
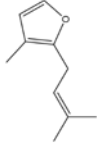
The Committee concluded that the 39 previously evaluated flavouring agents in this group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers that were re-evaluated at the present meeting (Nos 1491–1529 and 2103–2105) do not give rise to safety concerns. The Committee concluded that the additional genotoxicity data on members of this group allay the concerns that were raised at previous meetings.

An addendum to the monograph was prepared.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 10 February 2018).
2. 2015 IOFI Global Poundage Survey. Brussels: International Organization of the Flavor Industry; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
5. Bauter M. 2-Pentylfuran: A 90-day oral gavage study in rats. Product Safety Labs, Dayton NJ. Study no. 42348. Unpublished report to the International Organization of the Flavor Industry, 2017. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Lough R. A combined 28-day and 90-day toxicity study of four test articles [2-furyl methyl ketone, benzophenone, 3-(2-furyl)acrolein and isobutyl 3-(2-furyl) propionate] administered orally (in the diet) to the albino rat. Study no. 81238. Unpublished report from Bio-Research Laboratories, Ltd, Montréal, Quebec, Canada; 1985. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Long DW. TT 175: Acute oral toxicity and 3-month oral toxicity study in the rat. Study no. IFREB-R 770261. Unpublished report from Institut Français de Recherches et Essais Biologiques (IFREB), Les Oncins, France; 1977. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
8. Van Miller JP, Weaver EV. Fourteen-day dietary minimum toxicity screen (MTS) of 2-methyl-1-butanol blend, methyl-*o*-methoxy benzoate, 4,5,6,7-tetrahydro-3,6-dimethylbenzofuran, 3-actyl-2,5-dimethylfuran and furfuryl methyl ether in albino rats. Study no. 50-526. Unpublished report from

Table 14
Summary of the results of the safety evaluations of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers used as flavouring agents^{a,b,c,d}

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? ^e	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Structural class III						
2-Pentylfuran	1491	3777-69-3 	Yes, SPET: 180	Yes. The NOAEL of 30 mg/kg bw per day in a 90-day study in rats [5] is 10 000 times greater than the estimated dietary exposure to No. 1491 when used as a flavouring agent.	NR	No safety concern
2-Heptylfuran	1492	3777-71-7 	No, SPET: 6	NR	NR	No safety concern
2-Decylfuran	1493	83469-85-6 	Yes, SPET: 300	Yes. The NOAEL of 30 mg/kg bw per day in a 90-day study in rats [5] for the related substance 2-pentylfuran (No. 1491) is 6 000 times greater than the estimated dietary exposure to No. 1493 when used as a flavouring agent.	2-Pentylfuran (No. 1491) 	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	15186-51-3 	Yes, SPET: 300	Yes. The NOAEL of 45 mg/kg bw per day for the related substance 3-(2-furyl) acrolein (No. 1497) [6] is 9 000 times greater than the dietary exposure to No. 1494 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
2,3-Dimethylbenzofuran	1495	3782-00-1 	Yes, SPET: 100	Yes. The NOAEL of 0.6 mg/kg bw per day [7] is 360 times greater than the estimated dietary exposure to No. 1495 when used as a flavouring agent.	NR	No safety concern

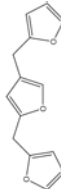
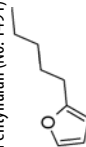
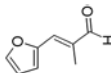
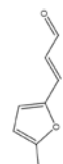
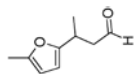
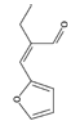
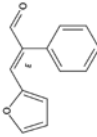
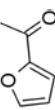
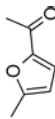
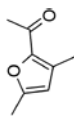
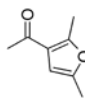
Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*				Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?		Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure		
Flavouring agent	No.	CAS no. and structure	Yes, SPET: 400	Yes, SPET: 500	Yes, SPET: 250	Yes, SPET: 1 000			
2,4-Difurfurylfuran	1496	64280-32-6 	Yes, SPET: 400				Yes. The NOAEL of 30 mg/kg bw per day [5] for the related substance 2-pentylfuran (No. 1491) is 4 500 times greater than the estimated dietary exposure to No. 1496 when used as a flavouring agent.	2-Pentylfuran (No. 1491) 	No safety concern
3-(2-Furyl)acrolein	1497	623-30-3 	Yes, SPET: 500				Yes. The NOAEL of 45 mg/kg bw per day [6] is 5 400 times greater than the dietary exposure to No. 1497 when used as a flavouring agent.	NR	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	874-66-8 	Yes, SPET: 250				Yes. The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 10 800 times greater than the dietary exposure to No. 1498 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	5555-90-8 	Yes, SPET: 1 000				Yes. The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 2 700 times greater than the estimated dietary exposure to No. 1499 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	31704-80-0 	No, SPET: 50				NR	NR	No safety concern
2-Furfurylidene-butyraldehyde	1501	770-27-4 	Yes, SPET: 250				Yes. The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 10 800 times greater than the estimated dietary exposure to No. 1501 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
2-Phenyl-3-(2-furyl)prop-2-enal	1502	65545-81-5 	No, SPET: 40	NR	NR	No safety concern
2-Furyl methyl ketone	1503	1192-62-7 	Yes, SPET: 350	Yes, The NOAEL of 25 mg/kg bw per day [6] is 4 300 times greater than the estimated dietary exposure to No. 1503 when used as a flavouring agent.	NR	No safety concern
2-Acetyl-5-methylfuran	1504	1193-79-9 	Yes, SPET: 100	Yes, The NOAEL of 10 mg/kg bw per day [8] for the related substance 3-acetyl-2,5-dimethylfuran (No. 1506) is 6 000 times greater than the estimated dietary exposure to No. 1504 when used as a flavouring agent.	3-Acetyl-2,5-dimethylfuran (No. 1506) 	No safety concern
2-Acetyl-3,5-dimethylfuran	1505	22940-86-9 	Yes, SPET: 1 000	Yes, The NOAEL of 10 mg/kg bw per day [8] for the related substance 3-acetyl-2,5-dimethylfuran (No. 1506) is 600 times greater than the estimated dietary exposure to No. 1505 when used as a flavouring agent.	3-Acetyl-2,5-dimethylfuran (No. 1506) 	No safety concern
3-Acetyl-2,5-dimethylfuran	1506	10599-70-9 	Yes, SPET: 200	Yes, The NOAEL of 10 mg/kg bw per day [8] is 3 000 times greater than the estimated dietary exposure to No. 1506 when used as a flavouring agent.	NR	No safety concern

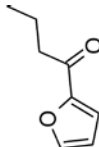
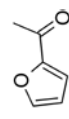
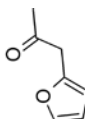
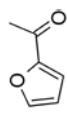
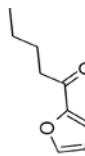
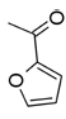
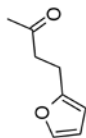
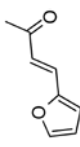
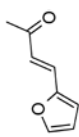
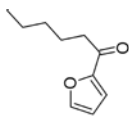
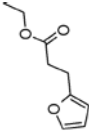
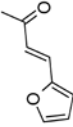
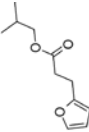
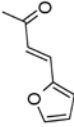
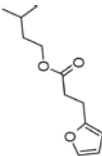
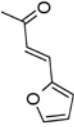
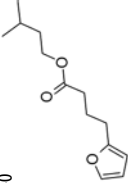
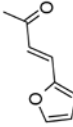
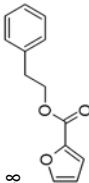
Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*				Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Flavouring agent	No.	CAS no. and structure				
2-Butyrylfuran	1507	100113-53-9 	Yes, SPET: 625	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 2 400 times greater than the estimated dietary exposure to No. 1507 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
(2-Furyl)-2-propanone	1508	6975-60-6 	Yes, SPET: 150	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 10 000 times greater than the estimated dietary exposure to No. 1508 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
2-Pentanoylfuran	1509	3194-17-0 	Yes, SPET: 1 000	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 1 500 times greater than the estimated dietary exposure to No. 1509 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
1-(2-Furyl)butan-3-one	1510	699-17-2 	Yes, SPET: 400	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1510 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	623-15-4 	Yes, SPET: 699	Yes. The NOAEL of 30 mg/kg bw per day [9] is 2 600 times greater than the estimated dietary exposure to No. 1511 when used as a flavouring agent.	NR	No safety concern
Pentyl 2-furyl ketone	1512	14360-50-0 	No, SPET: 15	NR	NR	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Ethyl 3-(2-furyl)propanoate	1513	10031-90-0 	Yes, SPET: 463	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 3 900 times greater than the estimated dietary exposure to No. 1513 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Isobutyl 3-(2-furan)propanoate	1514	105-01-1 	Yes, SPET: 400	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1514 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Isoamyl 3-(2-furan)propanoate	1515	7779-67-1 	Yes, SPET: 400	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1515 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Isoamyl 3-(2-furan)butyrate	1516	7779-66-0 	Yes, SPET: 150	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 12 000 times greater than the estimated dietary exposure to No. 1516 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Phenethyl 2-furoate	1517	7149-32-8 	No, MSDI: 2	NR	NR	No safety concern

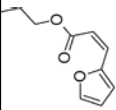
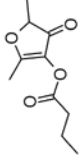
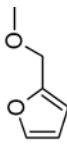
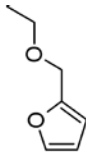
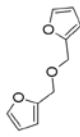
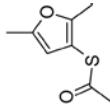
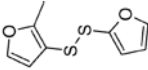
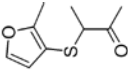
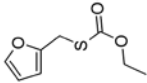
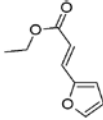
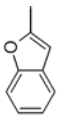
Step 4		Step 5		Conclusion based on current estimated dietary exposure
Flavouring agent	No.	Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?	Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	
Propyl 2-furanacrylate	1518	623-22-3 	NR	No safety concern
2,5-Dimethyl-3-oxo-(2H)-furan-4-yl butyrate	1519	114099-96-6 	Yes. The NOAEL of 200 mg/kg bw per day [10] for the related substance 2,5-dimethyl-4-hydroxyl-3(2H)-furanone (No. 1446) is 10 000 times greater than the estimated dietary exposure to No. 1519 when used as a flavouring agent.	No safety concern
Furfuryl methyl ether	1520	13679-46-4 	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 7 500 times greater than the estimated dietary exposure to No. 1520 when used as a flavouring agent.	No safety concern
Ethyl furfuryl ether	1521	6270-56-0 	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 12 000 times greater than the estimated dietary exposure to No. 1521 when used as a flavouring agent.	No safety concern
Difurfuryl ether	1522	4437-22-3 	NR	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523	55764-22-2 	NR	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? ^a	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Furfuryl(2-methyl-3-furyl) disulfide	1524	109537-55-5 	No, SPET: 10	NR	NR	No safety concern
3-[(2-Methyl-3-furyl)thio]-2- butanone	1525	61295-44-1 	No, SPET: 0.3	NR	NR	No safety concern
O-Ethyl S-(2-furylmethyl) thiocarbonate	1526	376595-42-5 	No, SPET: 9	NR	NR	No safety concern
(E)-Ethyl 3-(2-furyl)acrylate	2103	53282-12-5 	No, SPET: 20	NR	NR	No safety concern

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? ^a	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Di-2-furylmethane	2104	1197-40-6 	No, SPET: 40	NR	NR	No safety concern
2-Methylbenzofuran	2105	4265-25-2 	No, SPET: 3	NR	NR	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; no.: number; NOAEL: no-observed-adverse-effect level; NR: not required; SPET: single-portion exposure technique

^a The Committee re-evaluated 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides and ethers. All 39 flavouring agents have been considered previously by the Committee.

^b Step 1: Genotoxicity data on the flavouring agents in this group do not raise concerns for genotoxicity.

^c Step 2: All 39 flavouring agents belong to structural class III.

^d Step 3: Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported. Dietary exposure values are expressed in µg/day.

^e Step 4: The threshold for human dietary exposure for structural class III is 90 µg/day.

Bushy Run Research Center, Export, Pennsylvania, USA; 1987. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

9. Gill MW, Van Miller JP. Fourteen-day dietary minimum toxicity screen (MTS) in albino rats. Study no. 50-528. Unpublished report from Bushy Run Research Center, Export, Pennsylvania, USA to the Flavor and Extract Manufacturers Association, Washington, DC, USA; 1987. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
10. Kelly CM, Bolte HF. ST 07 C99: A 24-month dietary carcinogenicity study in rats. Unpublished report no. 99-2644. Huntingdon Life Sciences, East Millstone, NJ, USA to the Flavor and Extract Manufacturers Association, Washington, DC, USA; 2003. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

4.1.4 Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters

Introduction

The Committee evaluated two additional flavouring agents belonging to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters. One of the flavouring agents was a linear unsaturated aldehyde, *trans*-6-octenal (No. 2240), and the other was a branched unsaturated alcohol, 2,6-dimethyl-5-heptenol (No. 2241). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee evaluated 42 other members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), reference 137). For 41 of the 42 substances in this group, the Committee concluded that there were no safety concerns at estimated dietary exposures. The evaluation of the remaining substance, ethyl 2-methyl-3,4-pentadienoate (No. 353), was completed at the sixty-eighth meeting ([Annex 1](#), reference 187). The Committee evaluated 20 other members of this group of flavouring agents at its sixty-first meeting ([Annex 1](#), reference 166). The Committee concluded that there were no safety concerns at estimated dietary exposures with respect to all of these 20 flavouring agents. The Committee evaluated nine other members of this group of flavouring agents at its seventy-sixth meeting ([Annex 1](#), reference 211). The Committee concluded that all nine flavouring agents were of no safety concern at estimated dietary exposures.

Both of the additional flavouring agents (Nos 2240 and 2241) in this group have been reported to occur naturally and can be found in ginger [1].

A comprehensive literature search was conducted in PubMed; no additional relevant studies were identified.

Assessment of dietary exposure

The total annual volume of production of the two additional flavouring agents is 0.2 kg [2, 3]. The volume of the annual production in the USA is completely accounted for by *trans*-6-octenal (No. 2240). In Japan, the production volume is completely accounted for by 2,6-dimethyl-5-heptenol (No. 2241).

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 15. The higher estimated daily dietary exposure is for 2,6-dimethyl-5-heptenol (No. 2241), at 300 µg/day, the SPET value obtained from nonalcoholic soft beverages. For *trans*-6-octenal (No. 2240), the estimates of daily dietary exposures via the MSDI method and SPET are 0.03 and 40 µg/day, respectively.

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters has been described in the monographs of the fifty-first, sixty-first, sixty-eighth, and seventy-sixth meetings (Annex 1, references 138, 167, 188, 212).

The aliphatic esters are expected to hydrolyse to their analogous unsaturated aliphatic alcohol and carboxylic acids during passage through the gastrointestinal tract [4, 5, 6]. The resulting linear and branched-chain unsaturated primary alcohols are expected to be absorbed and further oxidized to their analogous aldehydes and acids, and rapidly absorbed [7, 8]. The absorbed aldehydes are oxidized to their analogous unsaturated carboxylic acids. These unsaturated carboxylic acids undergo further enzymatic conversion prior to entry into the β -oxidation pathway, where they are fully metabolized to carbon dioxide and water via the citric acid cycle.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no adequate chemical-specific genotoxicity studies on the additional flavouring agents. However, there are no structural alerts for these compounds. Data from related flavouring agents indicate that these do not show genotoxic potential based on uniformly negative genotoxicity data.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents to *trans*-6-octenal (No. 2240) and 2,6-dimethyl-5-heptenol (No. 2241), the Committee assigned both flavouring agents to structural class I [9].

Steps 3 and 4. Dietary exposures using both the MSDI method and SPET have been determined. The highest estimated dietary exposures of both flavouring agents in structural class I were below the threshold of concern (i.e. 1800 µg/person per day for class I). The Committee therefore concluded that both

flavouring agents (Nos 2240–2241) would not pose a safety concern at current estimated dietary exposures.

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters have low MSDIs (0.01–0.03 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of secondary components

One flavouring agent in this group, 2,6-dimethyl-5-heptenol (No. 2241), has a minimum assay value of less than 95% (see [Annex 3](#)). The major secondary component, 2,6-dimethyl-5-heptenal (No. 349), present at 1–6%, does not present a safety concern at estimated dietary exposures from the use of No. 2241 as a flavouring agent.

Consideration of additional data on previously evaluated flavouring agents

In the previous evaluation of substances in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters, studies of biochemistry, acute toxicity, short-term and long-term toxicity and genotoxicity were available ([Annex 1](#), references 137, 166, 187 and 211). None of the 71 flavouring agents in this group raised safety concerns.

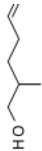
No adequate studies on the additional flavouring agents were available. For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 336, 1269, 1286 and 1640), studies of short-term toxicity (Nos 330, 332 and 333) and studies of genotoxicity (Nos 315, 329, 330, 333, 334, 346, 349, 1272, 1286 and 1637) were available. The studies available for the present evaluation support the conclusions drawn by previous safety evaluations.

Conclusions

The Committee concluded that the two flavouring agents *trans*-6-octenal (No. 2240) and 2,6-dimethyl-5-heptenol (No. 2241), which are additions to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters evaluated previously, do not give rise to any safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

Table 15
Summary of the results of the safety evaluations of two additional flavouring agents in the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of concern?	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
Structural class I					
<i>trans</i> -6-Octenal	2240	63196-63-4 	No, SPET: 40	NR	No safety concern
2,6-Dimethyl-5-heptenol	2241	4234-93-9 	No, SPET: 300	NR	No safety concern

CAS: Chemical Abstracts Service; NOAEL: no-observed-adverse-effect level; NR: not relevant; SPET: single-portion exposure technique

^a Seventy-one flavouring agents in this group were previously evaluated by the Committee (Annex 1, References 137, 166, 187 and 211).

^b Step 2: Two flavouring agents (Nos 2240–2241) are in structural class I.

^c The threshold for human dietary exposure for structural class I is 1800 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure, which was calculated using the SPET.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4: [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
2. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Gangolli SD, Shilling WH. Hydrolysis of esters by artificial gastric and pancreatic juices. Study no. 11/1968. Unpublished report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA; 1968. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Junge W, Heymann E. Characterization of the isoenzymes of pig-liver esterase: 2. Kinetic studies. *Eur J Biochem.* 1979;95(3):519–25.
6. Heymann E. Carboxylesterases and amidases. In: W. Jakoby, editor. *Enzymatic basis of detoxication*. New York: Academic Press; 1980.
7. Dawson AM, Holdsworth CD, Webb J. Absorption of short chain fatty acids in man. *Proc Soc Exp Biol Med.* 1964;117:97–100.
8. Gaillard D, Derache R. [Metabolism of different alcohols present in alcoholic beverages, in the rat]. *Trav Soc Pharm Montp.* 1965;25:51–62.
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

4.1.5 Maltol and related substances

Introduction

The Committee evaluated two flavouring agents belonging to the group of maltol and related substances: one additional flavouring agent, ethyl maltol isobutyrate (No. 2252), and one flavouring agent, maltol (No. 1480) that was being re-evaluated. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230). Ethyl maltol isobutyrate (No. 2252) has not been previously evaluated by the Committee. Maltol (No. 1480) was re-evaluated because new in vitro and in vivo genotoxicity data had become available.

The Committee previously evaluated seven members of this group of flavouring agents at its sixty-fifth meeting ([Annex 1](#), reference 178). The Committee concluded that all seven flavouring agents were of no safety concern at estimated dietary exposures and maintained the previously established ADIs of 0–1 mg/kg bw for maltol (No. 1480) and 0–2 mg/kg bw for ethyl maltol (No. 2252).

Maltol (No. 1480) was evaluated at the eleventh meeting ([Annex 1](#), reference 14), when a temporary ADI of 0–1 mg/kg bw was established because no long-term studies of toxicity were available. At the eighteenth meeting ([Annex 1](#), reference 35), the Committee withdrew the temporary ADI because the results of long-term studies of toxicity requested at the previous meeting had not been made available. At the twenty-second meeting ([Annex 1](#), reference 47), the Committee evaluated new data on toxicity and established a temporary ADI of 0–0.5 mg/kg bw. At its twenty-fifth meeting, the Committee evaluated additional data and established an ADI of 0–1 mg/kg bw for maltol on the basis of a NOEL of 100 mg/kg bw in rats ([Annex 1](#), reference 56).

Ethyl maltol (No. 1481) was evaluated at the fourteenth meeting ([Annex 1](#), reference 22), when the Committee established an ADI of 0–2 mg/kg bw. At its eighteenth meeting ([Annex 1](#), reference 35), the Committee re-evaluated ethyl maltol and re-affirmed the ADI of 0–2 mg/kg bw.

Maltol (No. 1480) has been reported to occur naturally in a wide variety of foods such as wheat and rye breads, milk, butter, uncured pork, beer, cocoa, coffee, peanuts, soy proteins, beans and clams [1]. During baking (for example, of bread or beans) and roasting (for example, of cocoa, coffee, peanuts), simple sugars are partly converted to maltol [1, 2].

A comprehensive literature search was performed in Scopus; two additional relevant studies were identified.

Assessment of dietary exposure

The total annual volume of production of the additional flavouring agent (ethyl maltol isobutyrate, No. 2252) is 29 kg in Japan [3, 4]. No production volume was reported for Europe, Latin America or the USA.

The total annual volume of production for the flavouring agent presented for re-evaluation, maltol (No. 1480), is approximately 83 200 kg in Europe, 87 600 kg in the USA, 12 500 kg in Japan and 47 600 kg in Latin America [3, 4].

Dietary exposure was estimated using both the SPET and the MSDI method, with the highest values reported in [Table 16](#). The estimated daily dietary exposure is highest for maltol (10 440 µg/day, SPET value for nonalcoholic “soft” beverages). The MSDI values for the four regions range from 2625 to 9091 µg/day. For ethyl maltol isobutyrate (No. 2252), the estimated daily dietary exposures were 8 µg/day (MSDI value) and 400 µg/day (SPET value).

Absorption, distribution, metabolism and excretion

Information on the ADME of flavouring agents belonging to the group of maltol and related substances is described in the monograph of the sixty-fifth meeting ([Annex 1](#), reference 179).

Chemically, maltol is classified as a γ -pyrone. It is a hydroxyl-substituted 4H-pyran-4-one and is anticipated to be metabolized like phenol, which primarily undergoes phase II conjugation of the free hydroxyl substituent. Maltol and ethyl maltol are predominantly metabolized to sulfate and glucuronic acid conjugates, which are then eliminated in the urine [5]. Ethyl maltol isobutyrate (No. 2252) is predicted to be hydrolysed to ethyl maltol and the corresponding simple aliphatic carboxylic acid (isobutyric acid) [6].

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for maltol (No. 1480) or ethyl maltol isobutyrate (No. 2252). Chemical-specific genotoxicity data available for maltol (No. 1480) do not indicate that this flavouring agent has genotoxic potential.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned one flavouring agent (No. 1480) to structural class II and one flavouring agent (No. 2252) to structural class III [7].

Step 3. The highest dietary exposures were estimated using the SPET for both flavouring agents.

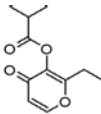
Step 4. The highest estimated dietary exposure for maltol (No. 1480) is above the threshold of toxicological concern (i.e. 540 $\mu\text{g/day}$ for class II). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

The highest estimated dietary exposure for ethyl maltol isobutyrate (No. 2252) is above the threshold of toxicological concern (i.e. 90 $\mu\text{g/day}$ for class III). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

Step 5. For maltol, the NOAEL of 125 mg/kg bw per day from a 90-day study in dogs [8] provides an MOE of 720 in relation to the estimated daily dietary exposure to No. 1480 (SPET = 10 440 $\mu\text{g/day}$ or 174 $\mu\text{g/kg bw per day}$) when used as a flavouring agent. The Committee therefore concluded that maltol (No. 1480) would not pose a safety concern at current estimated dietary exposures.

At its fourteenth meeting ([Annex 1](#), reference 22), the Committee established an ADI of 0–2 mg/kg bw for the structurally related substance ethyl maltol (No. 1481) on the basis of a NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats [8]. This ADI was maintained at the sixty-fifth meeting ([Annex 1](#), reference 178). The NOEL of 200 mg/kg bw per day provides an adequate MOE of 28 570 in relation to estimated daily dietary exposure to No. 2252 (SPET = 400 $\mu\text{g/day}$ or 7 $\mu\text{g/kg bw per day}$) when used as a flavouring agent. The Committee concluded that ethyl maltol isobutyrate (No. 2252) would not pose a safety concern at current estimated dietary exposures.

Table 16
Summary of the results of the safety evaluations of maltol and related substances used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4: Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 ^d Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Related structure name (No.) and structure	Conclusion based on current estimated dietary exposure
Structural class I						
Maltol ^e	1480	118-71-8 	Yes, SPET: 10 440	Yes. The NOAEL of 125 mg/kg bw per day from a 90-day study in dogs [8] is 720 times the estimated dietary exposure to No. 1480 when used as a flavouring agent.	Ethyl maltol ^f (No. 1481) 	No safety concern
Structural class III						
Ethyl maltol isobutyrate	2252	852997-28-5 	Yes, SPET: 400	Yes. The NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats for the structurally related substance ethyl maltol (No. 1481) [8] is 28 570 times the estimated dietary exposure to No. 2252 when used as a flavouring agent.	Ethyl maltol ^f (No. 1481) 	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; no.: number; NOAEL: no-observed-adverse-effect level; NOEL: no-observed-effect level; NR: not relevant; SPET: single-portion exposure technique

^a Seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 179).

^b Step 2: One flavouring agent is in structural class II and one flavouring agent is in structural class III.

^c The threshold of toxicological concern for human dietary exposure for structural class II is 540 µg/day and for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest estimated daily dietary exposures calculated using the SPET.

^d The margin of exposure was calculated based on the highest estimated daily dietary exposure calculated using the SPET.

^e The previously established ADI for maltol was withdrawn by the Committee.

^f The previously established ADI for ethyl maltol of 0–2 mg/kg bw was maintained.

Table 16 summarizes the evaluations of the two flavouring agents (Nos 1480 and 2252) belonging to this group of maltol and related substances.

Consideration of combined intakes from use as flavouring agents

The additional flavouring agent, ethyl maltol isobutyrate (No. 2252), in the group of maltol and related substances has a low MSDI (8 µg/day). The Committee concluded that consideration of combined intakes is not necessary because the additional flavouring agent would not contribute significantly to the combined intake of this flavouring group.

Consideration of secondary components

Ethyl maltol isobutyrate (No. 2252) has a minimum assay of less than 95% (see [Annex 3](#)). The major secondary component in No. 2252, present at 2–3%, is ethyl maltol (No. 1481). This flavouring agent had been previously evaluated by the Committee ([Annex 1](#), reference 178) and did not pose a safety concern at the estimated dietary exposure.

Consideration of additional data on previously evaluated flavouring agents

For two previously evaluated flavouring agents in this group, maltol (No. 1480) and maltyl isobutyrate (No. 1482), additional studies of acute toxicity (No. 1482) and genotoxicity (Nos 1480 and 1482) were available for the present evaluation. These additional data raised no safety concerns and support the previous safety evaluations.

However, the Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol at its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies as described in the monograph of that meeting ([Annex 1](#), reference 56). The Committee noted that a NOAEL of 125 mg/kg bw per day was identified in a published 90-day study in dogs [8]. This NOAEL was used to complete the re-evaluation of maltol as a flavouring agent.

Conclusions

In the previous evaluation of flavouring agents in this group of maltol and related substances, biochemical data and studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None of the seven previously evaluated flavouring agents raised safety concerns based on the estimated dietary exposures and the biochemical and toxicological data available ([Annex 1](#), reference 179).

The Committee concluded that the two flavouring agents (Nos. 2252 and 1480) under evaluation, one of which is an addition to the group of maltol

and related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures.

However, the Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting ([Annex 1](#), reference 57). The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the current ADI. The Committee therefore withdrew the ADI for maltol pending review of the appropriate data at a future meeting. The ADI for ethyl maltol was maintained.

An addendum to the monograph was prepared.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
2. Belitz H-D, Grosch W, Schieberle P. Food Chemistry, 4th ed. Berlin Heidelberg, Germany: Springer-Verlag; 2009.
3. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Rennhard HH. The metabolism of ethyl maltol and maltol in the dog. *J Agric Food Chem.* 1971;19(1):152–4.
6. Bennett C. Hydrolysis of maltol propionate via pancreatic secretion. Private communication to the International Organization of the Flavor Industry, Brussels; 1998. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
8. Gralla EJ, Stebbins RB, Coleman GL, Delahunt CS. Toxicity studies with ethyl maltol. *Toxicol Appl Pharmacol.* 1969;15(3):604–13.

4.1.6 Menthol and structurally related substances

Introduction

The Committee evaluated seven flavouring agents belonging to the group of menthol and structurally related substances, which was previously evaluated. The Committee re-evaluated menthol (No. 427) and evaluated six additional flavouring agents. These included four menthyl esters (menthyl formate [No. 2246], menthyl propionate [No. 2247], *l*-menthyl butyrate [No. 2248] and

dimenthyl glutarate [No. 2250]), *dl*-isomenthol (No. 2249) and one polyether alcohol, ($\pm$)-2-[(2-*p*-menthoxy)ethoxy]ethanol (No. 2251). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee previously evaluated menthol and 13 other members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), references 137) and 10 additional members of the group at its sixty-ninth meeting ([Annex 1](#), reference 190). The Committee concluded that all 24 flavouring agents were of no safety concern at estimated dietary exposures.

Menthol (No. 427) was first evaluated by the Committee at the eleventh meeting when an unconditional ADI of 0–0.2 mg/kg bw and a conditional⁴ ADI of 0.2–2 mg/kg bw was established ([Annex 1](#), reference 14). At the eighteenth meeting, an ADI of 0–0.2 mg/kg bw was established for menthol ([Annex 1](#), reference 35). This ADI was maintained at the twentieth meeting. At the twentieth meeting, the Committee requested additional long-term studies of toxicity and carcinogenicity in rats, information on the average and likely maximum dietary exposure to menthol, clinical observations of humans with higher than average dietary exposure to menthol and studies of metabolism ([Annex 1](#), reference 41). At the fifty-first meeting, an ADI of 0–4 mg/kg bw was established on the basis of the NOEL of 380 mg/kg bw per day in a long-term study in rats, applying a safety factor of 100 and rounding to one significant figure ([Annex 1](#), reference 137).

Menthol (No. 427), menthyl formate (No. 2246) and *dl*-isomenthol (No. 2249) have been reported to occur naturally in foods and can be mainly found in peppermint oil and other *Mentha* species oils. Menthol (10–70%) and menthone (No. 429; 7–40%) are the principal constituents of peppermint oil [2, [Annex 1](#), reference 137].

Menthol (No. 427) was re-evaluated because new data had become available since the previous evaluation. Menthol and the other flavourings were evaluated at the request of the Forty-eighth Session of the CCFA [3].

A comprehensive literature search was performed in Scopus; one additional reference was identified.

Assessment of dietary exposure

The total annual volume of production for menthol (No. 427) is 296 000 kg in Europe, 496 000 kg in the USA, 146 000 kg in Japan and 127 000 kg in Latin America [4, 5].

⁴ "Conditional ADI is a term no longer used by JECFA to signify a range above the 'unconditional ADI', which may signify an acceptable intake when special problems, different patterns of dietary intake, and special groups of the population that may require consideration are taken into account." [1]

The total annual volume of production of the six additional flavouring agents in the group of menthol and structurally related substances is 14 900 kg in Europe, 103 937 kg in the USA and 92 kg in Japan. No production volume was reported for Latin America [4, 5].

Of the seven flavouring agents under evaluation by this current Committee, menthol (No. 427) accounts for more than 95% of the total annual production volume in Europe and 82% in the USA. Dimethyl glutarate (No. 2250) accounts for almost all the remaining volume. Menthol (No. 427) accounts for more than 99% of the annual production volume in Japan and 100% in Latin America.

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 17. The highest estimated dietary exposure is for menthol (No. 427; 51 474 µg/day, MSDI value; the SPET value is 42 210 µg/day). For the other flavouring agents, the estimated daily dietary exposures range from 0.03 to 10 784 µg/day (MSDI values) and from 300 to 9000 µg/day (SPET values), with the SPET yielding the highest estimate in all but one case (dimethyl glutarate [No. 2250]).

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of menthol and structurally related substances has previously been described in the monographs of the eleventh, twentieth and fifty-first meetings (Annex 1, references 15, 42 and 138). Additional information was available for the current meeting.

The menthyl esters in this group (Nos 2246–2248 and 2250) can be expected to be readily hydrolysed to menthol and their respective carboxylic acids [6, 7]. Similar to menthol, *dl*-isomenthol (No. 2249) is expected to be conjugated with glucuronic acid and be eliminated in the urine or faeces [8, 9, 10]. The polyether alcohol conjugate of menthol, (±)-2-[(2-*p*-menthoxy)ethoxy] ethanol (No. 2251) is also expected to undergo conjugation with glucuronic acid and subsequent elimination in the urine and faeces.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for these flavouring agents. Chemical-specific genotoxicity data available for menthol (No. 427), *dl*-isomenthol (No. 2249) and (±)-2-[(2-*p*-menthoxy)ethoxy]ethanol (No. 2251) indicate that these flavouring agents are unlikely to be genotoxic based on the weight of evidence.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned six flavouring agents (Nos 427, 2246–2250) to structural class I and one flavouring agent (No. 2251) to structural class III [11].

Step 3. The highest dietary exposures were estimated using the SPET for five of the seven flavouring agents (Nos 2246–2249, 2251) and the MSDI method for menthol (No. 427) and dimethyl glutarate (Nos 2250).

Step 4. The highest estimated dietary exposures for three of the six flavouring agents in structural class I (Nos 2246–2248) are below the threshold of toxicological concern (i.e. 1800 µg/day for class I). The Committee therefore concluded that these flavouring agents would not pose a safety concern at current estimated dietary exposures.

The highest estimated dietary exposures for the remaining three flavouring agents in structural class I (Nos 427, 2249 and 2250) are above the threshold of toxicological concern. Therefore, the evaluation of these flavouring agents proceeded to Step 5.

The highest estimated dietary exposure for the one flavouring agent in structural class III (No. 2251) is above the threshold of toxicological concern (i.e. 90 µg/day for class III). Therefore, the evaluation of this flavouring agent proceeded to Step 5.

Step 5. For menthol (No. 427), an ADI of 0–4 mg/kg bw was allocated on the basis of the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats, applying a safety factor of 100 and rounding to one significant figure ([Annex 1](#), reference 137). The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] provides an adequate MOE of 440 in relation to the estimated dietary exposure to No. 427 (MSDI of 51 474 µg/day). The Committee therefore concluded that menthol (No. 427) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For *dl*-isomenthol (No. 2249), the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] of the structurally related substance menthol (No. 427) provides an adequate MOE of 7600 in relation to the estimated dietary exposure to No. 2249 (SPET value of 3000 µg/day). The Committee therefore concluded that *dl*-isomenthol (No. 2249) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For dimethyl glutarate (No. 2250), the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] for the structurally related substance menthol (No. 427) provides an adequate MOE of 2100 in relation to the estimated dietary exposure to No. 2250 (MSDI value of 10 784 µg/day). The Committee therefore concluded that dimethyl glutarate (No. 2250) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For (±)-2-[(2-*p*-methoxy)ethoxy]ethanol (No. 2251), the NOEL of 30 mg/kg bw per day from a 90-day study in rats for the structurally related substance 3-*l*-menthoxypropane-1,2-diol (No. 1408) [13] provides an adequate MOE of 1700 in relation to the estimated dietary exposure to No. 2251 (SPET value of 1000 µg/day). The Committee therefore concluded that (±)-2-[(2-*p*-methoxy)

ethoxy]ethanol (No. 2251) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

Table 17 summarizes the evaluations of the seven flavouring agents belonging to this group of menthol and structurally related substances (Nos 427 and 2246–2251).

Consideration of combined intakes from use as flavouring agents

With the exception of dimethyl glutarate (No. 2250), all additional flavouring agents to the group of menthol and structurally related substances have low MSDI values (range: 0.03–21 µg/day). The highest MSDI value for dimethyl glutarate (No. 2250) is 10 784 µg/day. No production volume for No. 2250 was reported for Japan and Latin America. The Committee concluded that consideration of combined intakes is not necessary for Japan and Latin America because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

In the unlikely event that the flavouring agents with the common metabolite menthol (No. 427) in this group were to be consumed together with menthol (No. 427) on a daily basis, the estimated combined intakes⁵ for the four flavouring agents (Nos 427, 429, 1414 and 2250) with the highest estimated dietary exposures (MSDI values) would be 24 622 µg/day in Europe and 65 627 µg/day in the USA. The estimated combined intake would therefore exceed the human threshold of toxicological concern (1800 µg/day for structural class I). However, the vast majority of the combined intake would be due to menthol alone, for which an ADI of 0–4 mg/kg bw was previously established. The estimated combined intake does not exceed this ADI, which is equal to 240 mg/day for a 60 kg person. Also, as the flavouring agents are likely to be metabolized efficiently, they would not saturate metabolic pathways.

Therefore, the Committee concluded that combined intake would not raise safety concerns.

Consideration of additional data on previously evaluated flavouring agents

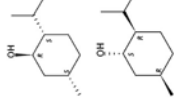
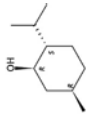
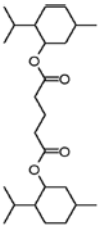
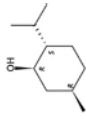
For eight previously evaluated flavouring agents in this group, additional studies of metabolism (No. 429); studies of acute toxicity (Nos 430, 1854 and 1856); and studies of genotoxicity (Nos 429, 430, 431, 445, 1856 and 1857) were available.

Conclusions

In the previous evaluations of substances in this group of menthol and structurally related substances, biochemical data; studies of acute toxicity, short-term toxicity,

⁵ Combined intake was calculated on a molar basis relative to the formation of a common metabolite. In this case, the common metabolite is menthol, with a relative molecular mass of 157.

Table 17
Summary of the results of safety evaluations of menthol and six additional structurally related substances used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 ^d Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Related structure name (No.) and structure	Conclusion based on current estimated dietary exposure
Structural class I						
Menthyl formate	2246	2230-90-2 	No, SPET: 400	NR	NR	No safety concern
Menthyl propionate	2247	86014-82-6 	No, SPET: 600	NR	NR	No safety concern
<i>l</i> -Menthyl butyrate	2248	68366-64-3 	No, SPET: 300	NR	NR	No safety concern
<i>dl</i> -Isomenthol	2249	3623-52-7 	Yes, SPET: 3 000	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats for the structurally related substance menthol [12] is 7 600 times the estimated dietary exposure to No. 2249 when used as a flavouring agent.	Menthol (No. 427) 	No safety concern
Dimenthyl glutarate	2250	406179-71-3 	Yes, MSDI: 10 784	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats for the structurally related substance menthol [12] is 2 100 times the estimated dietary exposure to No. 2250 when used as a flavouring agent.	Menthol (No. 427) 	No safety concern

Step 4: ^a		Step 5 ^d		Conclusion based on current estimated dietary exposure
Flavouring agent	No.	Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	
Menthol ^b	427	Yes, MSDI: 51 474	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] is 440 times the estimated dietary exposure to No. 427 when used as a flavouring agent.	No safety concern
Structural class III				
(±)-2-(2- <i>p</i> -Menthoxyl)ethanol	2251	Yes, SPET: 1 000	Yes. The NOEL of 30 mg/kg bw per day from a 90-day study in rats for the structurally related substance 3- <i>l</i> -menthoxypropane-1,2-diol [13] is 1 700 times the estimated dietary exposure to No. 2251 when used as a flavouring agent.	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; NOAEL: no-observed-adverse-effect-level; NOEL: no-observed-effect level; no.: number; NR: not relevant; SPET: single-portion exposure technique

^a Twenty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 137 and 190).

^b Step 2: Six flavouring agents (Nos 427, 2246–2250) are in structural class I and one flavouring agent (No. 2251) is in structural class III.

^c The thresholds for toxicological concern for structural class I and structural class III are 1800 µg/day and 90 µg/day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest daily dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case except for Nos 427 and 2250 where the MSDI value was higher.

^d The margin of exposure was calculated based on the highest estimated daily dietary exposure using either the SPET or the MSDI method.

^e The acceptable daily intake of menthol of 0–4 mg/kg bw established at the fifty-first meeting (Annex 1, reference 137) was maintained.

long-term toxicity and carcinogenicity, genotoxicity, developmental toxicity, immunotoxicity and sensitivity; and human data were available. None of the 24 previously evaluated flavouring agents raised safety concerns based on the estimated dietary exposures and the biochemical and toxicological data available (Annex 1, references 138 and 191).

For the flavouring agent under re-evaluation, menthol (No. 427), additional biochemical data, studies of acute toxicity and genotoxicity and a case study were available. Studies of genotoxicity were available for the *dl*-isomenthol (No. 2249) and $(\pm)$ -2-[(2-*p*-methoxy)ethoxy]ethanol (No. 2251).

The studies available for the present evaluation raised no safety concerns and support the previous safety evaluations. The additional data on menthol did not indicate a need to revise the ADI of menthol (No. 427).

The Committee concluded that the seven flavouring agents under evaluation, six of which are additions to the group of menthol and structurally related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures. The previously established ADI of 0–4 mg/kg bw for menthol was maintained.

An addendum to the monograph was prepared.

References

1. Principles and methods for the risk assessment of chemicals in food. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. Geneva: World Health Organization, International Programme on Chemical Safety; 2009 (Environmental Health Criteria 240).
2. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
3. Report of the Forty-eighth Session of the Codex Committee On Food Additives. Xi'an, China, 14–18 March 2016. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World; 2016 (REP16/FA).
4. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Heymann E. Carboxylesterases and amidases. In: Jakoby WB, editor, Enzymatic basis of detoxification. New York, NY, USA: Academic Press; 1980.
7. Anders MW. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson DH, Caldwell J, Paulson GD, editors. Intermediary xenobiotic metabolism in animals. New York, NY, USA: Taylor and Francis; 1989.

8. Williams RT. Studies in detoxication: The biological reduction of *l*-menthone to *d*-neomenthol and of *d*-isomenthone to *d*-isomenthol in the rabbit. The conjugation of *d*-neomenthol with glucuronic acid. *Biochem J.* 1940;34:690–7.
9. Atzl G, Bertel M, Daxenbichler G, Gleispach H. Determination of etheral oils from the urine by gas-liquid chromatography. *Chromatographia.* 1972;5(4):250–5.
10. Yamaguchi T, Caldwell J, Farmer PB. Metabolic fate of [3H]-*l*-menthol in the rat. *Drug Metab Dispos.* 1994;22(4):616–24.
11. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
12. Bioassay of *DL*-menthol for possible carcinogenicity. US National Cancer Institute Report NCI-CG-TR-98. US National Technical Information Services Report No. PB-288761, Bethesda, Maryland, USA; 1979 [cited in [Annex I](#), reference 138].
13. Wolfe GW. 91-Day subchronic oral toxicity study in rats with D1156.02 (Laboratory project identification HWA, study no. 297-648). Unpublished report from Hazelton Washington, Inc., Vienna, Virginia, USA; 1992. Submitted to WHO by the Flavor and Extract Manufacturers' Association of the United States [cited in [Annex I](#), reference 174].

4.1.7 Miscellaneous nitrogen-containing substances

Introduction

The Committee evaluated three additional flavouring agents belonging to the group of miscellaneous nitrogen-containing substances, which was evaluated previously. The additional flavouring agents included a triazole moiety with a thiopyridine side-chain, 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine (No. 2235); a benzothiadiazine moiety with a piperidinyl side-chain, (S)-1-(3-(((4-Amino-2,2-dioxido-1*H*-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one (No. 2236); and an *N*-pyrazole- and *N*-thiophene-substituted amide, 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(thiophen-2-ylmethyl)acetamide (No. 2237). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee considered whether (S)-1-(3-(((4-amino-2,2-dioxido-1*H*-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one (No. 2236) belonged to the group of aliphatic and aromatic amines and amides. This group includes the structurally related 3[(4-amino-2,2-dioxido-1*H*-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide (No. 2082), which was evaluated at the seventy-sixth meeting ([Annex 1](#), reference 211). The Committee concluded that the additional flavouring agent should remain in this group. All three flavouring agents that were evaluated at this meeting are reported to be flavour modifiers.

The Committee previously evaluated 16 additional members of this group of miscellaneous nitrogen-containing substances at its sixty-fifth meeting

(Annex 1, reference 178); 14 additional members at its sixty-ninth meeting (Annex 1, reference 190); two additional members at its seventy-sixth meeting (Annex 1, reference 211); and two additional members at its seventy-ninth meeting (Annex 1, reference 220). At each meeting, the Committee concluded that all of the evaluated flavouring agents were of no safety concern at estimated dietary exposures.

None of the additional flavouring agents (Nos 2235, 2236 and 2237) in this group have been reported to occur naturally.

A comprehensive literature search on data on the additional flavouring agents was conducted; no additional studies were identified.

Assessment of dietary exposure

The total annual volume of production of the three flavouring agents belonging to the group of miscellaneous nitrogen-containing substances is 0.3 kg in the USA. No data on intake were reported for Europe, Japan or Latin America [1, 2]. Each of these flavouring agents contributes equally to the annual production volume in the USA.

Dietary exposures were estimated using both SPET and the MSDI method. The highest values are reported in Table 18. The estimated daily dietary exposure is highest for 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine (No. 2235), at 800 µg/person per day, the SPET value obtained from soups and broths. For each of the three flavouring agents the estimate from the MSDI method was 0.01 µg/day. For Nos 2236 and 2237 the highest SPET estimates were 750 and 600 µg/person per day, respectively.

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to this group has previously been described in the monographs of the sixty-fifth, sixty-ninth, seventy-sixth and seventy-ninth meetings (Annex 1, references 179, 191, 212 and 221). Additional information was available for this meeting.

Metabolic studies show that Nos 2236 and 2237 have low bioavailability [3, 4]. The data for No. 2235 showed that it was bioavailable with a short half-life in rat plasma. No. 2235 was shown to be metabolized in toxicokinetic and in vivo metabolism studies in the rat [5]. All these additional flavouring agents undergo limited hydrolysis, sulfoxidation or oxidation of the heterocyclic rings or are expected to be excreted unchanged in the faeces or urine [3, 4, 5].

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for the three additional flavouring agents (Nos 2235–2237). Chemical-specific genotoxicity data available

for each of these flavouring agents indicate that they lack the potential to be genotoxic.

Step 2. All three flavouring agents (Nos 2235–2237) were assigned to structural class III [6].

Step 3. The highest dietary exposures were estimated using SPET (Table 18).

Step 4. The highest dietary exposure estimates of the three flavouring agents in structural class III were above the threshold of toxicological concern (i.e. 90 µg/person per day for class III). These flavouring agents proceeded to Step 5.

Step 5. For these flavouring agents, the NOAELs of 100 mg/kg bw per day in rats, the highest dose tested in 90-day oral toxicity studies [3, 5], provide adequate margins of exposure (7500, 8000 and 10 000, respectively) relative to the highest estimated dietary exposure to No. 2235 (SPET = 800 µg/day), No. 2236 (SPET = 750 µg/day) and No. 2237 (SPET = 600 µg/day) when used as flavouring agents. The Committee therefore concluded that Nos 2235–2237 would not pose safety concerns at current estimated dietary exposures.

Table 18 summarizes the evaluations of the three flavouring agents belonging to this group of miscellaneous nitrogen-containing substances (Nos 2235–2237).

Consideration of combined intakes from use as flavouring agents

The three additional flavouring agents in this group of miscellaneous nitrogen-containing substances have low MSDIs (0.01 µg/day). The Committee concluded that consideration of combined intakes is not necessary because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of additional data on previously evaluated flavouring agents

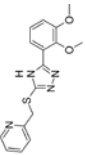
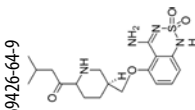
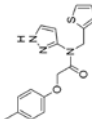
For previously evaluated flavouring agents in this group, studies of acute toxicity (No. 1566 and 1889), genotoxicity (No. 1566) and reproductive/developmental toxicity (No 2161) were available.

Conclusions

In previous evaluations of flavouring agents in this group of miscellaneous nitrogen-containing substances, biochemical data; studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and developmental and reproductive toxicity; and thyroid toxicity were available. The estimated dietary exposures and the biochemical and toxicological data available did not raise any safety concerns for the 34 previously evaluated flavouring agents in this group (Annex 1, references 178, 190, 211 and 220).

For the additional flavouring agents in this group, biochemical data (on Nos 2235–2237); studies of short-term toxicity (on Nos 2235–2237); studies on

Table 18
Summary of the results of the safety evaluations of miscellaneous nitrogen-containing substances^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 ^d Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
Structural class III					
2-(((3-(2,3-Dimethoxyphenyl)-1 <i>H</i> -1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	902136-79-2 	Yes, SPET: 800	Yes, The NOAEL of 100 mg/kg bw per day [5] is 7 500 times the estimated dietary exposure to No. 2235 when used as a flavouring agent.	No safety concern
(5 <i>S</i>)-1-(3-(((4-Amino-2,2-dioxido-1 <i>H</i> -benzol[1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	1469426-64-9 	Yes, SPET: 750	Yes, The NOAEL of 100 mg/kg bw per day [3] is 8 000 times the estimated dietary exposure to No. 2236 when used as a flavouring agent.	No safety concern
2-(4-Methoxyphenoxy)- <i>N</i> -(1 <i>H</i> -pyrazol-3-yl)- <i>N</i> -(thiophen-2-yl(methyl)acetamide	2237	1374760-95-8 	Yes, SPET: 600	Yes, The NOAEL of 100 mg/kg bw per day [4] is 10 000 times the estimated dietary exposure to No. 2237 when used as a flavouring agent.	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; NOAEL: no-observed-adverse-effect level; SPET: single-portion exposure technique

^a Thirty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 178, 190, 211 and 220).
^b Step 2: All three flavouring agents are in structural class III.
^c The threshold for toxicological concern for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using the SPET.
^d The margin of exposure was calculated based on estimated dietary exposure calculated by the SPET.

genotoxicity (on Nos 2235–2237 and a metabolite of 2237); and developmental studies (on Nos 2236 and 2237) were available.

The studies available on the previously evaluated and additional flavouring agents in this group available for the present evaluation raised no safety concerns and support the previous safety evaluations.

The Committee concluded that these three flavouring agents, which are additions to the group of miscellaneous nitrogen-containing substances evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

References

1. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
2. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Arthur AJ, Karanewsky DS, Luksic M, Goodfellow G, Daniels J. Toxicological evaluation of two flavors with modifying properties: 3-((4-amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)-2,2-dimethyl-*N*-propylpropanamide and (S)-1-(3-(((4-amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one. *Food Chem Toxicol*. 2015;76:33–45.
4. Karanewsky DS, Arthur AJ, Liu H, Chi B, Markison S. Toxicological evaluation of a novel cooling compound: 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide. *Toxicol Rep*. 2015;2:1291–309.
5. Karanewsky DS, Arthur AJ, Liu H, Chi B, Ida L, Markison S. Toxicological evaluation of a novel umami flavour compound: 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine. *Toxicol Rep*. 2016;3:501–12.
6. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol*. 1978;16(3):255–76.

4.1.8 Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids

The Committee evaluated two additional flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids that was evaluated previously. The additional flavouring agents were 8-methyldecanal (No. 2238) and 8-methylnonanal (No. 2239). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230). Neither of these substances had been previously evaluated.

The Committee evaluated 25 members of this group at its forty-ninth meeting ([Annex 1](#), reference 131). The Committee concluded that all 25 flavouring

agents did not raise any safety concerns at estimated dietary exposures. At its seventy-sixth meeting, the Committee evaluated four additional members of this group of flavouring agents and concluded that all four were of no safety concern at estimated dietary exposures ([Annex 1](#), reference 211).

8-Methylnonanal (No. 2239) was reported to occur naturally in citrus fruits. 8-Methyldecanal (No. 2238) is not reported to occur naturally [1].

A comprehensive literature search was conducted in PubMed; no additional relevant studies were identified.

Assessment of dietary exposure

The total annual volume of production of the two flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids is 0.1 kg in the USA, accounted for by 8-methyldecanal (No. 2238), and 0.1 kg in Japan, accounted for by 8-methylnonanal (No. 2239) [2, 3].

Dietary exposures were estimated using both the SPET and the MSDI method, with highest values reported in [Table 19](#). The highest estimated daily dietary exposure for 8-methyldecanal (No. 2238) is 0.1 µg/day (SPET value). The highest estimated daily dietary exposure of 8-methylnonanal (No. 2239) is 0.03 µg/day (MSDI value).

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids has previously been described in the monograph of the forty-ninth meeting ([Annex 1](#), references 132). Additional information on the ADME of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids was available for this meeting. The previously described information as well as the additional information available for this meeting, on the ADME of the flavouring agents belonging to this group, are summarized as follows.

The substances in this group share common metabolic pathways, and are expected to be absorbed into the gastrointestinal tract [4, 5]. Shorter branched-chain aliphatic alcohols, aldehydes and acids undergo β -oxidation cleavage, with intermediates metabolized to CO₂ via the tricarboxylic acid cycle. Longer alkyl chain length and branching increases susceptibility to both oxidation and glucuronidation in aliphatic alcohols, as there is an increase in the affinity for UDP-glucuronosyltransferases [6, 7]. Longer and more substituted alcohols produce polar metabolites after undergoing ω -, ω -1 and β -oxidation and selective dehydrogenation and hydration, resulting in chain-shortening [8]. The substances in this group are expected to be metabolized to innocuous products via common metabolic pathways, or be excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are neither structural alerts for genotoxicity nor chemical-specific genotoxicity data on the additional flavouring agents. New and previously evaluated data from other related flavouring agents indicate that these flavouring agents are not likely to be genotoxic. Therefore, the weight of evidence indicates that these additional saturated aliphatic acyclic branched-chain primary aldehyde flavouring agents are not likely to be genotoxic.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned both flavouring agents to structural class I [9].

Steps 3 and 4. Dietary exposures using both MSDI method and SPET have been determined. The highest estimated dietary exposures of both flavouring agents in structural class I were below the threshold of concern (i.e. 1800 µg/person per day for Class I). The Committee therefore concluded that both flavouring agents (Nos 2238 and 2239) would not pose a safety concern at current estimated dietary exposures.

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids have low MSDIs (0.01–0.03 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the exposure to this flavouring group.

Consideration of additional data on previously evaluated flavouring agents

In the previous evaluation of substances in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids, studies of biochemistry, acute toxicity, short-term and long-term toxicity, reproductive and developmental toxicity and genotoxicity were available ([Annex 1](#), references 132 and 211). None of the 29 flavouring agents of this group raised safety concerns.

For the present evaluation, no relevant studies were available for the two additional flavouring agents (Nos 2238 and 2239). For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 251, 252, 253, 254, 258, 260, 267, 268, 269 and 2176), studies of short-term toxicity (Nos 251, 252, 254, 258, 267, 269, 272 and 275), studies of long-term toxicity (No. 252), studies of genotoxicity (Nos 52, 251, 252, 253, 255, 259, 268, 270, 272 and 275), studies of reproductive and developmental toxicity (Nos 251, 252, 267, 268 and 272) and neurotoxicity (No. 251) were available.

There are positive genotoxicity data, not previously evaluated, for isobutyraldehyde (No. 252) in an in vitro and in vivo chromosomal aberration assay, an in vitro SCE assay and an in vitro forward mutation assay; and for

Table 19
Summary of the results of the safety evaluations of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
Structural class I					
8-Methyldecanal	2238	127793-88-8 	No, SPET: 0.1	NR	No safety concern
8-Methylnonanal	2239	3085-26-5 	No, MSDI: 0.03	NR	No safety concern

CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; no.: number; NOAEL: no-observed-adverse-effect level; NR: not required; SPET: single-portion exposure technique

^a Twenty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 132 and 211).

^b Step 2: Both flavouring agents are in structural class I.

^c The threshold for toxicological concern for structural class I is 1800 µg/day, respectively. All dietary exposure values are expressed in µg per day. The dietary exposure value listed represents the highest daily per capita intake calculated either by SPET or MSDI. The SPET gave the highest estimated dietary exposure for No. 2238 and the MSDI gave the highest estimated dietary exposure for No. 2239.

isobutyric acid (No. 253) in an in vitro forward mutation assay. The bacterial reverse mutation assays, and the in vivo micronucleus assays on isobutyraldehyde (No. 252), were negative. The in vivo chromosomal aberration assay using isobutyraldehyde (No. 252) was only positive at the highest dose, which produced notable signs of cytotoxicity. The negative in vivo results for isobutyraldehyde (No. 252) are consistent with the 2-year inhalational carcinogenicity mouse and rat assays that showed nasal toxicity but no carcinogenicity [10, 11]. Therefore, the weight of evidence indicates that these saturated aldehyde flavouring agents are not likely to be genotoxic. The results of the remainder of the genotoxicity assays on flavouring agents in this group are negative.

The studies available for the present evaluation support the previous safety evaluations.

Conclusions

The Committee concluded that these two flavouring agents, which are additions to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.3 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>).
2. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Dawson AM, Holdsworth CD, Webb J. Absorption of short chain fatty acids in man. *Proc Soc Exp Biol Med.* 1964;117:97–100.
5. Gaillard D, Derache R. [Metabolism of different alcohols present in alcoholic beverages, in the rat.] *Trav Soc Pharm Montp.* 1965;25:51–62.
6. Iwersen S, Schmoldt A. ADH-independent metabolism of aliphatic alcohols: Comparison of oxidation and glucuronidation. *Adv Forensic Sci.* 1995;4:19–22.
7. Jurowich S, Sticht G, Käferstein H. Glucuronidation of aliphatic alcohols in human liver microsomes in vitro. *Alcohol.* 2004;32:187–94.
8. Veenstra G, Webb C, Sanderson H, Belanger SE, Fisk P, Nielsen A et al. Human health risk assessment of long chain alcohols. *Ecotoxicol Environ Saf.* 2009;72(4):1016–30.
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

10. Abdo KM, Haseman JK, Nyska A. Isobutyraldehyde administered by inhalation (whole body exposure) for up to thirteen weeks or two years was a respiratory tract toxicant but was not carcinogenic in F344/N rats and B6C3F1 mice. *Toxicol Sci.* 1998;42(2):136–51.
11. NTP toxicology and carcinogenesis studies of isobutyraldehyde (CAS No. 78-84-2) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, North Carolina, USA: US Department of Health and Human Services, National Toxicology Program; 1999 (NTP Technical Report 472).

4.2 Specifications of identity and purity of flavouring agents

4.2.1 New and maintained specifications

The Committee received information related to specifications for 20 of the 21 new flavouring agents from the call for data for the present meeting. While carvone (Flavor and Extract Manufacturers Association of the United States [FEMA] No. 2249) was listed in the call for data as a new flavouring agent, the Committee noted that full specifications already existed for (+)-carvone (No. 380.1; *d*-carvone) and (–)-carvone (No. 380.2; *l*-carvone). As no new data on specifications were provided for (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), the existing specifications were maintained. Revisions were made to the chemical formulae for 3-[(2-methyl-3-furyl)thio]-2-butanone (No. 1525) and *O*-ethyl *S*-(2-furylmethyl)thiocarbonate (No. 1526), both of which remained as full specifications.

Full specifications were prepared for the 20 new flavouring agents for which data were provided. Forty-seven flavouring agents for which full specifications (Nos 427, 973–975, 980–982, 1480, 1491–1526 and 2103–2105) currently exist were considered by the Committee for toxicological re-evaluation at the current meeting (see sections 4.1.1, 4.1.3, 4.1.5 and 4.1.6). Thirty-nine of these flavouring agents (Nos. 1491–1526 and 2103–2105; see section 4.1.3) previously included a statement indicating that the safety evaluation for the flavouring agents had not been completed. As the toxicological evaluations of these 39 flavourings were completed at this meeting and no safety concerns were noted, the text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full.

A statement was added to the existing full specifications for *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) indicating that, at the current meeting, the safety evaluations for these flavouring agents were not completed. The specifications for *p*-mentha-1,8-dien-7-al (No. 973), (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) were maintained as full.

4.2.2 Revised specifications

The Committee received information in support of the revision of full specifications for three flavouring agents that were on the agenda of the present meeting (Nos 433, 619 and 2123).

The Committee revised specifications for *l*-menthyl lactate (No. 433) to reflect the specific isomeric composition of the flavouring agent in commerce. This was accomplished by changing the CAS number from 59259-38-0 to 61597-98-6 and revising the name to *l*-menthyl *l*-lactate.

The Committee revised specifications for *l*-malic acid (No. 619) by removing the specification for specific rotation based on results received, which indicate difficulty in standardizing this measurement for *l*-malic acid.

The Committee revised the specifications for glutamyl-valyl-glycine by revising the melting-point range of the flavouring.



5. Future work and recommendations

Specific food additives (other than flavouring agents)

Anionic methacrylate copolymer

The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid, one of the residual monomers of AMC. Further studies to clarify the in vivo carcinogenic potential are required to complete the evaluation of AMC.

Citric and fatty acid esters of glycerol

The specifications of CITREM were made tentative, pending a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.

The Committee noted that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform.

Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by **December 2019**.

Neutral methacrylate copolymer

The Committee noted that no data were submitted for a suitable method of assay. Tentative specifications for NMC were prepared pending a suitable validated method of assay.

Spirulina extract

The Committee received limited analytical data on spirulina extract. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested by **December 2019**:

- Full compositional characterization of commercial products in both liquid and powder forms;
- Full compositional characterization of the aqueous extract before formulation/standardization;
- Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data); and

- Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).

Modified starches

The Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in Hydroxypropyl starch (INS 1440) and Hydroxypropyl distarch phosphate (INS 1442) in order to consider lowering this limit.

The Committee requested suitable microbiological acceptance criteria and supporting data for all modified starches. Information required in the recommended annexes is summarized in [Table 9](#).

Flavouring agents

Carvone and structurally related substances

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within **3 years**.

For (–)-carvone (No. 380.2), the Committee concluded that toxicological data on (–)-carvone are necessary. Also, data are needed for an exposure assessment for the oral exposure to (–)-carvone from all sources.

Maltol and related substances

The Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol (No. 1480) during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting ([Annex 1](#), reference 57).

The Committee withdrew the ADI for maltol. The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the ADI.

The ADI for ethyl maltol was maintained.

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Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some

- antioxidants (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
 28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
 29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
 30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
 31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocabamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
 32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
 33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
 34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.
 35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
 36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
 37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
 38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
 39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
 40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.
43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.
46. Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.
49. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 7, 1978.
50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 14, 1980.
52. Specifications for identity and purity of food colours, flavouring agents, and other food additives. FAO Food and Nutrition Paper, No. 12, 1979.
53. Evaluation of certain food additives (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 15, 1980.
55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.
56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 16, 1981.
58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.
59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.
61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.
64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.
65. Guide to specifications – General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.
68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.
72. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
73. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
75. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 37, 1986.
76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pes.
77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.
78. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
79. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 38, 1988.
80. Evaluation of certain veterinary drug residues in food (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988.
83. Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. Evaluation of certain veterinary drug residues in food (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 25, 1990.
87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.
90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.
91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.
93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, 1991.
96. Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).
97. Evaluation of certain veterinary drug residues in food (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 29, 1991.
99. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, 1991.
100. Guide to specifications – General notices, general analytical techniques, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.
101. Evaluation of certain food additives and naturally occurring toxicants (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.

102. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series, No. 30, 1993.
103. Compendium of food additive specifications: addendum 1. FAO Food and Nutrition Paper, No. 52, 1992.
104. Evaluation of certain veterinary drug residues in food (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 31, 1993.
106. Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper, No. 41/5, 1993.
107. Evaluation of certain food additives and contaminants (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 32, 1993.
109. Compendium of food additive specifications: addendum 2. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. Evaluation of certain veterinary drug residues in food (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 33, 1994.
112. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/6, 1994.
113. Evaluation of certain veterinary drug residues in food (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 34, 1995.
115. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/7, 1995.
116. Evaluation of certain food additives and contaminants (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 35, 1996.
118. Compendium of food additive specifications: addendum 3. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. Evaluation of certain veterinary drug residues in food (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 36, 1996.
121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.
122. Evaluation of certain food additives and contaminants (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 37, 1996.

124. Compendium of food additive specifications, addendum 4. FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
125. Evaluation of certain veterinary drug residues in food (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 38, 1996.
127. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/9, 1997.
128. Evaluation of certain veterinary drug residues in food (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 39, 1997.
130. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/10, 1998.
131. Evaluation of certain food additives and contaminants (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.
133. Compendium of food additive specifications: addendum 5. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
134. Evaluation of certain veterinary drug residues in food (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.
135. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 41, 1998.
136. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/11, 1999.
137. Evaluation of certain food additives (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.
138. Safety evaluation of certain food additives. WHO Food Additives Series, No. 42, 1999.
139. Compendium of food additive specifications, addendum 6. FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. Evaluation of certain veterinary drug residues in food (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.
141. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 43, 2000.
142. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/12, 2000.
143. Evaluation of certain food additives and contaminants (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.
144. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 44, 2000.

145. Compendium of food additive specifications, addendum 7. FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. Evaluation of certain veterinary drug residues in food (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.
147. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 45, 2000.
148. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/13, 2000.
149. Evaluation of certain food additives and contaminants (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 901, 2001.
150. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 46, 2001.
151. Compendium of food additive specifications: addendum 8. FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
152. Evaluation of certain mycotoxins in food (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 906, 2002.
153. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper, No. 74, 2001.
154. Evaluation of certain food additives and contaminants (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.
155. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 48, 2002.
156. Compendium of food additive specifications: addendum 9. FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.
157. Evaluation of certain veterinary drug residues in food (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.
158. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 49, 2002.
159. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/14, 2002.
160. Evaluation of certain food additives and contaminants (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.
161. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 50, 2003.
162. Compendium of food additive specifications: addendum 10. FAO Food and Nutrition Paper, No. 52, Add. 10, 2002.
163. Evaluation of certain veterinary drug residues in food (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.
164. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 51, 2003.
165. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/15, 2003.

166. Evaluation of certain food additives and contaminants (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.
167. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 52, 2004.
168. Compendium of food additive specifications: addendum 11. FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.
169. Evaluation of certain veterinary drug residues in food (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.
170. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/16, 2004.
171. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 53, 2005.
172. Compendium of food additive specifications: addendum 12. FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.
173. Evaluation of certain food additives (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.
174. Safety evaluation of certain food additives. WHO Food Additives Series, No. 54, 2005.
175. Compendium of food additive specifications: addendum 13. FAO Food and Nutrition Paper, No. 52, Add. 13 (with Errata), 2005.
176. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.
177. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2006.
178. Evaluation of certain food additives (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934, 2006.
179. Safety evaluation of certain food additives. WHO Food Additives Series, No. 56, 2006.
180. Combined compendium of food additive specifications. FAO JECFA Monographs 1, Volumes 1–4, 2005, 2006.
181. Evaluation of certain veterinary drug residues in food (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006.
182. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 2, 2006.
183. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 57, 2006.
184. Evaluation of certain food additives and contaminants (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940, 2007.
185. Compendium of food additive specifications. FAO JECFA Monographs 3, 2006.
186. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 58, 2007.
187. Evaluation of certain food additives and contaminants (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.

188. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 59, 2008.
189. Compendium of food additive specifications. FAO JECFA Monographs 4, 2007.
190. Evaluation of certain food additives (Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 952, 2009.
191. Safety evaluation of certain food additives. WHO Food Additives Series, No. 60, 2009.
192. Compendium of food additive specifications. FAO JECFA Monographs 5, 2009.
193. Evaluation of certain veterinary drug residues in food (Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 954, 2009.
194. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 61, 2009.
195. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 6, 2009.
196. Evaluation of certain food additives (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.
197. Safety evaluation of certain food additives. WHO Food Additives Series, No. 62, 2010.
198. Compendium of food additive specifications. FAO JECFA Monographs 7, 2009.
199. Evaluation of certain contaminants in food (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959, 2011.
200. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 63/FAO JECFA Monographs 8, 2011.
201. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 9, 2010.
202. Evaluation of certain food additives and contaminants (Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960, 2011.
203. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 64, 2011.
204. Compendium of food additive specifications. FAO JECFA Monographs 10, 2010.
205. Evaluation of certain food additives and contaminants (Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 966, 2011.
206. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 65, 2011.
207. Compendium of food additive specifications. FAO JECFA Monographs 11, 2011.
208. Evaluation of certain veterinary drug residues in food (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 969, 2012.
209. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 66, 2012.
210. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 12, 2012.
211. Evaluation of certain food additives (Seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 974, 2012.
212. Safety evaluation of certain food additives. WHO Food Additives Series, No. 67, 2012.

213. Compendium of food additive specifications. FAO JECFA Monographs 13, 2012.
214. Evaluation of certain food additives and contaminants (Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 983, 2013.
215. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 68, 2013.
216. Compendium of food additive specifications. FAO JECFA Monographs 14, 2013.
217. Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 988, 2014.
218. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 69, 2014.
219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.
220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.
221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.
222. Compendium of food additive specifications. FAO JECFA Monographs 16, 2014.
223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.
224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.
225. Compendium of food additive specifications. FAO JECFA Monographs 17, 2015.
226. Evaluation of certain veterinary drug residues in food (Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 997, 2016.
227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.
228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 18, 2016.
229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.
230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.
231. Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.
232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.
233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1002, 2017.
234. Evaluation of certain food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1007, 2017.
235. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 74, 2018 (in press).
236. Compendium of food additive specifications. FAO JECFA Monographs 20, 2017.
237. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 75, 2018 (in press).

238. Evaluation of certain veterinary drug residues in food (Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1008, 2018.
239. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 21, 2018.
240. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 76, 2019 (in press).



ANNEX 2

Toxicological information and information on specifications

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Anionic methacrylate copolymer (AMC)	N, T ^a	The Committee was unable to complete the evaluation of AMC. While the copolymer itself is not of health concern, genotoxicity concerns remains for the residual monomer methacrylic acid. The specifications were made tentative pending the completion of the safety evaluation of AMC.
Basic methacrylate copolymer (BMC)	N	The Committee established an ADI “not specified” for basic methacrylate copolymer. The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification. The no-observed-adverse-effect level (NOAEL) for BMC ranged from 750 to 2 000 mg/kg body weight (bw) per day, which were the highest doses tested. The Committee evaluated exposure to BMC for the copolymer and its monomers (<i>n</i>-butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate). Estimated exposures to BMC range from 3.0 to 135 mg/kg bw per day. The total monomeric content of BMC is less than 0.3%. The Committee concluded that the toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.
Erythrosine	R ^b	The Committee concluded that the new data that have become available since the previous evaluation of erythrosine do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw. The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to for all age groups do not present a health concern. erythrosine for all age groups do not present a health concern.
Indigotine	R ^b	The Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies and concluded that there are no reasons to revise the ADI and confirmed the previous ADI of 0–5 mg/kg bw.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper bound of the ADI of 0–5 mg/kg bw. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.
Lutein	R ^{c,d}	Free lutein, lutein esters and free zeaxanthin including <i>meso</i>-zeaxanthin are biochemically and toxicologically equivalent. At the present meeting the Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from <i>Tagetes erecta</i>, synthetic zeaxanthin and <i>meso</i>-zeaxanthin. Free lutein, lutein esters and free zeaxanthin and <i>meso</i>-zeaxanthin are substances of low toxicity for which no adverse effects have been observed in a broad range of toxicological studies in laboratory animals and clinical studies in humans. Based on the absence of toxicity in a wide range of studies, the Committee established a group ADI “not specified” for lutein from <i>Tagetes erecta</i>, lutein esters from <i>Tagetes erecta</i> and zeaxanthin (synthetic). <i>Meso</i>-zeaxanthin was not included in this group ADI, as specifications are not currently available. The group ADI of 0–2 mg/kg bw for lutein from <i>Tagetes erecta</i> and zeaxanthin (synthetic) was withdrawn.
Neutral methacrylate copolymer (NMC)	N, T	The Committee established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative. The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes. The NOAELs for NMC ranged from 454 to 2 000 mg/kg bw per day, and these were the highest doses tested. The Committee evaluated exposure to NMC for the copolymer and its monomers (methyl methacrylate and ethyl acrylate). Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day. The total monomeric content of NMC is less than 0.01%. Toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.
Sorbitol syrup	—	Sorbitol syrup (INS 420(ii)) is currently included in the Codex General Standard for Food Additives (GSFA) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. The Committee was therefore requested to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances, and advise on the need for a separate evaluation of sorbitol syrup or if the ADI “not specified” for sorbitol is also applicable for sorbitol syrup.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		Based on the similarity of the chemical constituents of sorbitol syrup to the previously evaluated sorbitol, maltitol syrup and polyglycitol syrup, the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI “not specified” for sorbitol syrup.
Spirulina extract	N, T	The Committee established a temporary ADI “not specified” for spirulina extract. The ADI was based on the absence of toxicity in repeated-dose animal studies with spirulina extract and dried spirulina. The ADI “not specified” was made temporary due to the tentative nature of the specifications. Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour based on the Budget method and exposure to spirulina extract and dried spirulina from other dietary sources, including food ingredients, dietary supplements, and coatings of food supplements was 190 mg/kg bw for adults (60 kg/person) and 650 mg/kg bw for a child (15 kg/person). The Committee concluded that this dietary exposure does not present a health concern.

—: no specifications prepared; N: new specifications; R: existing specifications revised; T: tentative specifications

^a The specifications were made tentative pending the completion of the safety evaluation of AMC.

^b At the current meeting, high-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

^c The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

^d At the current meeting, the identity test for melting range was deleted, the identity tests for carotenoids and spectrophotometry were updated, the test for propylene glycol was incorporated verbatim and the previous reference removed, and the method of assay was updated.

Food additives considered for specifications only

Food additive	Specifications
Cassia gum	R ^a
Citric and fatty acid esters of glycerol	R, T ^b
Glycerol ester of wood rosin	R ^c
Modified starches	R ^d , T

R: existing specifications revised; T: tentative specifications

^a The Committee, at its current meeting, received analytical methods and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with *n*-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified. The specifications were revised and the tentative status was removed.

^b The Committee did not receive a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in its specifications monograph. The Committee noted further that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform. Specifications were revised and made tentative pending the availability of data. Specifications will be withdrawn if suitable information is not provided by December 2019.

^c The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species namely *Pinus halepensis* and *Pinus brutia* as source materials. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. Since the specifications monograph for GEWR does not contain an assay, the Committee recommends that the JECFA Secretariat be notified upon the development and validation of an appropriate assay. The existing specifications were revised.

^d The Committee reviewed data on the method of manufacture, identity, and purity of all 16 modified starches. Based on the information received, and available information the Committee noted the following:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered the application of a read-across approach to be appropriate for the toxicological evaluation of these substances.
- All 16 modified starches had been assigned an ADI of “not specified”.

- All modified starches can be additionally bleached or fragmented; therefore revision in the specifications of bleached or fragmented starches would imply the revision of all 16 monographs.
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals impurities content and microbiological considerations). Revision of those common specifications would affect all 16 monographs.
- As a result of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, potentially unavailable, and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.
- Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs should be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture, and the corresponding diversity of impurities. The Committee recommended that all modified starches be included in a modular monograph titled 'Modified Starches' that contains common requirements [General specifications for modified starches] consisting of specifications that apply to all 16 modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The Committee drafted a new modular specifications monograph titled "Modified starches" consisting of an explanatory introduction, "General specifications for modified starches," and eight annexes. The new modular specifications monograph for modified starches is printed in FAO Monograph 22, and will replace the 16 existing individual specifications for modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

The specification for lead included in the General specifications be decreased from 2 mg/kg to 0.2 mg/kg. The limit of lead for starch sodium octenylsuccinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added.

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

A. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Mixture of 1-Vinyl-3- cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde	2253	N	No safety concern
<i>p</i> -Mentha-1,8-dien-7-ol	974	N	No safety concern
<i>p</i> -Mentha-1,8-dien-7-yl acetate	975	N	No safety concern
Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	N	No safety concern
Myrtenol	981	N	No safety concern
Myrtenyl acetate	982	M	No safety concern
Structural class II			
(1-Methyl-2-(1,2,2- trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	N	No safety concern
Structural class III			
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	N	No safety concern
Flavouring agent excluded at Step 1 of the Procedure			
<i>p</i> -Mentha-1,8-dien-7-al (Perillaldehyde)	973	M	Genotoxicity data for <i>p</i> -mentha-1,8-dien-7-al raise concerns for potential genotoxicity

M: existing specifications maintained; N: new specifications

B. Carvone and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Pinocaryyl isobutyrate	2242	N	No safety concern
Carvyl palmitate	2243	N	No safety concern
Structural class III			
6-Hydroxycarvone	2244	N	No safety concern
Flavouring agents not evaluated according to the revised Procedure			
(+)-Carvone	380.1	M	The Committee did not re-evaluate (+)-carvone (No. 380.1) according to the revised Procedure given the lack of information on the oral exposure from all sources and the need to review the ADI. A review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.
(-)-Carvone	380.2	M	The Committee did not re-evaluate (-)-carvone (No. 380.2) according to the revised Procedure given the lack of information on the oral exposure from all sources and the lack of toxicological data.

M: existing specifications maintained; N: new specifications

C. Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-Pentylfuran	1491	M ^a	No safety concern
2-Heptylfuran	1492	M ^a	No safety concern
2-Decylfuran	1493	M ^a	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	M ^a	No safety concern
2,3-Dimethylbenzofuran	1495	M ^a	No safety concern
2,4-Difurfurylfuran	1496	M ^a	No safety concern
3-(2-Furyl)acrolein	1497	M ^a	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	M ^a	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	M ^a	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	M ^a	No safety concern
2-Furfurylidene-butyraldehyde	1501	M ^a	No safety concern

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
2-Phenyl-3-(2-furyl)prop-2-enal	1502	M ^a	No safety concern
2-Furyl methyl ketone	1503	M ^a	No safety concern
2-Acetyl-5-methylfuran	1504	M ^a	No safety concern
2-Acetyl-3,5-dimethylfuran	1505	M ^a	No safety concern
3-Acetyl-2,5-dimethylfuran	1506	M ^a	No safety concern
2-Butyrylfuran	1507	M ^a	No safety concern
(2-Furyl)-2-propanone	1508	M ^a	No safety concern
2-Pentanylfuran	1509	M ^a	No safety concern
1-(2-Furyl)butan-3-one	1510	M ^a	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	M ^a	No safety concern
Pentyl 2-furyl ketone	1512	M ^a	No safety concern
Ethyl 3-(2-furyl)propanoate	1513	M ^a	No safety concern
Isobutyl 3-(2-furan)propionate	1514	M ^a	No safety concern
Isoamyl 3-(2-furan)propionate	1515	M ^a	No safety concern
Isoamyl 3-(2-furan)butyrate	1516	M ^a	No safety concern
Phenethyl 2-furoate	1517	M ^a	No safety concern
Propyl 2-furanacrylate	1518	M ^a	No safety concern
2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	1519	M ^a	No safety concern
Furfuryl methyl ether	1520	M ^a	No safety concern
Ethyl furfuryl ether	1521	M ^a	No safety concern
Difurfuryl ether	1522	M ^a	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523	M ^a	No safety concern
Furfuryl 2-methyl-3-furyl disulfide	1524	M ^a	No safety concern
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	M ^a	No safety concern
O-Ethyl-S-(2-furylmethyl)thiocarbonate	1526	M ^a	No safety concern
(E)-Ethyl 3-(2-furyl)acrylate	2103	M ^a	No safety concern
di-2-Furylmethane	2104	M ^a	No safety concern
2-Methylbenzofuran	2105	M ^a	No safety concern

M: existing specifications maintained

^a The text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full.

D. Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
<i>trans</i> -6-Octenal	2240	N	No safety concern
2,6-Dimethyl-5-heptenol	2241	N	No safety concern

N: new specifications

E. Maltol and related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
Maltol	1480	M	No safety concern ^a
Structural class III			
Ethyl maltol isobutyrate	2252	N	No safety concern

M: existing specifications maintained; N: new specifications

^a The previously established ADI for maltol was withdrawn by the Committee.

F. Menthol and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Menthyl formate	2246	N	No safety concern
Menthyl propionate	2247	N	No safety concern
<i>l</i> -Menthyl butyrate	2248	N	No safety concern
<i>d,l</i> -Isomenthol	2249	N	No safety concern
Dimethyl glutarate	2250	N	No safety concern
Menthol	427	M	No safety concern
Structural class III			
(±)-2-[(2- <i>p</i> -Methoxy)ethoxy]ethanol	2251	N	No safety concern

M: existing specifications maintained; N: new specifications

^a The ADI of menthol of 0–4 mg/kg bw established at the fifty-first meeting was maintained.

G. Miscellaneous nitrogen-containing substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-(((3-(2,3-Dimethoxyphenyl)-1 <i>H</i> -1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	N	No safety concern
(<i>S</i>)-1-(3-(((4-Amino-2,2-dioxido-1 <i>H</i> -benzo[<i>c</i>][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	N	No safety concern
2-(4-Methylphenoxy)- <i>N</i> -(1 <i>H</i> -pyrazol-3-yl)- <i>N</i> -(thiophen-2-ylmethyl)acetamide	2237	N	No safety concern

N: new specifications

H.Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes, and acids

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
8-Methyldecanal	2238	N	No safety concern
8-Methylnonanal	2239	N	No safety concern

N: new specifications

Food additives considered for specifications only

Flavouring agent	No.	Specifications
L-Menthyl lactate	433	R ^a
L-Malic acid	619	R ^b
Glutamyl-valyl-glycine	2123	R ^c

R: existing specifications revised

^a The CAS number was changed from 59259-38-0 to 61597-98-6 and the name to *L*-menthyl *L*-lactate.

^b The specification for specific rotation were removed.

^c The melting point range was revised.

Annex 3

Summary of the safety evaluation of the secondary components for flavouring agents with minimum assay values of less than 95%

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers				
1519	2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	93%	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (1–3%) Butyric acid (No. 87) (1–3%)	The SPET value for No. 1519 is 1 200 µg/day, and 3% of this value is 36 µg/day, which is below the class III threshold of toxicological concern. Butyric acid (No. 87) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
1524	Furfuryl 2-methyl-3-furyl disulfide	90%	Di-(2-methyl-3-furyl) disulfide (6–7%)	The SPET value for No. 1524 is 10 µg/day, and 7% of this value is 0.7 µg/day, which is below the class III threshold of toxicological concern. The major secondary component of No. 1524 is therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1524 as a flavouring agent.
Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters				
2241	2,6-Dimethyl-5-heptenol	>90%	2,6-Dimethyl-5-heptenal (No. 349) (1–6%)	2,6-Dimethyl-5-heptenal (No. 349) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
Maltol and related substances				
2252	Ethyl maltol isobutyrate	93%	Ethyl maltol (No. 1481) (2–3%)	Ethyl maltol (No. 1481) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.



Annex 4

Meeting agenda



Food and Agriculture
Organization of the
United Nations



World Health
Organization

86th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
WHO Headquarters, Geneva 12 – 21 June 2018

Draft Agenda

1. Opening
2. Declarations of Interests (information by the Secretariat on any declared interests and discussion, update by experts)
3. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs
4. Adoption of Agenda
5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives (CCFA)
6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full committee)
7. Evaluations

Food Additives

7.1. Toxicological Evaluation, Exposure Assessment, and Establishment of Specifications:

- Basic methacrylate copolymer (INS 1205)
- Neutral methacrylate copolymer (INS 1206)
- Anionic methacrylate copolymer (INS 1207)
- Lutein from *Tagetes erecta* (INS 161b(i))
- Spirulina extract
- Erythrosine (INS 127)
- Indigotine (INS 132)

7.2. Food additives for revision of specifications and analytical methods:

- Citric and fatty acid esters of glycerol (INS 472 c)
- Glycerol ester of wood rosin (GEWR) (INS445(iii))
- Cassia gum
- Dextrin roasted starch (INS 1400)
- Acid treated starch (INS 1401)
- Alkaline treated starch (INS 1402)
- Bleached starch (INS1403)
- Enzyme-treated starch (INS 1405)
- Monostarch phosphate (INS 1410)
- Distarch phosphate (INS 1412)
- Phosphated distarch phosphate (INS 1413)
- Acetylated distarch phosphate (INS 1414)
- Acetylated distarch adipate (INS 1422)
- Hydroxypropyl starch (INS 1440)
- Hydroxypropyl distarch phosphate (INS 1442)
- Starch sodium octenyl succinate (INS 1450)

Flavourings

7.3 Toxicological evaluation, exposure assessment and establishment of specifications for certain flavourings

- Miscellaneous nitrogen-containing substances
- Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes, and acids
- Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids, and related esters
- Carvone and structurally related substances
- Menthol and structurally related substances
- Maltol and related substances
- Alicyclic primary alcohols, aldehydes, acids and related esters (reevaluation)
- Furan substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers.(reevaluation)

8. Revision of specification for certain flavourings
9. Other matters to be considered (general considerations)
Update of EHC240:
 - Development on guidance on the evaluation of genotoxicity studies
 - Updated guidance on dose-response modelling for the use in risk assessment
 - Steviol glycosides and frame specifications/frame toxicological profiles
10. Other matters as may be brought forth by the Committee during discussions at the meeting.
11. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of certain veterinary drug residues in food

Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1008, 2017 (150 pages)

Safety Evaluation of Certain Food Additives

WHO Food Additives Series, No. 75, 2018 (244 pages)

Evaluation of Certain Food Additives

Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1007, 2017 (92 pages)

Safety Evaluation of Certain Contaminants in Food

WHO Food Additives Series, No. 74, 2018 (897 pages)

Evaluation of Certain Contaminants in Food

Eighty-third Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No.1002, 2017 (166 pages)

Evaluation of Certain Food Additives

Eighty-second Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1000, 2016 (162 pages)

Evaluation of Certain Veterinary Drug Residues in Food

Eighty-first Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 997, 2016 (110 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food

Eighty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 72, 2016 (162 pages)

Evaluation of Certain Food Additives and Contaminants

Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 995, 2016 (114 pages)

Safety Evaluation of Certain Food Additives and Contaminants

Eightieth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 71, 2015 (132 pages)

Further information on these and other WHO publications can be obtained from
WHO Press, World Health Organization ■ 1211 Geneva 27, Switzerland ■ www.who.int/bookorders
tel.: +41 22 791 3264; **fax:** +41 22 791 4857; **email:** bookorders@who.int

Evaluation of certain food additives

This report represents the conclusions of a Joint FAO/WHO Expert Committee (JECFA) convened to evaluate the safety of various food additives, including flavouring agents, with a view to concluding on safety concerns and to prepare specifications for the identity and purity of the food additives.

The first part of the report includes updates on the work of the Codex Committee on Food Additives (CCFA) since the eighty-fourth meeting of JECFA and on activities relevant to JECFA with regard to the *Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food* (EHC 240). Following is a summary of the Committee's evaluations of technical, toxicological and dietary exposure data for eight food additives other than flavouring agents – anionic methacrylate copolymer; basic methacrylate copolymer; erythrosine; indigotine; lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic); neutral methacrylate copolymer; sorbitol syrup; and spirulina extract – and eight groups of flavouring agents – alicyclic primary alcohols, aldehydes, acids and related esters; carvone and structurally related substances; furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers; linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters; maltol and related substances; menthol and structurally related substances; miscellaneous nitrogen-containing substances; and saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids.

Specifications and analytical methods were revised for the following food additives other than flavouring agents: cassia gum; citric and fatty acid esters of glycerol (CITREM); glycerol ester of wood rosin (GEWR); and modified starches.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to all of the food additives as well as toxicological information, dietary exposures and information on specifications.

ISBN 978 92 4 121023 2



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FOREWORD

INTRODUCTION

MENTHOLS

CASN°:2216-51-5, 15356-60-2, 89-78-1, 1490-04-6

SIDS Initial Assessment Report

For

SIAM 16

Paris, 27-30 May 2003

- 1. Chemical Category:** Menthols
- 2. CAS Number:** Menthols Category:
L-Menthol CAS No: 2216-51-5
D-Menthol CAS No: 15356-60-2
D/L-Menthol CAS No: 89-78-1
Menthol CAS No: 1490-04-6
- 3. Sponsor Country:** Germany
Contact Point:
BMU (Bundesministerium für Umwelt, Naturschutz und
Reaktorsicherheit)
Prof. Dr. Ulrich Schlottmann
Postfach 12 06 29
D- 53048 Bonn-Bad Godesberg
- 4. Shared Partnership with:**
- 5. Roles/Responsibilities of the Partners:**
 - Name of industry sponsor /consortium Bayer AG, Germany
Contact person:
Dr. Burkhardt Stock
D-51368 Leverkusen
Gebäude 9115
 - Process used See next page
- 6. Sponsorship History**
 - How was the chemical or category brought into the OECD HPV Chemicals Progran? by ICCA-Initiative
- 7. Review Process Prior to the SIAM:** last literature search (update):
9 June 2002 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms
27 June 2002 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms
- 8. Quality check process:** As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA.
- 9. Date of Submission:** 20 August 2002
- 10. Date of last Update:** August 2003

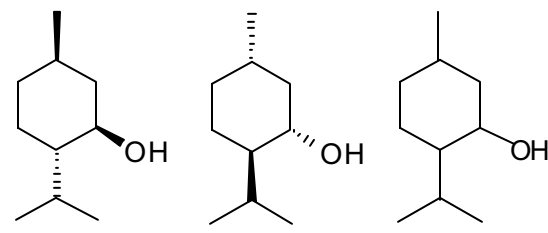
11. Comments:**OECD/ICCA - The BUA^{*} Peer Review Process**

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability (4), i.e. reliability not assignable)
- Review of validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing

^{*} BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	2216-51-5	15356-60-2	89-78-1 (former CAS No. 15356-70-4)	1490-04-6
Chemical Name	L-Menthol	D-Menthol	D/L-Menthol	Menthol
Structural Formula	 L-Menthol D-Menthol Menthol			

SUMMARY CONCLUSIONS OF THE SIAR

Category Rationale

The menthols category is comprised of the isomers L-menthol, D-menthol, the racemate and menthol (unspecified isomers). The menthols can be considered as a category because of their similarity in physico-chemical, toxicological, ecotoxicological and environmental fate properties.

Human Health

L-, D/L- and the unspecified menthol isomer are well absorbed by the oral route of exposure and are mainly excreted as glucuronides. In rats an extensive enterohepatic circulation additionally leads to various hydroxylated degradation products. Glucuronides and degradation products are eliminated mainly via urine, minor quantities via the faeces.

All menthol isomers are of very low acute oral toxicity with LD₅₀ values normally greater than 2000 mg/kg bw. Clinical signs of intoxication are unspecific, and included apathy and reduced activity. Based on old and limited studies for the racemate and the unspecified isomer, it can be assumed that the acute dermal toxicity of the menthol isomers is low.

All studied isomers of menthol are moderately irritating to the skin and slightly irritating to the eye. The skin sensitization potency of menthol isomers in animals and humans is low.

In rats given = 200 mg/kg bw/d of L-menthol in soybean oil by gavage for 28 days, increased liver weights and a non dose-related vacuolization of hepatocytes were reported. The relevance of these findings remains unclear and a NOAEL could not be derived from this study. No toxicity was observed in rats receiving diets providing up to 200 mg/kg bw/d of either L- or D/L menthol for 5.5 weeks. Therefore for L-menthol and the racemate D/L-menthol a NOAEL of 200 mg/kg bw/d can be deduced from this study. Irritant effects on lungs and trachea, but no systemic effects were found in rats that were whole body exposed to L-menthol vapour for 71-79 days.

D/L-menthol administered with the diet for 13 weeks to rats (up to 937/998 mg/kg bw/d for males/females) and mice (up to 3913/4773 mg/kg bw/d for males/females) did not induce any effects on organ weights. Microscopic examination of a comprehensive range of tissues revealed a slight increase in the severity of spontaneous interstitial nephritis in the male rats at the highest dose level. The only effect seen in mice of both sexes was a reduction in body weight gain in the highest dose group. The NOAELs derived from these studies were 937 mg/kg bw/d for the male rat, 998 mg/kg bw/d for the female rat and 1956 mg/kg bw/d for the male mouse and 2386 mg/kg bw/d for the female

mouse.

In a 103-week feeding study in rats with D/L menthol (about 188 and 375 mg/kg bw/d), the only effect was a slight increase in spontaneous, chronic inflammation of the kidney in male rats of both dose groups, and a slightly reduced body weight in female rats. The NOAELs in this study were 375 mg/kg bw/d for male rats, and 188 mg/kg bw/d for female rats. In a 103-week feeding study in mice with D/L menthol (about 334 and 667 mg/kg bw/d), the NOAEL for both sexes was 667 mg/kg bw/d.

Because the racemate D/L-menthol contains the D- and L-isomers in equal proportions, the study results with the racemate are considered adequate for the evaluation of the D-isomer and of the L-isomers. This view is further supported by the FAO/WHO 1999 safety evaluation on menthol, where the FAO/WHO expert committee had concluded that "the limited data that allow comparisons of metabolism and toxicity provide no indication of a difference in the toxicity of L-menthol and D/L-menthol". Overall it can therefore be concluded that the D-, L- and D/L- menthol isomers induce no specific systemic effects and are well tolerated after repeated oral administration.

The menthol isomers are considered non-genotoxic in *in vitro* bacterial and mammalian test systems. *In vivo*, L- and D/L-menthol have demonstrated no mutagenic potential in adequately performed dominant lethal and cytogenetic tests and in a bone marrow micronucleus test in mice.

D/L-Menthol showed no evidence of carcinogenic activity in 2-year studies performed in accordance with current standards in rats and mice (highest tested dose levels in rats approx. 375 mg/kg bw/d, in mice approx. 667 mg/kg bw/d).

There is no fertility study available. Histopathological examinations of the reproduction organs of rats and mice showed no changes in repeated dose toxicity studies with D/L-menthol and also in carcinogenicity studies with D/L-menthol. Hence there is no indication of a potential of D/L-menthol to interfere adversely with reproduction.

L-Menthol was not embryo- or fetotoxic and had no teratogenic properties in well performed gavage studies in various species (rat, mouse, rabbit, hamster) at not maternally toxic doses (185-425 mg/kg bw/d). No maternally toxic dose levels were used in these studies.

In summary, the available toxicity data indicate very similar toxicity profiles for all of the menthol isomers investigated.

Environment

Menthols have a melting point of ca. 40 °C, a density of about 0.9 g/cm³ (20 - 25 °C). A vapor pressure of 8.5 Pa (25 °C) was measured for L-menthol and an unspecified isomer mixture. This value was also used for the other two category members. The measured water solubilities were in the range of 420 - 500 mg/l (20 °C). The log Kow is measured to 3.4 for L-menthol and D/L-menthol. This value can be read-across to the other two category members.

According to a Mackay Level I model calculation, the main target compartments for menthols are air (39.5 - 44.2%) and water (40.5 - 43.8 %). In the atmosphere menthols are indirectly photodegradable by hydroxyl radicals with $t_{1/2}$ = 16 hours. The calculated Henry's law constant of 2.6 - 3.2 Pa·m³/mol indicates the menthol isomers to be volatile from aqueous solution. Under environmental conditions, neither hydrolysis nor direct photolysis of menthols is to be expected. The ready biodegradability of menthols was shown in two recently performed Closed Bottle Tests for L- and D-menthol (L-menthol: 79-92 % after 28 d, D-menthol: 76-92 % after 28 d, 10d-window for both isomers was fulfilled). Experimentally determined BCF values in the range of <0.5-15 l/kg indicate no significant bioaccumulation potential of menthols.

For the toxicity of menthols on aquatic species experimental results from tests with fish, daphnids and algae are available for L-menthol and D/L-menthol. The data for the two category members within each trophic level are in the same order of magnitude. D/L-menthol contains the D- and L- isomers, thus effect values obtained with this mixture should cover the toxicity of D-menthol and the unspecific isomer mixture. Therefore, all available effect values can be regarded together for the assessment of this category. In acute toxicity tests the following results were obtained:

fish (3 species):	48- 96h LC50 = 15.6 – 26 mg/l;
invertebrates (<i>Daphnia magna</i>):	24h LC50 = 37.7 - 71 mg/l; 48h LC50 = 26.6 mg/l
algae (<i>Scenedesmus subspicatus</i>):	72h ErC50 = 16.2 – 21.4 mg/l, 72h NOEC = 5 - 9.65 mg/l.

Applying an assessment factor of 1000 to the lowest ErC50 for algae, a PNEC_{water} of 16.2 µg/l is calculated. This

PNEC is valid for the whole category. Tests on long-term toxicity on aquatic species as well as on terrestrial species are not available. Two tests on sludge respiration are available with EC10 values of 117 and 51 mg/l.

Exposure

About 13,600 tonnes of menthols were produced worldwide in 2001. About 75 % of the menthol output is of biotic and 25 % of synthetic origin. L-Menthol, D/L-menthol and menthol liquid are widely used in oral care products, pharmaceuticals, flavors, tobacco and others. D-menthol is not commonly distributed and only used for scientific purposes. The major route of occupational exposure to menthol isomers is supposed to be inhalation. The most significant routes of consumer exposure are likely to be dermal and oral.

RECOMMENDATION

The chemicals in the Menthols category are currently of low priority for further work.

**RATIONALE FOR THE RECOMMENDATION AND
NATURE OF FURTHER WORK RECOMMENDED**

Human Health: The chemicals in the menthols category are currently of low priority for further work because of their low hazard potential. However, skin and eye irritation is noted.

Environment: The chemicals in the menthols category are currently of low priority for further work. The chemicals possess properties indicating a hazard for the environment. Although these hazards do not warrant further work as they are related to acute toxicity which may become evident only at very high exposure levels, they should nevertheless be noted by chemical safety professionals and users.

SIDS Initial Assessment Report

1 IDENTITY

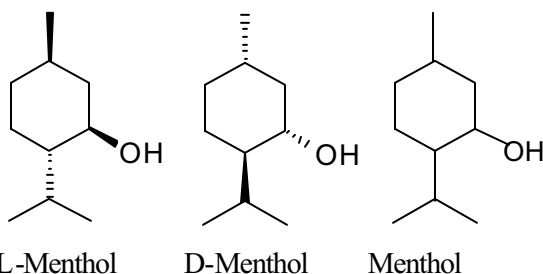
1.1 Identification of the Substance

CAS Number: L-Menthol CAS No: 2216-51-5
 D-Menthol CAS No: 15356-60-2
 D/L-Menthol CAS No: 89-78-1
 Menthol CAS No: 1490-04-6

IUPAC Name: cyclohexanol, 5-methyl-2-(1-methylethyl)-

Molecular Formula: C₁₀H₂₀O

Structural Formula:



Molecular Weight: 156.27 g/mol

The menthol molecule has 3 stereo centers, i.e. there are 8 possible stereoisomers. In nature the compound occurs generally as L-menthol as a component of e.g. peppermint oil, *Mentha piperita*, *Mentha* oil etc. Peppermint oil contains about 35 – 60 % menthol (menthone (15 - 30 %), methylacetate (4 -14 %), and small amounts of cineole and other terpenes) (Nair, 2001)

There are 4 menthol products of technical importance:

Substance	Synonyms	CAS-No.	Remark
L-Menthol	(-)-Menthol Menthol, (1R, 3R, 4S)-(-)-	2216-51-5	Natural or synthetic menthol
D-Menthol	(+)-Menthol	15356-60-2	non marketed by-product
D/L-Menthol	Racemate, "D/L-Menthol pure"	89-78-1 former CAS-No.: 15356-70-4	Synthetic product
Menthol	"D/L-Menthol raw"	1490-04-6	Unspecified mixture of isomers

1.2 Physico-Chemical properties

The menthol products are white solids with a minty odour. The physico-chemical properties of the products are (for references cf. IUCLID datasets):

Substance	L-Menthol	D-Menthol	D/L-Menthol	Menthol
CAS-No.	2216-51-5	15356-60-2	89-78-1	1490-04-6
Melting point	Ca. 42 °C	43 °C	30-32 °C	
Boiling point (1013 hPa)	212 °C	216.5°C	216 °C	215.5 °C
Density	0.89 g/cm ³ (20 °C)		0.895 g/cm ³ (20 °C)	0.898 g/cm ³ (25 °C)
Vapour pressure	0.085 hPa (25 °C)		1.3 hPa (55 °C)	0.085 hPa (25 °C)
Log Kow	3.4	3.4 (read-across from value for L-menthol and D/L-menthol)	3.4	3.4 (read-across from value for L-menthol and D/L-menthol)
Water solubility	431 mg/l (20 °C)		508 mg/l (20 °C)	420 mg/l (20 °C)

The enantiomeric menthols have identical physical properties (apart from their specific rotation), but the racemates differ from the optically active forms in, for example, their melting points (Ullmann 2002). The slight differences in the cited data are within the range of uncertainty of laboratory tests.

Of particular importance for the environmental behaviour and ecotoxicity are the values for partition coefficient (log Kow), vapour pressure and water solubility. The partition coefficient (log Kow) was measured for L-menthol and D/L-menthol to be 3.40 for both, and thus can be calculated for D-menthol and any mixture of D-menthol and L-menthol. Water solubility was determined for three substances. Due to the similar molecular structures, no significant differences in these parameter can be expected, thus the values are acceptable for D-menthol.

The vapour pressure at environmental relevant temperatures was determined for L-menthol and an unspecified isomer mixture. As well as for the parameters mentioned above, similar values for vapour pressure can be expected for D-menthol and the racemate, thus the values are acceptable as well.

1.3 Category Justification

See Annex 1

2 GENERAL INFORMATION ON EXPOSURE

World-wide production capacity of menthol was estimated to be about 13,600 tonnes in 2001. Menthol is a naturally occurring compound of plant origin, which gives plants of the mentha species (*Mentha piperita*, *Mentha arvensis*) the typical flavour. Two general ways of manufacturing menthol isomers exist: each menthol isomer can be generated synthetically and L-menthol may be produced via plant extraction. 25 % of the yearly menthol output is of synthetic and 75 % of biotic origin (Haarmann and Reimer, 2002). The world-wide production figures can be split up as follows:

Western Europe	1 producer	10 %
USA	1 producer	9 %
Russia		0 %
China	10 - 20 producers	34 %
India	10 big to medium size producers and several dozens small producers	35 %
Japan	3 producers	7 %
Other Asia	approx. 5 producers	3 %
Other World	approx. 5 producers	2 %

In Western Europe, menthol is currently produced only in Germany, realised in a batch process. D/L-menthol is produced via reaction of m-cresol with propene to thymol, and hydrogenation of thymol, resulting in 4 isomers: D/L-neomenthol, D/L-neoisomenthol, D/L-menthol and D/L-isomenthol. D/L-menthol is isolated by fractional distillation and is dispatched to the production site of L-menthol. Neomenthol, isomenthol and neoisomenthol are epimerized and given back to the distillation process.

To produce L-menthol, D/L-menthol is transesterificated with methylbenzoate and further manufactured. Resulting products are L- and D-menthol. The raw L-menthol is filled in barrels or containers. After crystallisation the solid L-menthol is filled in air-tight packages. D-Menthol as a side-product of this process is filled in rail tank cars and transported to the plant, where D/L-menthol is produced. There it is isomerized (Haarmann and Reimer, 2002). All intermediate products arising during the production process of L-menthol are interim stored in tanks. In China, Japan, India and other Asian countries L-menthol is supposed to be mainly produced from commint oil (*Mentha arvensis*), which contains 70 - 90 % menthol, via crystallisation. The Mentha oils are extracted from the plants by steam distillation. No exposure information for this production procedure is available.

L-Menthol, D/L-menthol and menthol liquid are widely used as flavoring, disinfectant and cooling compounds in confectionery products, liqueurs, chewing gums, toothpastes, cosmetics and common cold ointments and medications for human purposes (Haarmann and Reimer, 2002, Gestis Stoffdatenbank [Information on hazardous substances of the Berufsgenossenschaften - German Institutions for statutory accident insurance and prevention]). D-Menthol is not commonly distributed and only used for scientific purposes. L-Menthol is marketed in solid form and isomeric mixtures of menthol in liquid form.

The estimated use pattern for L-menthol is as follows (Haarmann and Reimer, 2002):

- 36 % Oral Care
- 22 % Pharma
- 17 % Flavors
- 12 % Tobacco

7 % Chewing Tobacco

6 % Others

In Canada menthol is registered for control of mites (*Acarapis woodi*) in apiculture (Westcott and Winston, 1999).

In the Swiss Product register (2002), 68 products, among them 49 consumer products containing D/L-menthol are listed with concentrations up to 10 %. Product types are paints and lacquers, adhesives, metal care products, cleaning products, shoe- and leather-care products, disinfectants, solvents, cosmetics, odor improvers, repellents and animal care products.

In the Danish Product register (2002) menthols are listed in a total of 95 products in amounts up to 50 %. Product types are cosmetics and odor agents with menthol concentrations up to 20 %. The most frequent industry groups are farming of cattle and other animals, manufacture of foodstuffs, manufacture of pharmaceutical and medicinal chemicals and of cleaning products.

The Swedish product register (2002) lists 37 products, 18 of those are consumer products containing menthol up to 5 %. The main uses are in cosmetics, hygienic articles and veterinary medicine.

Uses identified in sources available to the US, include the following (Westat, 1987 a,b,c):

Product (No identified)	Weight fraction
Non-prescription decongestants (1)	0.4
External analgesics and counterirritants (8)	0.018 – 0.165
Pharmaceutical skin preparations (1)	0.1
Aftershave (4)	0.001
Shampoo (2)	0.005
Oral hygiene products (6)	0.001 – 0.013
Cosmetics and toiletries (1)	0.001
Facial scrubs and masks (1)	0.001
Shaving soap and cream (1)	0.001

Release during production

Easily accessible information on exposure from production of the chemical in the sponsor country is available at Bayer AG.

The Bayer menthol synthesis plant continuously produces an isomer mixture of D/L-menthol. The exhausts from menthol production are connected to a thermal exhaust purification (in other menthol plants, exhaust air of the manufacture process is usually collected and purified in a gas washer). Some tanks are equipped with activated carbon filters to enable tank respiration. Thus during normal operation no menthols are emitted.

The production process and the filling of the product are executed in a closed system. In general there is no sewage leaving the menthol production. Extremely low quantities of menthols are released into the wastewater due to maintenance, e.g. from cleaning of changed parts. The wastewater of the menthol production unit is generally checked for TC at the production plant before reaching the industrial wastewater treatment plant.

The comparison of influent and effluent concentrations of the industrial wastewater treatment plant in Uerdingen is not possible due to low influent and effluent concentrations. The effluent

concentrations of menthols were always below the detection limit of 0.01 mg/l. This equals a maximum emission of 80 kg/a, however, the true emission is far below this maximum.

For the receiving water a maximum PEC of 14×10^{-3} µg/l is calculated taking in account the 10 percentile of the river flow (1050 m³/s), the dilution factor of 700 (derived from effluent volume of 1.5 m³/s), and the detection limit (10 µg/l; Bayer AG, 2002a)

Releases from products

Consumer use of oral care products, pharmaceuticals and flavours containing menthol also leads to environmental releases, mainly into the hydrosphere. There are no data available on emissions of menthol into the environment from consumer use.

Releases into the environment may also occur from the use in agents that contain menthol in veterinary agents or in the acaricidal treatment of honey bees against parasitic mites. However, a quantification is not possible.

2.1 Environmental Exposure and Fate

Distribution

As the main physico-chemical properties of the menthol isomers are in the same order of magnitude, the environmental distribution behaviour is expected to be similar.

The distribution of menthols in a “unit world” was calculated according to the Mackay fugacity model level I (Mackay, 1991), considering the values for vapour pressure (8.5 Pa), log Kow (3.4) and water solubility (431 mg/l for L-menthol and D-menthol, 508 mg/l for D/L-menthol and 420 mg/l for menthol). The main target compartments were estimated to be air (39.5 – 44.2 %) and water (40.5 – 43.8%), whereas soil (8.0 - 8.7 %) and sediment (7.3 - 8.1 %) are expected to be of minor importance.

The distribution of menthols between aqueous solutions and air can be calculated from water solubility and vapour pressure. Using solubilities of 420 - 508 mg/l and a vapour pressure of 8.5 Pa (25°C), Henry's law constants of 2.61 - 3.16 Pa.m³/mol are obtained, indicating the menthol isomers to be volatile from aqueous solution according to the criteria of Thomas (1990).

Using a fragment constant estimation method (not further specified), a Henry's law constant of 1.5 Pa.m³/mol was calculated for L-menthol, corresponding to volatilization half-lives of 2 days for a model river (1 m deep, flow-rate 1m/s, wind velocity 3 m/s) and 18 days for a model lake (1 m deep, flow-rate 0.05 m/s, wind velocity 0.5 m/s) (HSDB, 2001). Because of the structural similarities these values are expected to be similar for the other menthol isomers.

The distribution between the organic phase of soil or sediment solids and porewater can be calculated from the octanol/water partitioning coefficient. Using a log Kow of 3.4 and the equation $\log K_{oc} = 0.52 \log K_{ow} + 1.02$ (EC, 1996) a Koc value of 614 l/kg can be calculated, indicating a moderate sorption potential of the menthol isomers to soil organic matter according to the criteria of Blume (1990).

Degradation

A calculation of the indirect photodegradability of menthols in the atmosphere by hydroxyl radicals according to a structure estimation method revealed a rate constant of 2.4×10^{-11} cm³/molecule/s. Based on an atmospheric concentration of 500,000 OH-radicals/cm³, a half-life of about 16 hours was estimated (SRC-AOPWIN v. 1.90). Based on the chemical structure menthols are not expected to undergo direct photolytical degradation in the hydrosphere because of the lack of a chromophore

group. Furthermore, hydrolytic degradation of menthol is not to be expected based on the chemical structure.

Several studies on the ready biodegradability of menthols are available. In a modified OECD screening test (OECD 301E) using L-menthol as test substance the DOC decrease was determined to be 53% after 7 days, 93% after 14 days and 100 % after 28 days (Bayer AG, 1992a). No information on possible volatilisation and/or adsorption of the test substance is available. Therefore, it cannot be excluded that a significant amount of L-menthol was removed from the test system by this processes and the test is regarded as invalid. No conclusion on the ready biodegradability of this substance can be drawn.

On the other hand, a MITI I test (not specified, whether L- or D/L-menthol was used; in the literature source both CAS-numbers are referred) resulted in 0% oxygen consumption after 28 days of incubation (MITI, 1992). In tests with activated sludge EC50 values in the range of 237 – 306 mg/l have been found for L-menthol and D/L-menthol. Therefore, it cannot be excluded that the inoculum was (partly) inhibited by the employed test concentration of 100 mg/l and also this test is regarded as invalid.

A test on inherent biodegradability was conducted by Pitter (1976). The test design is comparable to the Zahn-Wellens-test. The test substance “menthol” (not further specified) was the sole source of carbon. Based on COD measurement a removal of 95% within 5 days was obtained in an open system after 20 days of adaptation. Again, no information is available about possible volatilisation and/or adsorption.

The positive result of the test conducted by Pitter indicates that an unspecified menthol isomer may be inherently biodegradable. It remains unclear, however, whether menthols are readily biodegradable.

To enable the assessment on the ready biodegradation of menthols, two tests according to OECD 301D (Closed Bottle Tests) were performed with both D-menthol and L-menthol. Two concentrations were tested in each test. For L-menthol a biodegradation of 92 % after 28 d was obtained using a concentration of 0.84 mg/l. With a concentration of 2 mg/l a biodegradation of 79 % after 28 days was found. For both concentrations the 10d window-criterion was fulfilled (TNO, 2003a). For D-menthol nearly the same result was found. At a concentration of 0.84 mg/l a biodegradation of 92 % after 28 days was found and at a concentration of 2 mg/l the biodegradation after 28 days was 76 %. For both concentrations the 10d window-criterion was fulfilled (TNO, 2003b). From these studies it can be concluded that both D-menthol and L-menthol are readily biodegradable.

Bioaccumulation

The bioaccumulation of menthols in fish (*Cyprinus carpio*) was determined in a test according to OECD guideline 305 C. It is unclear whether D- or L-menthol was used, in the literature source both CAS-numbers are referred. BCFs in the range of <0.5 - 15 l/kg with 0.2 mg menthol/l resp. < 4.6 - 11 l/kg with 0.02 mg menthol/l were reported (MITI, 1992) indicating no significant bioaccumulation potential. The variation of results may be partially explained by the variation of the lipid content in fish (2 - 6 %).

Summary of Environmental Fate

The available data reveal that menthols released into the atmosphere are rapidly degraded by OH-radicals with an estimated half-life of 16 hours. For menthols released into the aquatic environment, the calculated Henry's law constants indicate evaporation from surface waters within 2 - 18 days. Biotic degradation in surface waters is a relevant removal mechanism. From newly performed tests

it can be concluded that menthols are readily biodegradable. The calculated K_{oc} of 614 l/kg indicates a moderate sorption potential of menthols onto sediment or suspended solids.

The results of an available fish test indicate no significant bioaccumulation potential of menthols.

2.2 Human Exposure

The major route of occupational exposure to menthol isomers is the inhalation route. Although dermal exposure may also occur, the volatility of menthol isomers is high enough to limit the extent of absorption through the skin (see Henry's law constant). The main sources of occupational exposure are menthol manufacture, intertank transfers and cleaning processes. Further sources of occupational exposure in the processing industry are the following: manufacture of cosmetics, medical ointments, toothpastes, manufacture of sweets, liqueurs, and chewing gums (Haarmann and Reimer, 2002).

The Bayer menthol synthesis plant continuously produces an isomer mixture of D/L-menthol from m-cresol and propene via thymol in a closed system. The exhausts from menthol production are connected to a thermal exhaust purification. Some tanks are equipped with activated carbon filters to enable tank respiration.

Although for menthol and thymol there are no occupational exposure limits (OELs) like MAK, to ensure protection of workers at the workplace, thymol was used as an indicator for menthol and other expositions in the Bayer menthol manufacturing plant in 1990/91. Thymol has chemical properties similar to menthol, e.g. a melting point of about 50 °C and a boiling point of 233 °C. The results of the thymol measurements were < 0,5 mg/m³ and < 0,8 mg/m³ in the Bayer menthol manufacturing factory (Bayer AG, 2002a).

Since menthol is a food ingredient (FAO/WHO) and generally recognized as safe (US FDA), it was decided not to monitor menthols at the workplace in the Bayer production plant (Bayer AG, 2002a). In the Haarmann and Reimer menthol processing plants e.g. from olfaction and measurements of solvents used in the manufacturing process it is assumed that the menthol concentrations at their workplaces are very low.

The most significant routes of consumer exposure to menthol isomers from the use of mentholated products are likely to be dermal and oral. Consumers may also be exposed to menthol through the inhalational route, e.g. by smoking mentholated cigarettes, or by odour improving agents. Consumer exposure to menthol isomers is possible from a number of different sources including oral care products, pharmaceuticals, food and flavouring products, tobacco, and others.

In a survey of flavoring usage levels, the Flavoring Extract Manufacturing Association has summarized "average maximum use levels" on which an expert panel based its judgements that the substances are generally recognized as safe (Hall and Oser 1965). For menthol these levels are (in ppm):

Beverages	35
Ice cream	68
Candy	400
Baked goods	130
Chewing gums	1100

Recommended levels for the addition of L-menthol to products are e.g. (Hopp 1993)

Oral care products (concentrated mouthwashes)	up to 2 %
Pharmaceuticals (medicated oils)	up to 4 %
Menthol cigarettes	up to 0.45 %
Pipe tobacco	up to 0.3 %
Perfumed products (e.g. refreshing towels, cooling gels)	up to 1 %

Additional information of identified uses from the US are given under section 2, “General Information on Exposure”.

Information on consumer products from Product Registers revealed that the highest menthol concentrations are reported for cosmetics (2-20%), and odor agents (2-20%) (Danish Product Register, 2002). Concentrations in the range between 1 and 10% may be found in paints, animal care products, veterinary medicines, repellents, and cleaning products (Danish Product Register, 2002; Swedish Product Register, 2002; Swiss Product Register, 2002). Menthol concentrations of max. 1 % are reported for shoe- and leather care products and disinfectants (Swiss Product Register, 2002).

The Joint FAO/WHO Expert Committee on Food Additives derived in their 51st meeting in 1998 an acceptable daily intake (ADI) for L-menthol and D/L-menthol in the range of 0 - 4 mg/kg bodyweight (FAO/WHO 1999).

Exposure to menthol also occurs through the use of peppermint oil, since menthol is the primary component of peppermint oil (35 - 60 %) (Nair, 2001). Peppermint oil is used in cosmetic formulations, in the manufacture of chewing gum, confectionery, toothpastes and pharmaceutical products.

Potential exposure to menthol isomers from drinking water and ambient air is expected to be negligible (Haarmann and Reimer, 2002).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics

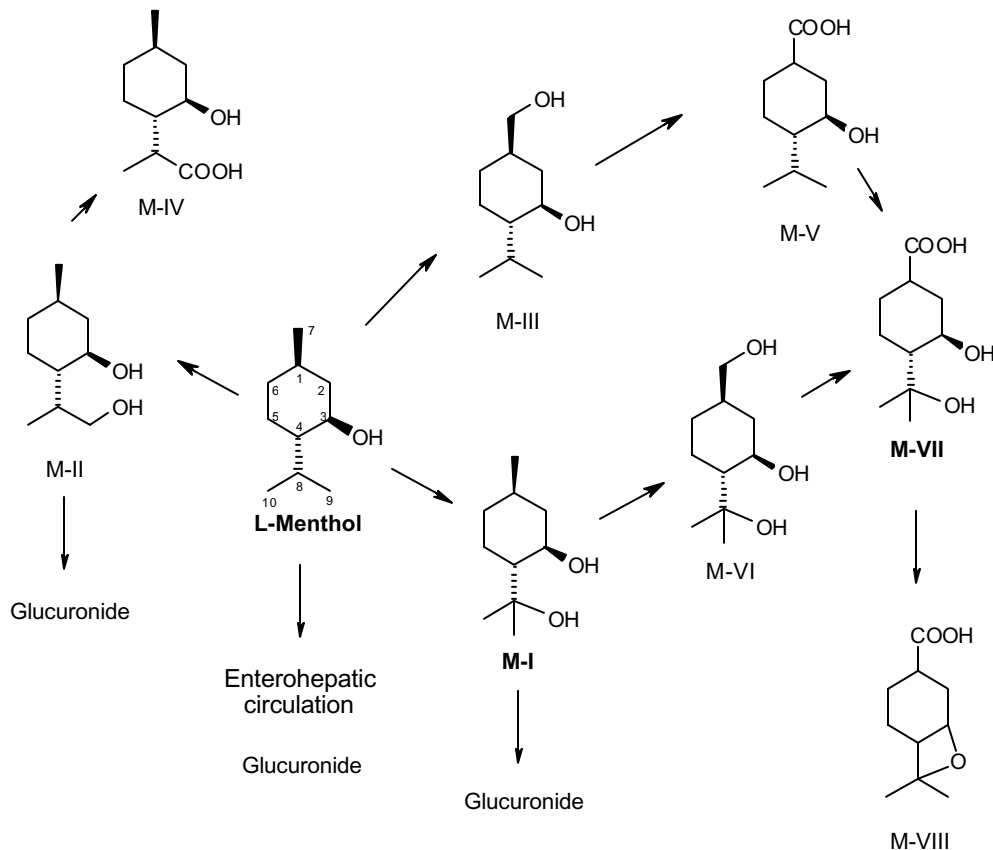
Absorption

From the studies on metabolism with L-, D/L- and the unspecified menthol isomer mixture it can be concluded that menthol is well absorbed by the oral route (Madhava Madyastha and Srivatsan, 1988; Yamaguchi, et al., 1994, Williams, 1938, Atzl et al., 1972). Dermal absorption is slower than oral absorption (Atzl et al., 1972). No quantitative data are available. From case reports it also can be concluded, that absorption by inhalation is very efficient (Atzl et al., 1972).

Metabolic transformation

The metabolic pathways of L-menthol have been studied in detail in two investigations with rats (Madhava Madyastha and Srivatsan, 1988; Yamaguchi, et al., 1994). In the first investigation IISc rats were treated by gavage with 800 mg L-menthol/kg bw/d for 20 days, urine was collected daily and metabolites in urine were analyzed. In the second investigation 3-tritium-L-menthol was administered by gavage to intact and bile duct-cannulated male Fischer 344 rats at a dose level of 500 mg/kg and metabolites in urine, faeces and bile were analyzed up to 48 h after administration. The investigations on intact rats show that menthol is rapidly glucuronidated and excreted in urine and faeces. In the studies with bile duct-cannulated rats it was shown that biliary excretion is rapid and extensive and that menthol undergoes an intensive enterohepatic circulation. After cleavage of the glucuronide and reabsorption in the small intestine it is further metabolized in the liver. It is proposed, that the first step is hydroxylation at the C-8 position, followed by oxidation of the C-1 methyl group (C7) to a carboxylic group. Further it is hydroxylated at the C-9 position. p-Menthane-3,8-diol (M-I) and 3,8-dihydroxy-p-menthane-7-carboxylic acid (M-VII) were identified as major metabolites (not further quantitated) in the urine in both studies. Further (minor urinary) metabolites were p-menthane-3,9-diol (M-II), 3,8-oxy-p-menthane-7-carboxylic acid (M-VIII), 3-hydroxy-p-menthane-9-carboxylic acid (M-IV), 3-hydroxy-p-menthane-7-carboxylic acid (M-V), p-menthane-3,7-diol (M-III) and p-menthane-3,7,8-triol (M-VI) (Madhava Madyastha and Srivatsan, 1988; Yamaguchi, et al., 1994, see Figure 1). Part of these metabolites are excreted as glucuronides.

Fig. 1: Proposed metabolic pathway scheme of L-menthol (Yamaguchi, et al., 1994)



In the investigation of Madhava Madyastha it was further found, that repeated oral administration of L-menthol for 3 days induced cytochrome P450 and the NADPH-cytochrome c reductase activity in the liver of rats by nearly 80%. Further treatment (for 7 days) reduced their levels considerably, although the levels were still higher than the control values. The metabolism of L-menthol was induced in the rat liver by phenobarbital, but not by methylcholanthrene (Madhava Madyastha and Srivatsan, 1988).

In contrast to the results of studies with rats, L-menthyl glucuronide was detected in the urine of sheep fed with L-menthol in major amounts - within 24 hours after application, the excretion being almost complete (Wright, 1945). Similarly also rabbits excreted major amounts of L-menthol in the urine. 48 % conjugated L-menthol was recovered after 2 days as glucuronide in the urine from rabbits fed with 1000 mg/kg bw L-menthol (Williams, 1938).

Also humans excrete considerable amounts of L-menthol as the glucuronide. In an investigation with two human volunteers between 17 and 38 % of menthol was excreted as menthyl glucuronide in urine within 24 hours after 8 daily doses of 750 mg L-menthol (Eisenberg et al., 1955).

In the urine of rabbits fed 1 g/kg bw of D/L-menthol and L-menthol, respectively, D/L-menthol glucuronides were found in similar amounts (59 % of the dose) as L-menthol glucuronides (48 % of the dose) (Williams, 1938).

Glucuronide excretion was investigated in the urine of several persons after oral ingestion of menthol (unspecified isomer) in a variety of studies. In older studies about 70% of the total dose (10

mg to 1560 mg) were found in the urine of humans as glucuronide 6 to 24 hours after ingestion (Quick, 1928; Atzl et al., 1972; Bolund et al., 1967). In a study of Somerville et al. (1984) 35 to 40% menthol was found in the urine of 6 volunteers 24 hours after ingestion of 180 to 190 mg menthol. In another study (Kaffenberger and Doyle, 1990) an average of 40 % menthol was recovered in the urine of 4 volunteers 14 hours after dosing with 72 mg menthol. In summary, the studies show that in humans the unspecified menthol isomer is rapidly glucuronidated and excreted mainly via urine.

Distribution

17 hours after oral administration of 470 mg/kg bw of [3-³H]-menthol (unspecified isomer) to rats 2.1 % of the dose was found in fat, 0.8 % in the liver, 0.2 % in the kidney, 0.3 % in serum and traces (< 0.1 %) in brain, and testes (Clegg et al., 1982).

Excretion

Investigations with male Fischer 344 rats showed that 48 h after administration of 500 mg/kg radiolabelled L-menthol > 70 % of the administered dose was found in urine and faeces (Yamaguchi, et al, 1994).

Rats were administered 470 mg/kg bw of [3-³H]-menthol (unspecified isomer) orally. After 17 hours 52 % of the administered radioactivity was found in the urine, 4.5 % and 3.5 % were found in the faeces and ileum (Clegg et al., 1982).

In humans urinary elimination of menthol after oral application was almost complete within about 12 to 24 hours (Atzl et al., 1972; Bolund et al., 1967; Kaffenberger and Doyle, 1990; Somerville et al., 1984).

Conclusion: L, D/L, and the unspecified menthol isomers are well absorbed via the oral route of exposure and are mainly excreted as glucuronic acid conjugates. In rats an extensive enterohepatic circulation leads in addition to various hydroxylated degradation products. Glucuronides and degradation products are mainly eliminated via urine, minor quantities via the faeces.

3.1.2 Acute Toxicity

Oral

The studies on acute oral toxicity were not performed according to guideline methods. However, the number of treated animals and the used protocols are scientifically acceptable to evaluate this endpoint sufficiently. All substances demonstrated an acute oral toxicity with LD50 values higher than 2000 mg/kg bw. The menthol isomers tested according to the same protocol (Haarmann and Reimer, 1974) are presented in Table 1. In an orientating study with 2 mice/dose no deaths occurred with doses up to and above 6000 mg/kg bw of the unspecified isomer mixture (Haarmann and Reimer, 1980). For L-menthol a LD50 value of 4380 mg/kg bw was determined in mice (FDA, 1977). Other studies with rats and mice gave similar results, indicating a low acute oral toxicity of the menthol isomers (Herken, 1961 in FAO/WHO, 1999; Mengs and Stotzem, 1989; FDA, 1975). Clinical signs of toxicity observed were a narcotic status and depressed activity (Haarmann and Reimer, 1974, Haarmann and Reimer, 1980, FDA, 1975). No information is given in the study reports at which doses these effects occurred.

A considerably lower LD50 of 940 mg/kg bw was observed in a single study with L-menthol in rats (FDA, 1975). In this study a severe irritation of the mucosal lining of the stomach and intestine was reported. Such effects have not been reported by the other investigators mentioned above.

The human lethal dose is reported to be in the range of 50 – 500 mg/kg bw (FAO/WHO, 1999).

Table 1: Results of studies on acute oral toxicity

Chemical	Species	Protocol	Result (LD ₅₀)	Reference
L-Menthol	Rat	Other*	2426 – 2615 mg/kg bw	Haarmann and Reimer, 1974
D-Menthol	Rat	Other*	2046 mg/kg bw	Haarmann and Reimer, 1974
D/L-Menthol	Rat	Other*	2602 mg/kg bw	Haarmann and Reimer, 1974

* LD₅₀ values were evaluated in the same laboratory, using the same protocol

Conclusion: All menthol isomers show low acute oral toxicity with LD₅₀ values normally greater than 2000 mg/kg bw (rats and mice). Clinical symptoms were unspecific, and included apathy and reduced activity. A considerably lower LD₅₀ of 940 mg/kg bw was determined in a single study with L-menthol in rats. Severe irritation of the mucosal lining of stomach and intestine was reported in this study. Such effects have not been reported by any other investigators, but may have contributed to the lower LD₅₀ value.

Inhalation

Although no experimental studies are available the low systemic toxicity of menthols (LD₅₀ > 2000 mg/kg bw) that is documented for oral application and single dermal contact can be expected also for the inhalation route.

Dermal

Only limited studies are available investigating dermal toxicity (Table 2). In one study the LD₅₀ of D/L-menthol in rabbits was above 5000 mg/kg bw. In a second investigation a dermal dose of 34500 mg menthol liquid / kg bw was lethal to a mouse.

Table 2 : Results of studies on acute dermal toxicity

Chemical	Species	Protocol	Result	Reference
D/L-Menthol	rabbit	other	LD ₅₀ >5000 mg/kg bw	Levenstein, 1973 in Opdyke, 1976
Menthol liquid (unspec. isomer)	mouse	other*	LD = 34500 mg/kg bw	Macht, 1939

*orientating study, 1 mouse was treated

Conclusions: It can be assumed that the acute dermal toxicity of the menthol isomers is low, based on old and limited studies for the racemate and the unspecified isomer.

3.1.3 Irritation

Skin Irritation

All compounds were tested for skin irritation in rabbits according to the current OECD-guideline 404. All isomers were tested undiluted and in 50, 25, 5 and 1% concentrations. Diethylphthalate (DEP) was used as diluent. The investigations were performed in the same laboratories, using the same protocol; the results are given in Table 3. The undiluted compounds were irritating to the skin.

Dilution of the compounds led to a pronounced decrease in the irritating properties of the compounds. No skin reaction at all were observed for D-menthol and menthol liquid at 5 % dilution and for L- and D/L-menthol at 1 % dilution.

Table 3: Results of studies on skin irritation

Chemical	Undiluted		50 %		25 %		5 %		1 %		Reference
	Scores		Scores		Scores		Scores		Scores		
	eryt.	oed.	eryt.	oed.	eryt.	oed.	eryt.	oed.	eryt.	oed.	
L-Menthol	3.0	2.9	1.6	2.2	1.0	0.2	0.3	0.1	0.0	0.0	Haarmann and Reimer, 1989, No. 11874
D-Menthol	2.5	2.4	1.9	1.3	0.7	0.0	0.0	0.0	0.0	0.0	Haarmann and Reimer, 1989, No. 11875
D/L-Menthol	3.0	3.0	1.6	1.7	0.8	0.5	0.2	0.0	0.1	0.0	Haarmann and Reimer, 1989, No. 11877
Menthol liquid	2.8	2.4	1.8	1.0	0.8	0.0	0.0	0.0	0.0	0.0	Haarmann and Reimer, 1989, No. 11876

eryt.: erythema, oed.: oedema

Conclusion: All studied isomers of menthol are, if applied undiluted, moderately irritating to skin.

Eye Irritation

All compounds were tested for eye irritation according to the current OECD-guideline 405. All examinations were performed in the same institute according to the same protocol; the results (means of Draize scores after 24, 48, and 72 h) are given in Table 4 in detail. The vehicle diethylphthalate (DEP) alone, tested in the opposite eye of the animals, showed no irritating properties. In all studies with the test compounds only slight reactions of cornea and conjunctiva were observed, depending on the concentration tested. There was no reaction in the iris observed in all cases. After treatment with menthol liquid (100% and 71%) slight redness of conjunctiva was seen on day 7 in 1/4 and 2/4 animals, respectively. For the undiluted menthol liquid it was shown that these effects were completely reversible within 14 days. Data are summarized in Table 4.

In a study by Carpenter and Smyth (1946) undiluted, 1% and 5% solutions of menthol (unspecified isomer and purity, no definite vehicle mentioned) are reported to affect the eyes of rabbits. Only the overall result is available in tabulated format and no details are available with regard to the individual experiments, the scores obtained in single animals and the number of animals used in the test with menthol. In summary the injuries were graded 9 on a scale of maximum 10.

In a quantitative structure-activity relationship (QSAR) analysis eye irritating properties have been predicted for unspecified menthol (Barratt, 1997).

Table 4: Results of studies on eye irritation

Chemical	Conc.	Scores*		Reference	
		Cornea opacity	Conjunctivae		
			Redness	Chemosis	
L-Menthol	29 %	0.2	0.6	0.1	Haarmann and Reimer, 1989, No. 11754
L-Menthol	64 %	1.0	2.0	0.6	Haarmann and Reimer, 1989, No. 11870
D-Menthol	29 %	0.4	1.3	0.4	Haarmann and Reimer, 1989, No. 11755
D-Menthol	64 %	0.9	2.1	0.3	Haarmann and Reimer, 1989, No. 11871
D/L-Menthol	40 %	0.8	1.5	0.4	Haarmann and Reimer, 1989, No. 11753
D/L-Menthol	64 %	1.0	2.1	0.3	Haarmann and Reimer, 1989, No. 11873
Menthol liquid	100 %	1.0	2.2	0.7	Haarmann and Reimer, 1989, No. 11872
Menthol liquid	71 %	1.0	2.2	0.7	Haarmann and Reimer, 1989, No. 11756

*scores are based on the results from the 24-, 48- and 72-hours reading 83/467/EEC of July 29, 1983

Conclusion: In studies performed according to OECD TG 405, concentrations of 29 to 64 % of L-, D-, and D/L-menthol in diethylphthalate and undiluted menthol liquid were shown to be slightly irritating to the eye. In another rabbit study instillation of undiluted menthol (of unknown purity) and a 1% and 5% solution in a unknown vehicle were reported to result in eye injury (grade 9 on a scale of maximum 10; no details are available on the number of animals and the nature of the effects seen after menthol treatment). Overall, the menthol isomers are slightly irritating to the eye.

3.1.4 Sensitisation

In non-adjuvant tests for skin sensitization (Buehler Test, Haarmann and Reimer, 1991; local lymph node assay, Haarmann and Reimer, 1995) L-menthol gave no indication of a sensitizing effect in animals. In the induction and the challenge phase of the Buehler test 0.5 ml of a 25 % solution was applied occlusively to the skin of guinea pigs. The test procedure was in accordance with the OECD guideline 406. The local lymph node assay was performed after the protocol of Kimber and Weisenberger, who developed and established the assay. In a modified Draize test, a positive result was only obtained when induction and challenge were followed by a second induction/challenge procedure (Sharp, 1978). The results of the animal studies are shown in Table 5.

In a maximization test with 8 % D/L-menthol in petrolatum performed in 25 volunteers, there was no positive reaction (Kligman, 1975 in Opdyke, 1976).

No valid study is available testing the sensitizing potential of D-menthol.

There are several case reports and clinical studies describing patch-tests with not further specified menthol isomers in patients with dermatological lesions. Of in total 6227 patch-tested patients (out of 9 investigations) about 82 showed positive reactions (0.3 to 6.1 % positive reactions were reported in these studies). The results of the clinical studies are given in detail in Table 6.

The presence of menthol and menthol-containing flavour and fragrance oils in consumer products such as cigarettes, toothpaste, and topical medications can lead to sensitivity reactions in the oral and nasal cavity of susceptible persons (Morton et al. 1995, Camarasa and Alomar 1978, Shah et al., 1996).

Table 5: Results of animal studies on skin sensitization

Chemical	Species	Protocol	Result	Reference
L-Menthol	rabbit	Buehler	not sensitizing	Haarmann and Reimer, 1991, HR90/000102 No.
	mouse	Local lymph node assay (LLNA)	not sensitizing	Haarmann and Reimer, 1995, No.CTL/E/160
	guinea pig	modified Draize	ambiguous (positive only after rechallenge)	Sharp, 1978

Table 6: Results of studies on sensitization in humans

Chemical	Number of patients	Complaints	% positive	Reference
D/L-Menthol	25	Volunteers	0	(Kligman, 1975 in Opdyke, 1976)
Menthol (D/L-menthol or L-menthol)	228	Dermatoses	1.3	Baer, et al., 1955
	330	Eczematous lesions	6.1	Blondeel, et al., 1978
	1385	Dermatologic complaints	0.4	Jarisch and Sandor, 1978
	1070	Atopic eczema or dermatitis	0.9	Rudzki and Kleniewska, 1971
	1200	Contact dermatitis	1.0	Santucci, et al., 1987
	1077	Crural ulcerations and eczema	1.9	Legiec, et al., 1996
	512	Intraoral complaints	2.1	Morton, et al., 1995
	75	Patients with mucosa/skin reactions caused by dental products	0	Kanerva, et al., 2001
	350	Anal eczema (20.2 % atopic dermatitis)	0.3	Schnuch and Geier, 1995

Conclusion: Standard non-adjuvant animal tests with L-menthol were negative. In humans, a few cases of hypersensitivity reactions, including skin reactions and reactions in the oral and nasal cavity, have been reported. Based on the wide exposure of consumers to these substances and also on the results from clinical studies, which investigated a high number of subjects, the overall sensitizing potential of the menthol isomers is considered to be low.

3.1.5 Repeated Dose Toxicity

Valid studies investigating the repeated dose toxicity of menthol isomers were available for L-menthol and D/L-menthol.

L-Menthol

Oral exposure

Female and male Wistar rats (10/sex/dose) received 200, 400 and 800 mg/kg bw/d L-menthol in soybean oil daily by gavage for 28 days. This study was performed mainly according to OECD TG 407. It is reported that liver weights were significantly increased in male rats at doses of 200, 400 and 800 mg/kg bw/d and also in female rats at doses of 400 and 800 mg/kg bw/d. Additionally, rats of all menthol treatment groups showed vacuolization of hepatocytes (4/20; 5/17, 4/19; no distinction between sexes), which was not seen in the control animals. This effect was not dose related and may reflect an adaptation (Thorup, 1983). Since no information is available as to the magnitude and the incidence of increased liver weights in the various exposure groups, the relevance of this finding is questionable and a NOAEL or a LOAEL cannot be deduced from this study. In a feeding study, groups of 40 male and 40 female rats received 0, 100 or 200 mg/kg bw/d of either L-menthol or D/L-menthol in their diets for 5.5 weeks. There were no adverse effects on weight gain or excretion of glucuronide, water and electrolytes, nor was there any interference with central nervous system reactions to stimulants (Herken, 1961 in: FAO/WHO, 1999). Therefore for L-menthol and the racemate D/L-menthol a NOAEL of 200 mg/kg bw/d can be deduced from this study.

Exposure by inhalation

There is one detailed study from 1954 available with exposure to L-menthol by whole body vapour inhalation for 71 to 79 days with male and female Sherman rats (groups of 12). Although the authors attempted to measure the menthol concentrations in the gas phase, there was no adequate analytic method available. Therefore the exposure concentrations are given as weight of menthol vaporized divided by the volume of air circulated. The exposure concentrations were determined to be 0.087, 0.148 and 0.259 ppm (according to 0.57, 0.96 and 1.68 mg/m³). Besides this shortcoming, the study is of good design with numerous parameters investigated. Histopathological organ examinations showed toxic effects in the lungs only, ranging from tracheitis to severe congestion of the lungs at the highest dose, changes indicative of irritation (Rakieten et al., 1954). As the measurement of the exposure concentration does not seem reliable, this study cannot be used to derive a NOAEL. However, it points to the respiratory system as possible target organ after exposure by inhalation.

D/L-Menthol

Oral exposure

D/L-Menthol was applied in the feed at 930 to 15000 ppm in a subchronic dose-finding study (10 animals/sex/dose) for a carcinogenicity assay to F344 rats (Tracor Jitco, 1976, Project-No. 976-243) and to B6C3F1 mice (Tracor Jitco, 1976, Project-No. 976-223).

Duration	Species	Dose (ppm)	Dose (mg/kg bw) for males	Dose (mg/kg bw) for females
13 weeks	Rat	930	59	67
		1870	114	142
		3750	231	285
		7500	472	521
		15000	(NOAEL) 937	(NOAEL) 998
13 weeks	Mouse	930	243	290
		1870	488	595
		3750	978	1193
		7500	(NOAEL) 1956	(NOAEL) 2386
		15000	3913	4773

In rats the only effect recorded after 13 weeks of D/L-menthol exposure at 15000 ppm (corresponding to 937 mg/kg bw/d in males and 998 mg/kg/d in females) was a minimal increase in the severity of spontaneous interstitial nephritis in males, which was considered by the authors of the study as of questionable significance. Thus the NOAEL for this study is 937 mg/kg bw/d for male rats and 998 mg/kg bw/d for female rats.

In male and female mice a slight decrease in body weight gain was observed at 15000 ppm but not at 7500 ppm (corresponding to 1956 mg/kg bw/d in males and 2386 mg/kg bw/d in females). Histologic examination of tissues at the 7500 and 15000 ppm levels revealed no compound-related tissue alterations in any of the mice. The sections of lung in control and treated mice revealed early spontaneous respiratory disease lesions (peribronchial and perivascular lymphoid hyperplasia) and occasional focal areas of pneumonitis, which were unrelated to the treatment with the test substance. Also minimal focal interstitial nephritis was noted as a spontaneous lesion which was observed in control and treated mice, and not related to the treatment with D/L-menthol. The NOAEL therefore can be considered with 1956 mg/kg bw/d for male mice and 2386 mg/kg bw/d for female mice based on slightly reduced body weight gain.

In carcinogenicity feeding studies (103 weeks; NCI, 1979) F344 rats were administered 3750 and 7500 ppm D/L-menthol (intake calculated for rats with a mean body weight of 400 g and 20 g/day of food consumption: ca. 188 and 375 mg/kg bw/d) and B6C3F1 mice were administered 2000 and 4000 ppm (intake calculated for mice with a mean body weight of 30 g and 5 g/day of food consumption: ca. 334 and 667 mg/kg bw/d). The mean body weights of male and female rats and mice were slightly reduced at all dose levels. No statistical analysis has been performed on body weights. Estimated maximal body weight differences between control and high dose groups were < 10 % in male rats and male and female mice and < 14 % in female rats. In low dosed female rats body weights were reduced by maximal 10 %. In male rats chronic inflammation of the kidney was found with greater frequency in dosed males than in control males (controls: 29/49; low-dose: 41/50; high-dose 41/50). These findings were regarded as of questionable relevance by the authors, since such lesions are often found in aged male Fischer rats. In B6C3F1 mice, no effects were found at the highest dose tested. The NOAELs in these studies can be considered with 375 mg/kg bw/d for male rats and 667 mg/kg bw/d for male and female mice. For female rats the NOAEL is 188 mg/kg based on slightly reduced body weight at 375 mg/kg bw.

Menthol (unspecified isomers)

There are a number of repeat dose studies using menthol (unspecified isomers) as the test substance. However, these studies do not add to the assessment and are therefore not described here.

Conclusion: In rats given = 200 mg/kg bw of L-menthol in soybean oil by gavage, increased liver weights and a non dose-related vacuolization of hepatocytes were reported. The relevance of these findings remain unclear. No toxicity was observed in rats receiving diets providing up to 200 mg/kg bw/d of either L- or D/L menthol for 5.5 weeks. Therefore for L-menthol and the racemate D/L-menthol a NOAEL of 200 mg/kg bw/d can be deduced from this study. Irritant effects on lungs and trachea, but no systemic effects were found in rats that were whole body exposed to L-menthol vapour for 71-79 days.

D/L-menthol administered with the diet (up to 15,000 ppm) for 13 weeks to rats and mice did not induce any effects on organ weights. Microscopic examination of a comprehensive range of tissues revealed a slight increase in the severity of spontaneous interstitial nephritis in the male rats at the highest dose level. The only effect seen in mice of both sexes was a reduction in body weight gain in the highest dose group (NOAEL, rat: 15,000 ppm, corresponding to 937 mg/kg bw/d for the male and 998 mg/kg bw/d for the female rat; NOAEL, mouse: 7500 ppm, corresponding to 1956 mg/kg bw/d (males) and 2386 mg/kg bw/d (females).

In a 103-week study in rats with D/L menthol (3750 and 7500 ppm in the diet), the only effect was a slight increase in spontaneous, chronic inflammation of the kidney in male rats of both dose groups, and a slightly reduced body weight in female rats at the high dose level (NOAEL: 7500 ppm (approx. 375 mg/kg bw/d) for male rats, and 3750 ppm (188 mg/kg bw/d) for female rats). In 103-week studies in mice with D/L menthol (2000 and 4000 ppm in the diet), the NOAEL for both sexes was 4000 ppm (approx. 667 mg/kg bw/d).

Because the racemate D/L-menthol contains the D- and L-isomers in equal proportions, the study results with the racemate are considered adequate for the evaluation of the D-isomer and of the L-isomer. This view is further supported by the FAO/WHO 1999 safety evaluation on menthol, where the FAO/WHO expert committee had concluded that “the limited data that allow comparisons of metabolism and toxicity provide no indication of a difference in the toxicity of L-menthol and D/L-menthol”. Overall it can therefore be concluded that these menthol isomers induce no specific systemic effects and are well tolerated after repeated oral administration.

3.1.6 Mutagenicity

Genotoxicity in vitro

L-Menthol

L-Menthol was not mutagenic in Ames tests using the tester strains *S. typhimurium* TA 97a, TA 98, TA 100, TA 102, TA 1535, TA 1537, and TA 2637 with and without metabolic activation (Nohmi, et al., 1985, Andersen and Jensen, 1984, Gomes-Carneiro, et al., 1998). The tests were performed also at cytotoxic concentrations (800 µg/plate, Gomes-Carneiro et al., 1998). A reverse mutation assay with *E. coli* WP2 *uvrA* (*trp*⁻) was negative in concentrations up to 0.8 mg/plate; a recombination assay with *Bacillus subtilis* M 45 and H 17 gave positive results in doses up to 10 mg/disk (Yoo, 1986).

In a detailed study of Murthy et al. (1991) peripheral lymphocytes of 24 human donors were treated in vitro with L-menthol in the absence and in the presence of rat S9-mix. The concentration range tested was 0.1 to 10 mM L-menthol. The authors investigated chromosomal aberrations (in at least 100 cells per donor) and sister chromatid exchanges (in at least 25 second division metaphases per donor). In both test systems L-menthol did not induce chromosomal damage. In a further

cytogenetic assay the anaphase chromosome aberrations of human fibroblasts were investigated. The concentration range tested was 0.1 to 10 µg/ml. There was no indication for chromosomal aberrations induced by L-menthol (FDA, 1975).

Chromosomal aberration tests with Chinese hamster cells (CHL) tested in concentrations of 0.1 to 0.3 mg/ml L-menthol (Sofuni et al., 1985; Matsuoka et al., 1998) were negative with and without metabolic activation.

D-Menthol

Alkaline single gel tests (comet assay) were performed using V79 Chinese hamster cells and human lymphocytes respectively. Both assays were performed with and without metabolic activation. D-Menthol did not induce DNA single strand breaks in both cell types (Hartmann and Speit, 1997).

D/L-Menthol

D/L-Menthol was not mutagenic in the Ames test with the standard tester strains *Salmonella typhimurium* TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537, TA 2637 with and without metabolic activation and including cytotoxic concentrations (Nohmi et al., 1985; Ishidate et al., 1984; Zeiger et al., 1988).

A negative result was obtained for D/L-menthol in a mouse lymphoma assay with L5178Y mouse lymphoma cells with and without metabolic activation (Myhr and Caspary, 1991). The concentration range tested was 12.5 to 200 µg/ml; the lethal dose was 200 µg/ml (see Table 8).

An alkaline elution assay to detect DNA damage in primary rat hepatocytes – testing concentrations of 0.1, 0.3, 0.7, 1.0, 1.3 mM up to cytotoxic concentrations – was negative (Storer et al., 1996).

Chromosomal aberration tests performed with D/L-menthol show primarily negative results. Tests conducted with CHO cells in concentrations of 100, 150 and 200 µg/ml without metabolic activation and in concentrations of 50, 124 and 200 µg/ml with metabolic activation by Ivett et al. (1989) were negative. A cytogenetic assay with CHL cells performed by Sofuni et al. (1985) and Ishidate et al. (1984) showed a negative result without metabolic activation. The concentrations tested were 100, 150 and 200 µg/ml. A study of Hilliard et al. (1998) showed ambiguous results. Weak but statistically significant increases in chromosomal aberrations were observed in CHO cells and TK6 human lymphocytes after treatment with D/L-menthol in concentrations of 250 to 281 µg/ml (cell viability 47-33% of controls) and 128 to 187 µg/ml (cell viability at 187 µg/ml 20% of controls), respectively, without metabolic activation. However, in a second experiment this result could only be reproduced for the highest scorable concentration for CHO cells (250 µg/ml). Negative results were obtained in CHO cells with metabolic activation, but due to too high cytotoxicity of D/L-menthol only the concentration of 203 µg/ml could be evaluated. A further chromosome aberration test with CHO cells with limited documentation was positive, showing maximal 7% aberrant metaphases (Galloway et al., 1998). It is not published whether Galloway's investigations were performed with or without metabolic activation. It can be assumed that the data presented are originally from Hilliard et al. (see above). Test results in detail are given in Table 7.

Table 7: Results of chromosome aberration tests of D/L-menthol

Test system	Protocol	Concentrations		Results*		Reference
		Exp. [µg/ml]	Cytotox. [µg/ml] (% cell viability)	+ MA	- MA	
CHO	Exposure time: 8 hrs (-); 2 hrs (+) Harvest time: 10.50 (-), 12.50 (+) hrs	100, 150, 200 (- MA), 50, 124, 250 (+ MA)	200	-	-	Ivett, et al., 1989
CHL	Exposure time: 24, 48 hrs	100, 150, 200	200 = 50 % cell-growth inhibition	n.d.	-	Sofuni, et al., 1985, Ishidate, et al., 1984
CHO	Exposure time: 3 hrs Harvest time: 20 hrs	203, 219, 234	234 (45 %)	+* ¹		Galloway, et al., 1998
CHO	Exposure time: 3 hrs Harvest time: 20 hrs	46-297	(47%), 266 (39%), 281 (33%)	(+)* ²	(+)* ²	Hilliard, et al., 1998
TK6 human lymphocytes	Exposure time: 3 hrs Harvest time: 17-35 hrs	128-187	187 (20%)	n.d.	(+)* ²	Hilliard, et al., 1998

* summarized Chromosome aberrations are: Chromatid and chromosome breaks, triradials, chromatid and chromosome exchanges. Gaps and endoreduplications are not counted.

*¹ it is not defined, whether the test was performed with or without metabolic activation.

*² result positive only at cytotoxic concentration (≥ 250 µg/ml).

A sister chromatid exchange assay with chinese hamster ovary cells was judged negative with and without metabolic activation (Ivett et al., 1989).

Table 8: Results of different tests on *in vitro* genotoxicity of menthol isomers

	L-Menthol		D-Menthol	D/L-Menthol		Reference
Bacteria						
Ames Tests	+ MA: negative	- MA: negative	n.d.	+ MA: negative	- MA: negative	Nohmi et al. 1995, Andersen and Jensen, 1984, Gomes-Carneiro et al., 1998
Reverse mutation assay	E.coli WP2 uvrA (trp -): negative		n.d.	n.d.		Yoo et al., 1986
Recombination assay	Bacillus subtilis M45 H 17: positive		n.d.	n.d.		Yoo et al. 1986
Mammalian cells						
Gene mutation	n.d.		n.d.	L5178Y mouse lymphoma cells		Myhr and Caspary, 1991
				+ MA: negative	- MA: negative	
Cytogenetic assay	Human fibroblasts: Negative		n.d.	n.d.		FDA, 1975
Chromosomal aberration	CHL cells and human lymphocytes		n.d.	CHO/CHL: ambiguous (see Table 7)		Sofuni et al., 1985 Matsuoka et al., 1998 Murthy, et al., 1991
	+ MA: negative	- MA: negative				
DNA damage (alkaline elution)	n.d.		n.d.	Rat hepatocytes : negative		Storer, et al. 1996
DNA damage (comet assay)	n.d.		V79 CHL and human lymphocytes: negative	n.d.		Hartmann and Speit, 1997
Sister chromatid exchange	Human peripheral lymphocytes		n.d.	CHO		Murthy, et al., 1991 Ivett et al., 1989
	+ MA: negative	+ MA: negative		+ M A : negative	- M A : negative	

Conclusion: All menthol isomers were consistently tested negative in standard bacterial gene mutation tests, both in the presence and in the absence of metabolic activation and including cytotoxic concentrations. A slightly increased frequency of chromosomal aberrations was found in CHO cells and human lymphocytes at cytotoxic concentrations, but not in CHL cells or in human fibroblasts.

Overall, menthol and its isomers are considered non-genotoxic in *in vitro* bacterial and mammalian test systems.

Genotoxicity in Vivo

L-Menthol

L-menthol did not lead to an increased rate of chromosomal aberrations in the bone marrow of rats at oral doses up to 3000 mg/kg bw (single dose; sacrifice 6, 24 and 48 h after treatment) and 1150 mg/kg bw/d (5 applications; sacrifice 6 h after last dose). Besides the restriction that only 50 metaphases have been investigated per animal, the experiments were performed in accordance with current standards. L-menthol was not mutagenic in a dominant lethal test in rats (FDA, 1975) with doses up to 3000 mg/kg bw (single dose; providing 14 to 20 pregnant females per mating group) and 1150 mg/kg bw/d (5 applications; providing 13 to 19 pregnant females per mating group). The results are summarized in Table 9.

D/L-Menthol

The *in vivo* mutagenicity of D/L-menthol was investigated in two *Drosophila* SLRL tests and in a mouse bone marrow micronucleus assay.

Canton-S males of *Drosophila* were administered D/L-menthol via feed (3 days) or injection in concentrations of 50000 and 10000 ppm respectively. No genotoxicity for a total of 3 broods was observed (Foureman, et al., 1994).

In a micronucleus assay in bone marrow cells of B6C3F1 mice, which received daily intraperitoneal injections of 250, 500 or 1000 mg/kg bw/d D/L-menthol for 3 days, performed in accordance with current guidelines, no increase in micronuclei was observed. The data do not indicate cytotoxic effects on the bone marrow cells. However, at the highest dose level 3 out of 6 mice died prior to sacrifice. This dose was obviously exceeding the reported intraperitoneal LD50 of 750 mg/kg bw for rats (FAO/WHO, 1999).

Table 9: Results of *in vivo* mutagenicity tests of menthol isomers

Test system species, strain	L-Menthol		D/L-Menthol	Reference
<i>Drosophila</i> SLRL test	n.d.		10000 (injection) and 50000 (feed) ppm: negative	Foureman et al., 1994
Dominant lethal assay - rats	single dose (1.45, 14.5, 145 mg/kg bw and 500, 3000 mg/kg bw): negative	5 applications (1.45, 14.5, 145 mg/kg bw/d and 1150 mg/kg bw/d): negative	n.d.	FDA, 1975
Cytogenetic assay – bone marrow albino rats	Single dose (1.45, 14.5, 145 mg/kg bw and 500, 3000 mg/kg bw): negative	5 applications (1.45, 14.5, 145 mg/kg bw/d and 1150 mg/kg bw/d): negative	n.d.	FDA, 1975
Micronucleus assay B6C3F1 (Bone marrow cells) mice	n.d.		3 i.p. injections (250, 500, 1000 mg/kg bw/d): negative	Shelby, et al., 1993

Although the studies mentioned in Table 9 were not fully conducted in accordance with current test guidelines, taken together they allow drawing conclusions as to the mutagenic potential of L- and D/L-menthol *in vivo*.

Conclusion: L- and D/L-menthol have demonstrated no mutagenic potential in adequately performed dominant lethal and cytogenetic tests and in a bone marrow micronucleus test in mice.

3.1.7 Carcinogenicity

D/L-Menthol was tested in a well performed study for carcinogenicity (103 weeks) in doses of 3750 and 7500 ppm in the feed in F344 rats (intake calculated for rats with a mean body weight of 400 g and 20 g/day of food consumption: about 188 and 375 mg/kg bw/d) and of 2000 and 4000 ppm in the feed in B6C3F1 mice (intake calculated for mice with a mean body weight of 30 g and 5 g/day of food consumption: about 334 and 667 mg/kg bw/d). 50 animals per sex and dose were treated. In male and female rats the survival rate was not affected by treatment and no carcinogenic effects of D/L-menthol were found in any organ. In treated females, the incidences of chromophobe adenomas in the pituitary gland, of mammary gland fibroadenomas and adenocarcinomas were reduced, compared to the controls (NCI, 1979).

In male mice the survival rate was not affected (control 62%, high dose 70%). Female control mice showed a very high survival rate of 90%. However, the survival of high dosed females (72%) was in the range of control male mice and seems not to be affected by the test substance. In male mice the incidence of hepatocellular carcinoma was increased at the highest dose (8/47 controls, 8/49 low-dose, 14/48 high-dose), however, the incidence was not statistically significant and within the range of the laboratory historical-control groups of mice of this age and strain. It was therefore not considered to be relevant by the authors of the study. The incidence of alveolar/bronchial adenoma or carcinoma in female mice was also slightly increased (1/49, 3/47, 5/48), but not statistically significant. This type of neoplasm has been commonly seen at a similar low incidence in historical-control groups.

Conclusion: There was no evidence of carcinogenicity of D/L-menthol in rats and mice in a study performed in accordance with current standards (highest tested dose levels in rats approx. 375 mg/kg bw, in mice approx. 667 mg/kg bw). Since D/L-menthol contains the two relevant isomers in a 50:50 ratio it can be assumed that also L- and D-menthol have no carcinogenic properties.

3.1.8 Toxicity for Reproduction

Toxicity to fertility

There are no fertility studies with menthol or its isomers available. Examinations of reproductive organs in repeated dose studies can however be used to evaluate adverse effects on reproductive organs. These studies are reported in detail in chapters 3.1.6 (repeated dose toxicity) and 3.1.8 (carcinogenicity).

D/L-Menthol was applied in the feed at 930 to 15000 ppm in a subchronic 13 week-study to F344 rats (Tracor Jitco, 1976, Project No. 976-243) and to B6C3F1 mice (Tracor Jitco, 1976, Project No. 976-223). There were no changes observed in the histopathological examination of testes, prostate, uterus, ovaries, mammary glands and adrenals in rats and mice at any of the doses administered (male rats: up to 937 mg/kg bw/d, female rats: up to 998 mg/kg bw/d, male mice: up to 3913 mg/kg bw/d, female mice: up to 4773 mg/kg bw/d).

Also in a feeding carcinogenicity study with D/L-menthol in mice and rats (103 weeks), no changes in reproductive organs (testes, prostate, uterus, ovaries, mammary gland and adrenals) were observed in histopathological examinations at any of the doses administered (up to about 375 mg/kg bw/d in rats and 667 mg/kg bw/d in mice) (NCI, 1979).

Conclusion: There is no evidence indicating a potential of D/L-menthol to interfere adversely with reproduction. Histopathological examinations of the reproduction organs of rats and mice showed no changes in repeated dose toxicity studies with D/L-menthol and also in carcinogenicity studies with D/L-menthol.

*Developmental Toxicity***L-Menthol**

Teratogenicity studies with L-menthol were conducted in rats, mice, hamsters and rabbits (FDA,1973). In all four developmental toxicity studies, maternally toxic dose levels were not used. The dose levels were, however, sufficiently high to allow an initial assessment of this endpoint:

2.18, 10.15, 47.05 and 218.0 mg/kg bw/d were administered by gavage to Wistar rats from gestation day 6 to 15. There was no effect on maternal and fetal survival and the number of abnormalities in soft or skeletal tissues observed did not differ from sham treated control. No clinical signs of maternal toxicity were observed. Therefore the NOEL derived for maternal and fetal toxicity and teratogenicity in rats can be determined as 218.0 mg/kg bw/d.

Doses of 1.85, 8.59, 39.9 and 185.0 mg/kg bw/d were administered to CD-1 mice from gestation day 6 to 15. No effect on maternal and fetal survival and no dose-related increase in the number of abnormalities in soft or skeletal tissues was observed. No clinical signs of maternal toxicity were observed. The NOEL derived for maternal and fetal toxicity and teratogenicity was 185.0 mg/kg bw/d.

Rabbits were administered menthol from gestation day 6 to 18 in following doses: 4.25, 19.75, 91.7 and 425.0 mg/kg bw/d. Few of the rabbits died or aborted before day 29 (4.25/2 out of 13 animals, 19.75/3 out of 12 animals, 91.7/1 out of 11 animals, 425.0/4 out of 14 animals), however, these effects were not dose related and are not considered to be a consequence of test substance administration. Also in rabbits no effect on maternal and fetal survival and no dose-related increases in the number of abnormalities in soft or skeletal tissues were observed. No clinical signs of maternal toxicity were observed. The NOEL derived for maternal and fetal toxicity and teratogenicity was therefore 425.0 mg/kg bw/d.

4.05, 21.15, 98.2 and 405.0 mg/kg bw/d were administered to Syrian hamsters during gestation days 6-10. No effect on maternal and fetal survival and no dose-related number of abnormalities in soft or skeletal tissues were observed. The NOEL for maternal and fetal toxicity and teratogenicity was therefore 405.0 mg/kg bw/d.

No experimental data with the other menthol isomers is available with regard to this endpoint. Since there is no indication of a difference between the isomers in their toxicokinetics and metabolism, and since this is further supported by all other available toxicological data, which do not show any evident differences in the individual toxicological profiles, there is no reason to assume that the stereoisomeric properties may affect the toxicological properties of menthol isomers. Hence, a similar result in developmental toxicity studies would reasonably be expected from studies with D-menthol, the racemate or the unspecified menthol isomer.

Conclusion: L-Menthol was not embryo- or fetotoxic and had no teratogenic properties in well performed gavage studies in various species (rat, mouse, rabbit, hamster) at not maternally toxic doses (185-425 mg/kg bw).

3.1.9 Other relevant information*Human experiences*

Ingestion of high menthol doses may cause abdominal pain, convulsions, nausea, vomiting, vertigo, ataxia, drowsiness and coma (Dukes, 1980; Gleason & al., 1969) After drinking of about 200 – 250 mg menthol/kg bw a child became drowsy, somnolent, felt pain in the stomach and vomited. The symptoms were fully reversible within 4 days (Leiber, 1967). Oral intake of 8000 to 9000 mg of menthol (unspecified isomer) by three volunteers (corresponding approx. 120 mg menthol/kg bw)

led to a cold burning sensation in mouth, throat and esophagus, a cold sensation on the mucous membranes of the nose, on the skin of the hand and feet, and fatigue (Schwenkenbecher, 1908). About 20 mg menthol/kg bw led only to a mild abdominal discomfort (Bolund, et al., 1967).

Adverse CNS effects are described for a 13-year old boy after inhalation of a menthol containing oil. The estimated inhaled amount of menthol was 200 mg (O'Mullane et al., 1982). Similar symptoms are described after smoking mentholated cigarettes (after smoking 80 mentholated cigarettes for 3 months a woman developed insomnia, unsteady gait, mental confusion, depression, vomiting, and cramp in the legs (Luke, 1962).

In very few cases, all in children younger than 1 year, menthol applied to the nostrils or near the nose caused reflex apnea. Clinical signs were laryngospasm, spasm of the glottis or instant collapse, dyspnea, apnea, unconsciousness, cyanosis and hyperextensive extremities (Champeau, 1935, Kleinschmidt, 1935, Klinke, 1967, Lesoine, 1965, Leiber 1967, Martindale, 1982, Melis, 1989). This effect is assumed not to be a poisoning effect but a reflectory reaction of the nervus trigeminus (Kratschmer reflex) (Leiber, 1967).

Glucose-6-phosphate-dehydrogenase-deficiency in newborn babies may result in development of severe jaundice after menthol administration due to the inability of the neonates to conjugate menthol (Olowe and Ransome-Kuti, 1980).

Menthol has been tested in humans mainly for its pharmaceutical properties, such as enhancement of lung and airway volume (FAO/WHO, 1999).

3.2 Initial Assessment for Human Health

L-, D/L- and the unspecified menthol isomer are well absorbed by the oral route of exposure and are mainly excreted as glucuronides. In rats an extensive enterohepatic circulation additionally leads to various hydroxylated degradation products. Glucuronides and degradation products are eliminated mainly via urine, minor quantities via the faeces.

All menthol isomers are of very low acute oral toxicity with LD₅₀ values normally greater than 2000 mg/kg bw. Clinical signs of intoxication are unspecific, and included apathy and reduced activity. Based on old and limited studies for the racemate and the unspecified isomer, it can be assumed that the acute dermal toxicity of the menthol isomers is low.

All studied isomers of menthol are moderately irritating to the skin and slightly irritating to the eye.

The skin sensitization potency of menthol isomers in animals and humans is low.

In rats given = 200 mg/kg bw of L-menthol in soybean oil by gavage for 28 days, increased liver weights and a non dose-related vacuolization of hepatocytes were reported. The relevance of these findings remains unclear and a NOAEL could not be derived from this study. No toxicity was observed in rats receiving diets providing up to 200 mg/kg bw/d of either L- or D/L menthol for 5.5 weeks. Therefore for L-menthol and the racemate D/L-menthol a NOAEL of 200 mg/kg bw/d can be deduced from this study. Irritant effects on lungs and trachea, but no systemic effects were found in rats that were whole body exposed to L-menthol vapour for 71-79 days.

D/L-menthol administered with the diet for 13 weeks to rats (up to 937/998 mg/kg bw/d for males/females) and mice (up to 3913/4773 mg/kg bw/d for males/females) did not induce any effects on organ weights. Microscopic examination of a comprehensive range of tissues revealed a slight increase in the severity of spontaneous interstitial nephritis in the male rats at the highest dose level. The only effect seen in mice of both sexes was a reduction in body weight gain in the highest dose group. The NOAELs derived from these studies were 937 mg/kg bw/d for the male rat, 998

mg/kg bw/d for the female rat, and 1956 mg/kg bw/d for the male mouse and 2386 mg/kg bw/d for the female mouse.

In a 103-week feeding study in rats with D/L menthol (about 188 and 375 mg/kg bw/d), the only effect was a slight increase in spontaneous, chronic inflammation of the kidney in male rats of both dose groups, and a slightly reduced body weight in female rats. The NOAELs in this study were 375 mg/kg bw/d for male rats, and 188 mg/kg bw/d for female rats. In a 103-week feeding study in mice with D/L menthol (about 334 and 667 mg/kg bw/d), the NOAEL for both sexes was 667 mg/kg bw/d.

Because the racemate D/L-menthol contains the D- and L-isomers in equal proportions, the study results with the racemate are considered adequate for the evaluation of the D-isomer and of the L-isomer. This view is further supported by the FAO/WHO 1999 safety evaluation on menthol, where the FAO/WHO expert committee had concluded that “the limited data that allow comparisons of metabolism and toxicity provide no indication of a difference in the toxicity of L-menthol and D/L-menthol”. Overall it can therefore be concluded that the D-, L-, and D/L-menthol isomers induce no specific systemic effects and are well tolerated after repeated oral administration.

The menthol isomers are considered non-genotoxic in *in vitro* bacterial and mammalian test systems. *In vivo*, L- and D/L-menthol have demonstrated no mutagenic potential in adequately performed dominant lethal and cytogenetic tests and in a bone marrow micronucleus test in mice.

D/L-Menthol showed no evidence of carcinogenic activity in 2-year studies performed in accordance with current standards in rats and mice (highest tested dose levels in rats approx. 375 mg/kg bw/d, in mice approx. 667 mg/kg bw/d).

There is no fertility study available. Histopathological examinations of the reproduction organs of rats and mice showed no changes in repeated dose toxicity studies with D/L-menthol and also in carcinogenicity studies with D/L-menthol. Hence, there is no indication of a potential of D/L-menthol to interfere adversely with reproduction.

L-Menthol was not embryo- or fetotoxic and had no teratogenic properties in well performed gavage studies in various species (rat, mouse, rabbit, hamster) at not maternally toxic doses (185-425 mg/kg bw/d). No maternally toxic dose levels were used in these studies.

Application to nose, nostrils and near to the nose in newborns and children younger than two years of age may cause a severe and dangerous trigeminus reflex (apnea) and should be avoided.

In summary, the available toxicity data indicate very similar toxicity profiles for all of the menthol isomers investigated.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

For the effects assessment of menthols on aquatic organisms the volatility of the isomers from aqueous solutions has to be taken into account, particularly in tests with open systems and longer exposure periods. Toxicity tests with analytical monitoring of the applied menthols reveal that after 4 days the concentrations decreased to about 60 - 80 % of the nominal concentrations.

In most of the studies available on the toxicity of menthols on aquatic species the reported effect values are based on measured (effective) test substance concentrations. However, also the results of the tests with invertebrates and activated sludge (being the only tests without analytical monitoring) should more or less correspond to nominal test substance concentrations due to the short exposure periods (24 h resp. 3 h). Ecotoxicity data available for L-menthol and D/L-menthol are summarized in the following table:

Substance	L-Menthol	D/L-Menthol
CAS-No.	2216-51-5	89-78-1
Fish (<i>P. promelas</i>)	EC ₅₀ (96 h) = 18.4 mg/l (e)	
Fish (<i>D. rerio</i>)	LC ₅₀ (96 h) = 15.6 mg/l (e)	LC ₅₀ (96 h) = 17.6 mg/l (e)
Fish (<i>O. latipes</i>)	LC ₅₀ (48 h) = 26 mg/l (e)	
Invertebrates (<i>D. magna</i>)	EC ₅₀ (24 h) = 37.7 mg/l (n) EC ₅₀ (48 h) = 26.6 mg/l (e)	EC ₅₀ (24h) = 71 mg/l (n)
Algae (<i>S. subspicatus</i>)	ErC ₅₀ (72h) = 21.4 mg/l (e) NOEC (72h) = 9.65 (e)	ErC ₅₀ (72 h) = 16.2 mg/l (n) NOEC (72 h) = 5mg/l (n)
Sludge (Respiration inhibition)	EC ₁₀ = 51 mg/l (n) EC ₅₀ = 237 mg/l (n)	EC ₁₀ (3 h) = 117 mg/l (n) EC ₅₀ (3 h) = 306 mg/l (n)

Values based on effective (e) or nominal (n) TS concentrations

Fish

A flow-through test on the acute toxicity of L-menthol to *Pimephales promelas* was conducted by Geiger et al. (1988). The fish were exposed in Lake Superior water to 5 test substance concentrations in the range of 4.39 to 24.6 mg/l. Analytical measurements revealed that the menthol concentrations were >80 % of the nominal during the test period. Based on measured concentrations a 96h-LC₅₀ of 18.9 mg/l was obtained. The affected fish lost schooling behaviour, were hyperactive and underreactive to external stimuli. They had increased respiration, were darkly coloured and lost equilibrium prior to death. Considering these sub-lethal effects, a 96h-EC₅₀ of 18.4 mg/l was determined.

A static test on the acute toxicity of L-menthol to *Danio rerio* was conducted according to the OECD guideline 203 (Bayer AG, 1992b). The fish were exposed to 5 nominal test substance concentrations in the range of 7.8 to 31 mg/l. In all test media, undissolved substance particles remained on the water surface. Analytical measurements revealed that the test substance concentrations decreased below 80 % of the nominal within 96 h, probably due to volatilization.

Based on the mean measured concentrations, a LC_{50} of 15.6 mg/l was calculated using adjusted probit analysis. At a nominal concentration of 11 mg/l, all fish had a slow and inactive swimming behaviour even after 2 hours; the behaviour at the lowest concentration is not reported. The same test conducted with D/L-Menthol (Bayer AG, 1990a) resulted in a LC_{50} of 17.6 mg/l based on mean measured concentrations. The same sub-lethal effects as for L-menthol are reported at the nominal concentration of 11 mg/l.

A static or semistatic test on the acute toxicity to *Oryzias latipes* in accordance with the Japanese Industrial Standard resulted in a 48h- LC_{50} of 26 mg/l (MITI, 1992). It remains unclear whether D/L or L-menthol was used, in the literature source both CAS-numbers are referred.

Invertebrates

The acute toxicity of D/L-menthol to *Daphnia magna* was determined in a static test conducted according to a proposal of the German Federal Environmental Agency after an exposure period of 24 h. The organisms were exposed to 8 test substance concentrations between 2.0 and 250 mg/l, analytical control was not performed. The nominal LC_{50} was calculated to be 71 mg/l (Bayer AG, 1990b).

In a recent test the acute toxicity of L-menthol to *Daphnia magna* was studied in a static test conducted according to method 92/69/EEC Annex V C2. The organisms were exposed to 6 test concentrations in the range of 3.2 to 100 mg/l. At start and end of the test the test substance concentration was measured with GC. Measured concentrations ranged from 93.1 to 104 % of nominal values at test begin and from 81.3 to 91.6 % after 48 hours. The test results were expressed in terms of nominal concentrations at 24 hours and in terms of mean measured concentrations at 48 hours. A 24h- EC_{50} of 37.7 mg/l and a 48h- EC_{50} of 26.6 mg/l was found (Bayer AG, 2002b).

Algae

The growth inhibition of D/L-menthol to the alga *Scenedesmus subspicatus* was tested by Bayer AG (2000) according to OECD guideline 201. The algae were exposed to 6 nominal concentrations between 1.25 and 40 mg/l. Analytical control (TOC measurements) revealed that the test concentrations have not decreased below 80 % of the nominal. Based on nominal concentrations the 72h- ErC_{50} value was 16.2 mg/l. The NOEC resulting from the Dunnett test was 5mg/l.

In a recent test the acute toxicity of L-menthol to the green algae *Desmodesmus subspicatus* was studied in a static test conducted according to method 92/69/EEC Annex V C3. The algae were exposed to 4 test concentrations in the range of 5 mg/l to 40 mg/l. At start and end of the test the test substance concentration was measured with GC. Measured concentrations ranged from 92 to 102.5 % of nominal values at test begin and from 88 to 105 % after 72 hours. The test results were expressed in terms of mean measured concentrations. A 72h- ErC_{50} of 21.4 mg/l and a 72h-NOEC of 9.65 mg/l was found (Bayer AG, 2002c).

Summary of aquatic effects

Ecotoxicity data for fish, invertebrates and algae are available for L- and D/L-menthol. The data for the two category members within each trophic level are in the same order of magnitude and are within the uncertainty range of laboratory effect tests. D/L-menthol contains the D- and L- isomers, thus effect values obtained with this mixture should cover the toxicity of D-menthol and the unspecific isomer mixture. Therefore, all available effect values can be regarded together for the assessment of this category.

Determination of PNECaqua

Tests on acute aquatic toxicity for 3 trophic levels are available for L-menthol and D/L-menthol. Most tests were conducted with analytical control. The lowest effect value was found in an algal growth inhibition test (*Scenedesmus subspicatus*) with an ErC_{50} of 16.2 mg/l.

Applying an assessment factor of 1000 to the algae ErC_{50} , a PNECaqua of 16.2 µg/l is calculated. This PNEC is valid for the whole category.

Tests on long-term toxicity are not available.

Microorganisms

A respiration inhibition test on sludge from a laboratory facility was conducted with D/L-menthol according to OECD guideline 209 (Bayer AG, 1989). The nominal 3h- EC_{10} was 117 mg/l and the EC_{50} 306 mg/l.

In a similar test according ISO 8192 with L-menthol, an EC_{10} of 51 mg/l and an EC_{50} of 237 mg/l was determined (Bayer AG, 1992c).

4.2 Terrestrial Effects

No results from standard soil toxicity tests are available. There are some data e.g. from studies with insects (bees, caterpillars) and from menthol-treated bee-hives, which cannot be related to relevant environmental conditions (Lee et al., 1999; Kevan et al., 1999; Westcott and Winston, 1999).

4.3 Other Environmental Effects

No reliable data available.

4.4 Initial Assessment for the EnvironmentEnvironmental behaviour:

According to a Mackay Level I model calculation menthols are mainly distributed to air (39.5 - 44.2 %) and water (40.5 - 43.8 %), followed by soil (8.0 - 8.7%) and sediment (7.3 - 8.1 %). The calculated Henry's law constant indicates evaporation from surface waters within 2-18 days.

In the atmosphere, indirect photodegradation by hydroxyl radicals is expected with an estimated half-life of 16 hours.

Under environmental conditions, neither hydrolysis nor direct photolysis are to be expected due to the chemical structure. From recently performed tests it can be concluded that menthols are readily biodegradable. The results of one laboratory test indicate no significant bioaccumulation potential of menthols.

Environmental effects:

Ecotoxicity data for fish, invertebrates and algae are available for L-menthol and D/L-menthol. The data for both isomers within each trophic level are in the same order of magnitude and are within the uncertainty range of laboratory effect tests. D/L-menthol contains the D- and L- isomers, thus effect values obtained with this mixture should cover the toxicity of D-menthol and the unspecific isomer mixture. Therefore, all available effect values can be regarded together for the assessment of this category. Tests on acute aquatic toxicity for 3 trophic levels are available for L-menthol and D/L-menthol. For *Danio rerio* an LC_{50} (96 h) of 17.6 mg/l was found. For the acute toxicity on *Daphnia magna*, an EC_{50} (48 h) of 26.6 mg/l was obtained. The most sensitive organism tested is

the alga *Scenedesmus subspicatus* with an ErC50 (72 h) of 16.2 mg/l. Using an assessment factor of 1000, a PNECaqua of 16.2 µg/l is derived. This PNEC is valid for the whole category.

Two tests on sludge respiration inhibition are available for L-menthol and D/L-menthol with EC10 values of 51 and 117 mg/l.

Tests on long-term toxicity on aquatic species as well as on terrestrial species are not available.

5 RECOMMENDATIONS

Environment:

The chemicals in the menthols category are currently of low priority for further work. The chemicals possess properties indicating a hazard for the environment. Although these hazards do not warrant further work as they are related to acute toxicity which may become evident only at very high exposure levels, they should nevertheless be noted by chemical safety professionals and users.

Human Health:

The chemicals in the menthols category are currently of low priority for further work because of their low hazard potential. However, skin and eye irritation is noted.

6 REFERENCES

- Andersen P and Jensen N (1984) Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test. *Mutat Res* 138: 17 - 20
- Atzl G, Bertl M, Daxenbichler G and Gleispach H (1972) Determination of etheral oils from the urine by gas-liquid chromatography. *Chromatographia* 5: 250 - 255
- Baer RL, Serri F and Weissenbach- Vial C (1955) Studies on allergic sensitization to certain topical therapeutic agents. *Arch Dermatol* 71: 19 - 23
- Barratt MD (1997) QSARS for the eye irritation potential of neutral organic chemicals. *Toxicology in Vitro* 11: 1 - 8
- Bayer AG: Acute toxicity of D/L-Menthol in the respiration inhibition test according to OECD Guideline 209, Internal Study, Report No. 136 A/89 B (1989)
- Bayer AG: Acute toxicity of D/L-Menthol on *Brachydanio rerio*. Internal Study, Report No. 136 A/89 F (1990a)
- Bayer AG: Acute toxicity of D/L-Menthol on *Daphnia magna*. Internal Study, Report No. 136 A/89 D (1990b)
- Bayer AG: Biodegradability of L-Menthol in the modified OECD Screening Test according to Guideline OECD 301E. Internal Study, Report No. 342 A/92 (1992a)
- Bayer AG: Acute toxicity of L-Menthol on *Brachydanio rerio*. Internal Study, Report No. 370 A/92 (1992b)
- Bayer AG: Acute toxicity of L-Menthol in the respiration inhibition test according to ISO 8192, Internal Study, Report No. 370 A/92 B (1992c)
- Bayer AG: Acute toxicity of D/L-Menthol on the alga *Scenedesmus subspicatus*. Internal Study, Report No. 959 A/00 (2000)
- Bayer AG: Menthols Category - Internal report on production, emissions and workplace settings (2002a)
- Bayer AG: Menthol L H and R: Acute Daphnia toxicity. Internal study, Study No. 1242 A/02 D (2002b)
- Bayer AG: Menthol L H and R: Alga, growth inhibition test. Internal study, Study No. 1242 A/02 A1 (2002c)
- Blondeel A, Oleffe J and Achten G (1978) Contact allergy in 330 dermatological patients. *Contact Dermatitis* 4: 270 - 276
- Blume, H.-P. (ed.) *Handbuch des Bodenschutzes: Bodenökologie und –belastung; vorbeugende und abwehrende Schutzmaßnahmen*, ecomed-Verlag, Landsberg-Lech (1990)
- Bolund S, Falus F and Jorgensen K (1967) A menthol loading test for glucuronide synthesis normal values. *Scand J Clin Lab Invest* 19: 288 - 290
- Camarasa G and Alomar A (1978) Menthol dermatitis from cigarettes. *Contact Dermatitis* 4: 169 - 170
- Carpenter CP and Smyth HF (1946) Chemical burns of the rabbit cornea. *Am J Ophthalmol.* 29, 1363 - 1372

Champeau M (1935) Accidents graves attribues a l'ingestion de 6 milligrammes de menthol chez une enfant de quatre ans et demi. Strasb Med 95: 553 - 554

Clegg RJ, Middleton B, Bell GD and White DA (1982) The mechanism of cyclic monoterpene inhibition of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase in vivo in the rat. J Biol Chem 257: 2294 - 2299

Danish product register, communication from June 2002

Dukes M.G (1980) Meyler's side effects of drugs: an encyclopedia of adverse reactions and interactions; 9th ed. Amsterdam - Oxford - Princeton, Excerpta Medica 279

EC, Technical guidance document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 1488/94 on risk assessment for existing substances. Ispra, European Chemicals Bureau (1996)

Eisenberg F, Field JB and Stetten D (1955) Studies on glyceronide conjugation in man. Arch Biochem Biophys 59: 297 - 299

FAO/WHO (1999) Menthol. In: WHO food additives series: 42: Safety evaluation of certain food additives. Geneva, World Health Organization 57 - 76

FDA (1973) Teratologic evaluation of FDA 71-57 (menthol natural, brazilian) Prepared for: Washington, D.C., U.S. Food and Drug Administration , PB-223 815 // June, 1973, 1 - 55

FDA (1975) Mutagenic evaluation of compound FDA 71-57, menthol Prepared for: Washington, D.C., U.S. Food and Drug Administration, PB-245 444 // January, 1975, 1 - 152

Fouremant P, Mason J, Valencia R and Zimmering S (1994) Chemical mutagenesis testing in Drosophila. IX. Results of 50 coded compounds tested for the national toxicology program. Environ Mol Mutagen 23: 51 - 63

Galloway SM, Miller JE, Armstrong MJ, Bean CL, Skopek TR and Nichols WW (1998) DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens. Mutat Res 400 (1 - 2): 169 - 186

Geiger, D.L. et al., Acute Toxicities of Organic Chemicals to Fathead Minnows (*Pimephales promelas*), Vol. IV. Center for Lake Superior Environmental Studies, University of Wisconsin-Superior (1988)

Gleason M, Gosselin R, Hodge H and Smith R (1969) Clinical toxicology of commercial products: acute poisoning; 3rd ed. Baltimore, The Williams and Wilkins Co.

Gomes-Carneiro M, Felzenszwalb I and Paumgartten F.R (1998) Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay. Mutat Res 416(1-2): 129 - 136

Haarmann and Reimer GmbH (1995) Local lymph node assay; Menthol L HandR Prepared for: 3450 Holzminden , Report No. CTL/E/160, 1 - 18

Haarmann and Reimer (1974c) Subacute toxicity of synthetic l-menthol , December 20, 1974, 1-8

Haarmann and Reimer GmbH (1974) Untersuchung der akuten oralen Toxizitäten der vier Menthol-Quantitäten (Menthol brasilianisch, D-Menthol dest., Menthol racemisch 100, L-Menthol HandR) Prepared for: Holzminden , 1 - 4

- Haarmann and Reimer GmbH (1980) Menthol flüssig - Akute Toxizität an Mäusen Prepared for: 3450 Holzminden , 1
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/131136 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11873, 1 - 11
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/131136 in rabbits Prepared for: 3450 Holzminden , Lab. No. 11753, 1 - 10
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/620006 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11872, 1 - 9
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/620001 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11870, 1 - 11
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/620005 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11755, 1 - 10
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/620001 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11754, 1 - 10
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/620006 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11756, 1 - 10
- Haarmann and Reimer GmbH (1989) Assessment of the eye irritant effect of HR 89/620005 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11871, 1 - 11
- Haarmann and Reimer GmbH (1989) Assessment of the skin irritant effect of HR 89/620006 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11876, 1 - 9
- Haarmann and Reimer GmbH (1989) Assessment of the skin irritant effect of HR 89/131136 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11877, 1 - 9
- Haarmann and Reimer GmbH (1989) Assessment of the skin irritant effect of HR 89/620001 in rabbits. Prepared for: 3450 Holzminden , Lab. No. 11874, 1 - 9
- Haarmann and Reimer GmbH (1989) Assessment of the skin irritant effect of HR 89/920005 in rabbits - Menthol-D. Prepared for: 3450 Holzminden , Lab. No. 11875, 1 - 9
- Haarmann and Reimer GmbH (1991) HR90/000102 Buehler sensitization test in guinea pigs , IRI-Inveresk Research International Limited, prepared for: Haarmann and Reimer GmbH, Holzminden, 6870 05/07/91, 1 - 20
- Haarmann and Reimer: Internal report: Menthols Category (2002) 6 pages
- Hall, R. L., Oser, B.L., Recent Progress in the Consideration of Flavoring Ingredients under the Food Additives Admendment. III GRAS Substances, Food Technology Feb. 1965, 253- 282 (1965)
- Hartmann A and Speit G (1997) The contribution of cytotoxicity to DNA-effects in the single cell gel test (comet assay). Toxicol Lett 90: 183 - 188
- Herken, H (1961) : Pharmacological expertise on tolerance to natural and synthetic menthol. Unpublished report from Pharmakologischen Institut der Freien Universität, Berlin, Dahlem. Submitted to WHO by Schering AG, Berlin (in German). As cited in: FAO/WHO (1999) Menthol. In: WHO food additives series: 42: Safety evaluation of certain food additives. Geneva, World Health Organization 57 - 76

- Hilliard C, Armstrong M, Bradt C, Hill R, Greenwood S and Galloway S (1998) Chromosome aberrations in vitro related to cytotoxicity of nonmutagenic chemicals and metabolic poisons. *Environ Mol Mutagen* 31(4): 316 - 326
- Hopp R (1993) Menthol : Its origins, chemistry, physiology and toxicological properties. *Recent Advances Tobacco Sci* 19 :3 - 46
- HSDB: Hazardous Substances Data Bank, data sheet for l-Menthol from 09/05/2001 (2001)
- Ishidate M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M: and Matsuoka A (1984) Primary mutagenicity screening of food additives currently used in Japan. *Fd Chem Toxic* 22(8): 623 - 636
- Ivett JL, Brown B, Rodgers C, Anderson BE, Resnick MA and Zeiger E (1989) Chromosomal aberrations and sister chromatid exchange tests in chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. *Environ Mol Mutagen* 14(3): 165 - 187
- Jarisch R and Sandor I (1978) Epicutanstandardtestung: Ergebnisse aus 5 Jahren und ihre Auswirkungen auf zukünftige Untersuchungen. *Z Hautkr* 53(13): 462 - 470
- Kaffenberger RM and Doyle MJ (1990) Determination of menthol and mentholglucuronide in human urine by gas chromatography using an enzyme-sensitive internal standard and flame ionization detection. *J Chromatogr* 527: 59 - 66
- Kanerva, L. et al. (2001) A Multicenter study patch test reactions with dental screening series. *American Journal of Contact Dermatitis* 12, 83-87
- Kevan, S.D. et al. (1999), Feeding menthol to honeybees (Hymenoptera: Apidae): Entry and persistence in Haemolymph without causing mortality, *The canadian Entomologist* 131, 279-281
- Kleinschmidt H (1935) Mentholschädigungen bei Säuglingen. *Med Welt* 23: 843 - 844
- Klinke K (1967) Klinische Beobachtungen nach Verabreichung von WickVapoRub bei Kleinkindern. In: Dost F and Leiber B (ed.) *Menthol and menthol-containing external remedies - Use, mode of effect and tolerance in children*. Stuttgart, Georg Thieme Verlag 82 - 86
- Lee, S. et al.: *J. Econ. Entomol.* 92(1), 56-67 (1999)
- Legiec C, Olpinska-Tomczyk I and Bzdulska-Doskocz B (1996) Contact drug allergy coexisting with crural ulceration and crural eczema. *Przegląd Dermatologiczny* 83(4): 371 - 375
- Leiber B (1967) Menthol-eine kritische Bestandsaufnahme. In: Dost F and Leiber B (ed.) *Menthol and menthol-containing external remedies*. Stuttgart, Georg Thieme Verlag 7 - 32
- Lesoine W (1965) Gefahren und Komplikationen bei der Anwendung mentholhaltiger Präparate in der HNO-Heilkunde. *HNO für die ärztliche Praxis* 13: 238 - 239
- Luke E (1962) Addiction to mentholated cigarettes. *Lancet* 1: 110 - 111
- Macht D (1939) Comparative pharmacology of menthol and its isomers. *Arch Pharmacodyn* 63: 43 - 58
- Mackay, Multimedia Environmental Models: The Fugacity Approach. Lewis Publishers Inc. Chelsea, Michigan (1991)
- Madhava Madyastha K and Srivatsan V (1988) Studies on the metabolism of L-menthol in rats. *Drug Metab Dispos* 16 (5): 765 - 772

- Martindale W (1982) Menthol. In: Reynolds (ed.) The extra pharmacopoeia 352
- Matsuoka-Atsuko, Hayashi-Makoto and Sofuni-Toshio (1998) In vitro clastogenicity of 19 organic chemicals found in contaminated water and 7 structurally related chemicals. *Environ Mutagen Res Commun* 20(3): 159 - 165
- Melis K, Janssens G and Bochner A (1989) Accidental nasal eucalyptol and menthol instillation. *Acta Clin Belg suppl.* 13: 101 - 102
- Mengs U and Stotzem C (1989) Toxicological evaluation of peppermint oil in rodents and dogs. *Med Sci Res* 17: 499 - 500
- MITI, Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Compiled under the Supervision of Chemical Products Safety Division, Basic Industries Bureau MITI, Ed. by CITI, October 1992. Published by Japan Chemical Industry Ecology-Toxicology and Information Center (1992)
- Morton C, Garioch J, Todd P and Lamey P F, (1995) Contact sensitivity to menthol and peppermint in patients with intra-oral symptoms. *Contact Dermatitis* 32: 281 - 284
- Murthy P.K, Ahmed M and Regu K (1991) Lack of genotoxicity of menthol in chromosome aberration and sister chromatid exchange assays using human lymphocytes in vitro. *Toxicol in Vitro* 5(4): 337 - 340
- Myhr B and Caspary W (1991) Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program. *Environ Mol Mutagen* 18: 51 - 83
- Nair (2001), *International Journal of Toxicology*, 20 (Suppl. 3): 61-73
- NCI (1979) National Cancer Institute: Bioassay of D/L-menthol for possible carcinogenicity. Technical Report Series No. 98, Bethesda, Maryland: 1 - 112
- Nohmi T, Miyata R, Yoshikawa K and Ishidate M (1985) Mutagenicity tests on organic chemical contaminants in city water and related compounds I. Bacterial mutagenicity tests. *Bull Nat Inst Hyg Sci* 0(103): 60 - 64
- O'Mullane NM, Joyce P and Kamath SV (1982) Adverse CNS effects of menthol- containing oilbas oil. *Lancet* 1/8281: 1121
- OECD, OECD Guidelines for Testing of Chemicals – Ready Biodegradability, adopted by the Council on 17th July 1992, Paris (1992)
- Olowe SA and Ransome-Kuti O (1980) The risk of jaundice in glucose-6-phosphate dehydrogenase deficient babies exposed to menthol. *Acta Paediatr Scand* 69: 341 - 345
- Opdyke D.J (1976) Monographs on fragrance raw materials. *Fd Cosmet Toxic* 14:473-474
- Pitter, P., *Water Res.* 10, 231 - 235 (1976)
- Quick AJ (1928) Quantitative studies of beta-oxidation IV. The metabolism of conjugated glycuronic acids. *J Biol Chem* 80: 535 - 541
- Rakieten N, Rakieten M and Boykin M (1954) Effects of menthol vapor on the intact animal with special reference to the upper respiratory tract. *J Am Pharm Ass* 43: 390 - 392
- Roempp, *Lexikon der Chemie* (10th. ed.), vol 4, p 2589 - 2590 (1998)

- Rudzki E and Kleniewska D (1971) Kontaktallergie auf einige Lokalthérapeutika und Konservierungsmittel. *Dermatologica* 143: 36 - 42
- Santucci B, Cristaudo A and Picardo M (1987) Contact dermatitis to fragrances. *Contact Dermatitis* 16: 93 - 95
- Schnuch A and Geier J (1995) Epikutantestung mit dem DKG-Analblock. *Dermatosen* 43: 81 - 82
- Schwenkenbecher A (1908) Über Mentholvergiftung des Menschen. *Münch Med Wschr* 55: 1495 - 1496
- Shah M, Lewis M and Gawkodger DJ (1996) Contact allergy in patients with oral symptoms: a study of 47 patients. *American Journal of Contact Dermatitis* 7: 146-151
- Sharp D (1978) The sensitization potential of some perfume ingredients tested using a modified dnaize procedure. *Toxicology* 9: 261 - 271
- Shelby M and et al. (1993) Evaluation of a Three-Exposure Mouse Bone Marrow Micronucleus Protocol: Results with 49 chemicals. *Environ Mol Mutagen* 21: 160 - 179
- Sofuni T, Hayashi M, Matsuoka A, Sawada M, Hatanaka M and Ishidate M (1985) Mutagenicity tests on organic chemical contaminants in city water and related compounds II. Chromosome aberration tests in cultured mammalian cells. *Eisei Shikensho Hokoku* 103: 64 - 75
- Sommerville KW, Richmond CR and Bell GD (1984) Delayed release peppermint oil capsules (Colpermin) for the spastic colon syndrome: A pharmacokinetic study. *Br J Clin Pharmacol* 18: 638 - 640
- Storer R, McKelvey T, Kraynak A, Elia M, Barnum J, Harmon L, Nichols W and DeLuca J (1996) Revalidation of the in vitro alkaline elution rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. *Mutat Res* 368: 59 - 101
- Swedish Product Register, communication from June, 2002
- Swiss Product Register, communication from June 5, 2002
- Thomas, R.G., Volatilization from water. In: *Handbook of chemical property estimation methods*; Lyman, W.J., Reehl, W.F., Rosenblatt, D.H. (Eds.), McGraw-Hill Book Company, New York, 15 - 16 (1990)
- Thorup I, Würtzen G, Carstensen J and Olsen P (1983a) Short term toxicity study in rats dosed with pulegone and menthol. *Toxicol Lett* 19: 207 - 210
- TNO: Determination of the ready biodegradability of Menthol L Dist. in a Closed Bottle Test. Report No. V4106/01, 2003a
- TNO: Determination of the ready biodegradability of Menthol D Dist. in a Closed Bottle Test. Report No. V4107/01, 2003b
- Tracor Jitco, I. (1976) Final report: 13-week subchronic toxicity - mice , Hazleton Laboratories America, Inc. Prepared for: Tracor Jitco, Inc. , Project No. 976-223, 1 - 17
- Tracor Jitco, I. (1976) Final report: 13-week subchronic toxicity - rats , Hazleton Laboratories America, Inc. Prepared for: Tracor Jitco, Inc. , Project No. 976-243, 1 - 16
- Ullmann's Encyclopedia of Industrial Chemistry, Flavours and Fragrances, Sixth Edition, 2002 Electronic release

- Uno Y, Takasawa H, Miyagawa M, Inoue Y, Murata T. and Yoshikawa K (1994) An in vivo - in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens. *Mutat Res* 320: 189 - 205
- Westat Inc. (1987a) Household solvent products: A national usage survey. Washington D.C.: Environmental Protection Agency, Office of Pollution Prevention and Toxics. EPA Contract No. 68-02-4243. July 1987
- Westat Inc. (1987b) National household survey of interior painters. Final report. Washington D.C.: Environmental Protection Agency, Office of Pollution Prevention and Toxics. July 1987
- Westat Inc. (1987c) National usage survey of household cleaning products. Final report. Washington D.C.: Environmental Protection Agency, Office of Pollution Prevention and Toxics. July 1987
- Westcott, L. and Winston, ML (1999), *The Canadian Entomologist* 131, 363-371
- Williams RT(1938) 239. Studies in detoxication II.(a) The conjugation of isomeric 3-Menthanols with glucuronic acid and the asymmetric conjugation of D/L-menthol, L-menthol and D/L-isomenthol in the rabbit. (b) D-isomenthylglucuronide, a new conjugated glucuronic acid. *Biochem J* 32: 1849 - 1855
- Wright S (1945) Etoxication mechanism in the sheep. *Univ Queens Papers* 1(25): 1 - 10
- Yamaguchi T, Caldwell J and Farmer P (1994) Metabolic fate of [3H]-L-menthol in the rat. *Drug Metab Dispos* 22 (4): 616 - 624
- Yoo Y(1986) Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. *J Osaka City Med Cent* 34: 267 - 288
- Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K (1988) Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ Mol Mutagen Suppl* 11(S12): 1 - 18

Annex 1: Menthols Category Justification

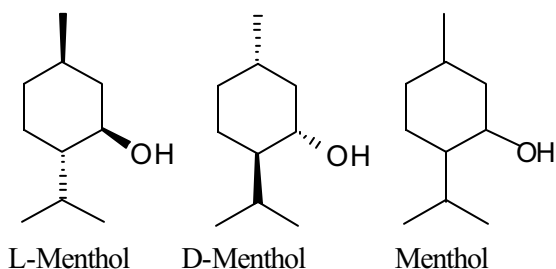
Identity:

Chemical name: cyclohexanol, 5-methyl-2-(1-methylethyl)-

Empirical Formula: $C_{10}H_{20}O$

Molecular weight: 156.27 g/mol

Structural Formula:



The molecule has 3 stereo centers, i.e. there are 8 possible stereoisomers (menthols, neomenthols, isomenthols, neoisomenthols). In nature the compound occurs generally as L- menthol, the main component of e.g. peppermint oil, mentha piperita, mentha oil etc. D/L-menthol, the racemic mixture of the L- and D- isomer, is exclusively of synthetic origin. D-menthol is a non-marketed side-product in the production process of L-menthol. However, as a main component of the widely used D/L-menthol it was included in this Category Approach.

The unspecified mixture of menthol isomers (CAS-No. 1490-04-6; here called menthol or menthol liquid) contains the L- and D- isomer in unspecified relation and can contain additional not specified stereoisomers. This mixture was included in the Menthol Category Approach because of the following reasons:

- The correct assignment of CAS-Nos. and chemical identities is very confusing for the group of menthol-isomers (16 different CAS- and EINECS Nos.). As a consequence, many authors used this CAS-No. in their publications not knowing the correct assignment.
- The data base for the unspecified isomer mixture is very large (see IUCLID). Not to include this mixture would lead to a loss of important knowledge on the physico-chemical, toxicological and environmental properties of the menthols.

L-menthol, D/L-menthol and menthol liquid are widely used as flavourings, disinfectant and cooling compounds.

The other stereoisomers (neomenthols, isomenthols, neoisomenthols) are of minor importance. These isomers are formed in the production of D/L-menthol and are re-introduced into the process after epimerization.

Below see the 4 menthol products of technical importance selected for the category approach:

Substance	Synonyms	CAS-No.	Remark
L-Menthol	(-)-Menthol Menthol, (1R, 3R, 4S)-(-)-	2216-51-5	Natural or synthetic menthol
D-Menthol	(+)-Menthol	15356-60-2	Non marketed by-product
D/L-Menthol	Racemate, "D/L-Menthol pure"	89-78-1	Synthetic product
Menthol or Menthol liquid	"D/L-Menthol raw"	1490-04-6	Unspecified mixture of isomers

In the following data summaries information will be presented that indicate these materials share similar physico-chemical properties, environmental fate characteristics, ecotoxicity, and mammalian toxicity.

Scientific literature was searched and summarized. Each study on category materials was evaluated for adequacy. Robust summaries were developed for each study addressing specific SIDS endpoints. Summaries were also developed for studies either considered not adequate but providing information of relevance for hazard identification and evaluation, or which covered non-SIDS endpoints.

Category Justification

As structural isomers, the members of the menthol category share the same molecular weight. Of particular importance to environmental effects are the values for partition coefficient (log Kow), vapour pressure and water solubility.

Available Physico-Chemical Data for Menthols:

Substance	L-Menthol	D-Menthol	D/L-Menthol	Menthol
CAS-No.	2216-51-5	15356-60-2	89-78-1	1490-04-6
Vapour Pressure	0.085 hPa (25°C)		1.3 hPa (55°C)	0.085 hPa (25°C)
Log Kow	3.4	3.4 (read-across from value for L-menthol and D/L-menthol)	3.4	3.4 (read-across from value for L-menthol and D/L-menthol)
Water Solubility	431 mg/l (20°C)		508 mg/l (20°C)	420 mg/l (20°C)

The enantiomeric menthols have identical physical properties (apart from their specific rotation), but the racemates differ from the optically active forms in, for example, their melting points (Ullmann 2002). The slight differences in the cited data are within the range of uncertainty range of laboratory tests.

The water solubility was determined for three products. Due to the similar molecular structures, no significant differences in the solubility are expected. The vapour pressure at environmental relevant temperatures was determined for L-menthol and an unspecified isomer mixture. As well as for the parameters above, similar values are expected for D-menthol and the racemate.

Available Data on Ready Biodegradability

Substance	L-Menthol	D-Menthol	D/L-Menthol	Menthol
CAS-No.	2216-51-5	15356-60-2	89-78-1	1490-04-6
OECD 301E	100 % after 28 d;			
MITI I	0% after 28d		0% after 28d	
OECD 301D	79 – 92 % after 28 d	76 – 92 % after 28 d		

The OECD 301E test using L-menthol as test substance showed a rapid decrease of DOC. However, there is no information about possible volatilisation and /or adsorption. The MITI (I) test (not clearly specified whether L- or D/L-menthol was used, in the literature source both CAS-numbers are referred) resulted in 0% oxygen consumption. It cannot be excluded that the employed substance concentration partly inhibited the inoculum. The two recently performed Closed-Bottle tests with D-menthol and L-menthol show clearly that both isomers are readily biodegradable.

Available Ecotoxicity Data

Substance	L-Menthol	D-Menthol	D/L-Menthol	Menthol
CAS-No.	2216-51-5	15356-60-2	89-78-1	1490-04-6
Fish (<i>P. Promelas</i>)	EC50 = 18.4 mg/l (e)			
Fish (<i>B. rerio</i>)	LC50 = 15.6 mg/l (e)		LC50 = 17.2 mg/l (e)	
Fish (<i>O. latipes</i>)	LC50 = 26 mg/l (e)			
Invertebrates (D. magna)	EC50 (24 h) = 37.7 mg/l (n) EC50 (48 h) = 26.6 mg/l (e)		EC50 (24 h) = 44.3 mg/l (n)	
Algae (<i>S. subspicatus</i>)	ErC50 = 21.4 mg/l (e) NOEC = 9.65 (e)		ErC50 = 16.2 mg/l (n) NOEC = 5 mg/l (n)	
Sludge (Respiration Inhibition)	EC10 = 51 mg/l (n) EC50 = 237 mg/l (n)		EC10 (3 h) = 117 mg/l (n) EC50 (3 h) = 306 mg/l (n)	

(e): effective concentration

(n): nominal concentration

For the toxicity of menthols on aquatic species experimental results from tests with fish, daphnis and algae are available for L-menthol and D/L-menthol. The available effect values for the two

category members indicate a similar range of toxicity within each trophic level, thus the available test results can be considered as being representative for all menthol isomers. Results are within the uncertainty range of laboratory effect tests.

Available Toxicity Data (Human Health)

The following data were identified for materials in the category:

Substance	L-Menthol	D-Menthol	D/L Menthol	Menthol
CAS-No.	2216-51-5	15356-60-2	89-78-1	1490-04-6
Acute toxicity oral dermal	v / +	v / +	v / + v / +	v / + v / -
Irritation skin eye	v / + v / +	v / + v / +	v / + v / +	v / + v / +
Sensitization	v / +		v / +	
Repeated dose toxicity	v / +		v / +	
Genetic toxicity in vitro in vivo	v / + v / +	v / -	v / + v / +	
Carcinogenicity			v / +	
Toxicity to fertility Developmental toxicity Toxicity to reproduction	v / + X*		X*	

v / + Adequate data available

v / - Data available, but not adequate

X* Data taken from repeated dose toxicity studies

Investigations on toxicokinetics show that L-, D/L- and the unspecified menthol are well absorbed via the oral route. For all of the isomers, elimination is rapid and mainly occurs as glucuronic acid conjugates via urine, minor amounts via faeces. Significant differences in toxicokinetic properties of menthol isomers were not reported.

The available toxicity data indicate very similar toxicity profiles for D-, L-, D/L-menthol and the unspecified menthol isomer mixture. In mammalian species the low toxicity is manifested in LD₅₀ values generally greater than 2000 mg/kg bw in acute studies, limited toxicity in repeated dose studies, and no effects in teratology evaluations. Irritation to skin and eyes was slight to moderate. The low hazard potential is not unexpected, since the FDA regulates menthol as a GRAS (generally recognized as safe) component and an acceptable daily intake (ADI) of 0-4 mg/kg bw for L-menthol and D/L-menthol was adopted in 1999 by the Joint FAO/WHO Committee.

All of the products have been tested for acute oral toxicity, skin and eye irritation in rodents, often following identical test protocols.

Data for sensitization, repeated dose toxicity, genetic toxicity, fertility, and carcinogenicity are available for D/L-menthol and mostly for L-menthol as well.

D/L-menthol is a racemic mixture of the D- and L- isomers and contains both isomers in equal proportion. Data gaps for D-menthol and the unspecified isomer mixture can therefore be filled by

the respective results with the racemic mixture and the doses for each isomer might be equivalent to half of the total tested D/L-dose.

L-menthol showed no embryotoxic or teratogenic properties at not maternally toxic dose levels (maternally toxic dose levels were not tested). No experimental data with the other menthol isomers is available with regard to developmental toxicity. Since there is no indication of a relevant difference between the isomers in their toxicokinetics and metabolism, and since this is further supported by all other available toxicological data, which do not show any evident differences in the respective toxicological profiles, there is no reason to assume that the stereoisomeric properties may affect the toxicological properties of the menthol isomers. Hence, a similar result in developmental toxicity studies would reasonably be expected from studies with D-menthol, the racemate or the unspecified menthol isomer.

Because of the low hazard potential of the chemicals in the menthols category, no further toxicity tests are recommended.

Menthol containing mixtures - peppermint oil:

Peppermint oil contains about 35 – 60 % menthol (menthone (15 - 30 %), menthylacetate (4 -14 %), and small amounts of cineole and other terpenes) (Nair, 2001). Hence, adverse effects after administration of peppermint oil cannot be associated with menthol. The peppermint oil studies are therefore considered to be not relevant for the hazard assessment of menthol.

SPECIES		PROTOCOL	RESULTS			
			L-Menthol	D-Menthol	D,L-Menthol	Unspecified mixture of Menthol isomers
CAS NO:			2216-51-5	15356-60-2	89-78-1 former CAS-No.: 15356-70-4	1490-04-6
PHYSICAL-CHEMICAL						
2.1	Melting Point		Ca. 42 °C	43 °C	30-32 °C	
2.2	Boiling Point (1013 hPa)		212°C	216.5 °C	216 °C	215.5 °C
2.3	Density		0.89 g/cm ³ (20°C)		0.895 g/cm ³ (20°C)	0.898 g/cm ³ (25°C)
2.4	Vapour Pressure		0.085 hPa (25°C)		1.3 hPa (55°C)	0.085 hPa (25°C)
2.5	Partition Coefficient (Log Kow)		3.4	3.4	3.4	3.4
2.6 A.	Water Solubility		431 mg/l (20°C)		508 mg/l (20°C)	420 mg/l (20°C)
B.	pH					
	pKa					
2.12	Oxidation: Reduction potential					

SPECIES		PROTOCOL	RESULTS			
CAS NO:			2216-51-5	15356-60-2	89-78-1	1490-04-6
ENVIRONMENTAL FATE AND PATHWAY						
3.1.1	Photodegradation	Calculated	In air $T_{1/2} = 16 \text{ h } (0.5 * 10^6 \text{ OH/cm}^3)$			
3.1.2	Stability in Water		Neither hydrolytic nor photolytic degradation expected			
3.1.3	Stability in Soil					
3.2	Monitoring Data					0.0093 - 0.139 µg/l
3.3	Transport and Distribution	Henry-constant (calculated) Calculated distribution (Fugacity Level I acc. to Mackay) Koc (calculated)	3.08 (Pa x m ³ /mol) In Air: 43.2 % In Water: 40.6 % In Soil: 8.0 % In Sediment: 8.1 % Biota: <0.1 % 614 l/kg	3.08 (Pa x m ³ /mol) In Air: 43.2 % In Water: 40.6 % In Soil: 8.0 % In Sediment: 8.1 % Biota: <0.1 % 614 l/kg	2.62 (Pa x m ³ /mol) In Air: 39.5 % In Water: 43.8 % In Soil: 8.7 % In Sediment: 7.9 % Biota: <0.1 % 614 l/kg	3.16 (Pa x m ³ /mol) In Air: 44.2 % In Water: 40.4 % In Soil: 8.0 % In Sediment: 7.3 % Biota: <0.1 % 614 l/kg
3.5	Biodegradation	Mixed activated sludge, non-adapted Mixed activated sludge, non-adapted activated sludge, adapted	OECD 301 E OECD 301 C (MITI I) Comparable to OECD 302 B OECD 301 D (Closed Bottle Test)	53% after 7 days 93% after 14 days 100 % after 28 days 0% after 28 days 79 – 92 % after 28 days	0% after 28 days	95.1% after 5 days
3.7	Bioaccumulation	<i>Cyprinus carpio</i>	OECD 305 C	BCF < 0.5 - 15 after 6-8 weeks		

		SPECIES	PROTOCOL	RESULTS			
CAS NO:				2216-51-5	15356-60-2	89-78-1	1490-04-6
ECOTOXICOLOGY							
4.1	Acute/Prolonged Toxicity to Fish	<i>Pimephales promelas</i> <i>Brachydanio rerio</i> <i>Oryzias latipes</i>	flow-through OECD 203 JIS K 0102-1986-71 (Japan)	EC50 (96 h) = 18.4 mg/l (effective) LC50 (96 h) = 15.6 mg/l (effective) LC50 (48 h) = 26 mg/l (effective)		LC50 (96 h) = 17.6 mg/l (effective)	
4.2	Acute Toxicity to Aquatic Invertebrates	<i>Daphnia magna</i>	Static	EC50 (24 h) = 37.7 mg/l (nominal) EC50 (48 h) = 26.6 mg/l (effective)		EC50 (24 h) = 71 mg/l (nominal)	
4.3	Toxicity to Aquatic Plants e.g. Algae	<i>Scenedesmus subspicatus</i>	OECD 201	ErC50 (72h) = 21.4 mg/l (effective) NOEC (72h) = 9.65 (effective)		ErC50 (72 h) = 16.2 mg/l (nominal) NOEC (72 h) = 5 mg/l (nominal) (Concentrations were measured but did not fall < 80 % of the nominal concentration)	
4.4	Toxicity to Microorganisms e.g. Bacteria	activated sludge	OECD 209	EC10 (3 h) = 51 mg/l (nominal) EC50 (3 h) = 237 mg/l (nominal)		EC10 = 117 mg/l (nominal) EC50 = 306 mg/l (nominal)	

SPECIES		PROTOCOL	RESULTS			
CAS NO:			2216-51-5 - L-Menthol	15356-60-2 - D-Menthol	89-78-1 - D/L-Menthol	1490-04-6 - Menthol, unspecified isomer
TOXICOLOGY						
5.1	Acute Oral Toxicity	Rat (f) Mouse (m)				
			Mainly according to OECD 401	LD50 = 2046 mg/kg bw	LD50 = 2602 mg/kg bw	No data LD50 >> 2000 mg/kg bw
5.1.2	Acute Inhalation Toxicity			No data	No data	No data
5.1.3	Acute Dermal Toxicity	Rabbit Mouse	No data orientating study (only 1 animal treated)	No data	LD50 > 5000 mg/kg bw	No data LD = 34 500 mg/kg bw
5.2	Corrosiveness and Irritation					
5.2.1	Skin Irritation	Rabbit	OECD 404	Moderately irritating	Moderately irritating	Moderately irritating
5.2.2	Eye Irritation	Rabbit	OECD 405 (vehicle: DEP) 1%, 5%,undiluted (vehicle: no data)	Slightly irritating	Slightly irritating	Slightly irritating
5.3	Sensitization	Rabbit Mouse Guinea pig Human	Buehler LLNA Modified Draize Patch Case reports	Not sensitizing Not sensitizing Ambiguous (positive after rechallenge) No data Sensitizing	No data No data No data Not sensitizing Sensitizing	No data No data No data No data Sensitizing

SPECIES		PROTOCOL	RESULTS			
CAS NO:			2216-51-5 - L-Menthhol	15356-60-2 - D-Menthhol	89-78-1 - D/L-Menthhol	1490-04-6 - Menthol, unspecified isomer
5.4	Repeated Dose Toxicity	Rat (m/f)	Gavage, 4 w	NOAEL/LOAEL not assignable	No data	No data
		Rat (m/f)	Feed, 5.5 w	NOAEL: 200 mg/kg bw/d (highest tested dose)	NOAEL: 200 mg/kg bw d (highest tested dose)	No data
		Rat (m/f)	Feed, 13 w	No data	NOAEL m = 937 mg/kg bw/d; f = 998 mg/kg bw/d	No data
		Mouse (m/f)	Feed, 13 w	No data	NOAEL m = 1956 mg/kg bw/d, f = 2386 mg/kg bw/d	No data
		Rat (m/f)	Feed, 103 w	No data	NOAEL m = 375 mg/kg bw/d; f = 188 mg/kg bw/d	No data
		Mouse (m/f)	Feed, 103 wk	No data	NOAEL m/f = 667 mg/kg bw/d	No data
		Rat (m/f)	Vapour Inhalation, 71-79 days	Irritation (lung, trachea)	No data	No data
5.6	Genetic Toxicity in Vitro					
Bacteria						
	Bacterial (Gene mutation)	Salmonella typhimurium TA 92, 94, 97a, 98, 100, 102, 1535, 1537, 2637	Ames Test	TA 97a, 98, 100, 102, 1535, 1537, 2637: negative (+/- MA)	No data	TA 92, 94, 98, 100, 1535, 1537, 2637: negative (+/- MA)
	Bacterial (Reverse Mutation)	E. coli WP2 uvrA (trp ⁻)	Ames Test	Negative	No data	No data
	Bacterial (Recombination assay)	M45 and H17	Recombination assay	Positive	No data	No data

SPECIES		PROTOCOL	RESULTS			
CAS NO:			2216-51-5 - L-Menthol	15356-60-2 - D-Menthol	89-78-1 - D/L-Menthol	1490-04-6 - Menthol, unspecified isomer
Mammalian cells						
Gene mutation	L5178Y mouse lymphoma cells	Mouse lymphoma assay	No data	No data	Negative (+/- MA)	No data
Cytogenetic Assay	Human Tissue cells	Chromosomal aberration test	Negative	No data	No data	No data
Cytogenetic Assay	CHO TK6 human lymphoblasts	Chromosomal aberration test	No data	No data	Negative (+/- MA); Positive (+ MA)	No data
	CHL Human lymphocytes		No data	No data	Negative (- MA)	No data
			Negative (+/- MA)		No data	
DNA -damage	Primary rat hepatocytes	Alkaline elution assay	No data	No data	Negative	No data
DNA -damage	V79 CHL	Comet assay	No data	Negative (+/- MA)	No data	No data
	Human lymphocytes			Negative (+/- MA)		
DNA -damage	CHO	Sister chromatid exchange assay	No data	No data	Negative (+/- MA)	No data
	Human lymphocytes		Negative (+/- MA)		No data	
5.6 Genetic Toxicity in Vivo						
SLRL Assay	<i>Drosophila melanogaster</i>	Feed Injection	No data	No data	Negative Negative	No data
Dominant lethal Assay	Rat	Single dose (gavage) 5 applications (gavage)	Negative Negative	No data	No data	No data

	SPECIES	PROTOCOL	RESULTS			
CAS NO:			2216-51-5 - L-Menthol	15356-60-2 - D-Menthol	89-78-1 - D/L-Menthol	1490-04-6 - Menthol, unspecified isomer
	Cytogenetic Assay	Bone marrow albino rats	Single dose (gavage) 5 applications (gavage)	Negative Negative	No data	No data
	Micronucleus assay	Bone marrow B6C3F1 mice	3 applications (injection)	No data	Negative	No data
5.7	Carcinogenicity	Rat (m/f) Mouse (m/f)	Feed, 103 w Feed, 103 w	No data	No evidence of carcinogenicity No evidence of carcinogenicity	No data
5.8.1	Toxicity to Fertility	Rat	Feed, 13 w Feed, 103 w	No data	No evidence of adverse effects on reproduction organs	No data
5.8.2	Developmental Toxicity/Teratogenicity	Mouse	Feed, 13 w Feed, 103 w	No data	No evidence of adverse effects on reproduction organs	No data
		Rats	Gavage, gd 6-15	NOEL (maternal and foetal) = 218 mg/kg bw/d	No data	No data
		Mouse	Gavage, gd 6-15	NOEL (maternal and foetal) = 185 mg/kg bw/d	No data	No data
		Rabbits	Gavage, gd 6-18	NOEL (maternal and foetal) = 425 mg/kg bw/d	No data	No data
		Syrian hamster	Gavage, gd 6-10	NOEL (maternal and foetal) = 405 mg/kg bw/d	No data	No data

SPECIES		PROTOCOL	RESULTS			
CAS NO:						
5.9	Specific Investigations					
5.10	Exposure Experience	Humans children under 1 year	Pharmaceutical properties			
						enhancement of lung and airway volume Menthol applied to the nostrils or near the nose in few cases caused reflex apnea

I U C L I D Data Set

Existing Chemical : ID: 2216-51-5
CAS No. : 2216-51-5
EINECS Name : L-Menthol
EC No. : 218-690-9
TSCA Name : Cyclohexanol, 5-methyl-2-(1-methylethyl)-
Molecular Formula : C₁₀H₂₀O

Producer related part

Company : Bayer AG
Creation date : 17.10.2001

Substance related part

Company : Bayer AG
Creation date : 17.10.2001

Status :
Memo : ICCA Bayer AG

Printing date : 10.06.2003
Revision date :
Date of last update : 18.03.2003

Number of pages : 1

Chapter (profile) : Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile) : Reliability: without reliability, 1, 2, 3, 4
Flags (profile) : Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information

Id	2216-51-5
Date	10.06.2003

1.0.1 APPLICANT AND COMPANY INFORMATION**1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR****1.0.3 IDENTITY OF RECIPIENTS****1.0.4 DETAILS ON CATEGORY/TEMPLATE****1.1.0 SUBSTANCE IDENTIFICATION****1.1.1 GENERAL SUBSTANCE INFORMATION**

Purity type	: typical for marketed substance
Substance type	: organic
Physical status	: solid
Purity	: >= 99.7
Colour	: white
Odour	: minty
Remark	: maximum of 0.3 %: other menthol isomers
Flag	: Critical study for SIDS endpoint
07.08.2002	

1.1.2 SPECTRA**1.2 SYNONYMS AND TRADENAMES****(-)-Menthol**

Flag	: Critical study for SIDS endpoint
17.10.2001	

1-Menthol (natural)

Flag	: Critical study for SIDS endpoint
17.10.2001	

5-METHYL-2-(1-ETHYLETHYL)-CYCLOHEXANOL

Flag	: Critical study for SIDS endpoint
03.06.2002	

Cyclohexanol, 5-methyl-2-(1-methylethyl)-

Flag	: Critical study for SIDS endpoint
17.10.2001	

1. General Information

Id	2216-51-5
Date	10.06.2003

Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1R- (1alpha, 2beta, 5alpha)

Flag : Critical study for SIDS endpoint
03.06.2002

(1)

L-MENTHOL

Flag : Critical study for SIDS endpoint
03.06.2002

Levomenthol

Flag : Critical study for SIDS endpoint
17.10.2001

MENTHOL L

Flag : Critical study for SIDS endpoint
03.06.2002

Menthol, (1R, 3R, 4S)-(-)-

Flag : Critical study for SIDS endpoint
17.10.2001

1.3 IMPURITIES**1.4 ADDITIVES****1.5 TOTAL QUANTITY****1.6.1 LABELLING**

Labelling : provisionally by manufacturer/importer
Specific limits :
Symbols : Xi, , ,
Nota : , ,
R-Phrases : (38) Irritating to skin
S-Phrases : (25) Avoid contact with eyes

Flag : Critical study for SIDS endpoint
17.07.2002

1.6.2 CLASSIFICATION

Classified : provisionally by manufacturer/importer
Class of danger : irritating
R-Phrases : (38) Irritating to skin
Specific limits :
Flag : Critical study for SIDS endpoint

1. General Information

Id	2216-51-5
Date	10.06.2003

03.06.2002

1.6.3 PACKAGING**1.7 USE PATTERN**

Type of use	: type
Category	: Wide dispersive use

Remark	: L-Menthol, D/L-menthol and menthol liquid are widely used as flavoring, disinfectant and cooling compounds in confectionery products, liqueurs, chewing gums, toothpastes, cosmetics and common cold ointments and medications and veterinary activities

Flag	: Critical study for SIDS endpoint

23.07.2002

1.7.1 DETAILED USE PATTERN**1.7.2 METHODS OF MANUFACTURE**

Origin of substance	: Synthesis
Type	: Production

Remark	: L-Menthol is produced via reaction of m-cresol with propen to thymol, and hydrogenation of thymol, resulting in 4 isomers: D/L-neomenthol, D/L-neoisomenthol, D/L-menthol and D/L-isomenthol. D/L-menthol is isolated by fractional distillation. To produce L-menthol, D/L-menthol is transesterificated with methylbenzoate and further manufactured.

03.06.2002

1.8 REGULATORY MEASURES**1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES****1.8.2 ACCEPTABLE RESIDUES LEVELS****1.8.3 WATER POLLUTION****1.8.4 MAJOR ACCIDENT HAZARDS****1.8.5 AIR POLLUTION**

1. General Information

Id	2216-51-5
Date	10.06.2003

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : other: Registry of Toxic Effects of Chemical Substances, NIOSH, USA
Additional information :

03.06.2002

(2)

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS**1.9.2 COMPONENTS****1.10 SOURCE OF EXPOSURE****1.11 ADDITIONAL REMARKS****1.12 LAST LITERATURE SEARCH**

Type of search : Internal and External
Chapters covered : 5
Date of search : 01.09.2001

Remark : Human Health: last literature search September 2001: CAS number search in external and internal databases, e.g. Biosis, Embase, Toxline, Scisearch
Flag : Critical study for SIDS endpoint
 10.07.2002

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 14.01.2002

Remark : Physico-chemical properties / Environment / Ecotoxicology : last literature search January 2002: CAS number search in external and internal databases, e.g. HSDB, Aquire.
Flag : Critical study for SIDS endpoint
 29.07.2002

1.13 REVIEWS

Memo : Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties
Flag : Critical study for SIDS endpoint
 03.06.2002

(1)

2. Physico-Chemical Data

Id 2216-51-5
Date 10.06.2003

2.1 MELTING POINT

Value : ca. 42 °C
Sublimation :
Method : other: PIQ-Method DS061 modified
Year : 2000
GLP :
Test substance : other TS: purity > 99.7 %

Flag : Critical study for SIDS endpoint
18.03.2003

(2) (3)

Value : 41 - 43 °C
Sublimation :
Method :
Year :
GLP : no data
Test substance : other TS: no data

14.03.2003

(4) (5)

Value : 43 °C
Sublimation :
Method :
Year : 1993
GLP : no data
Test substance :

14.03.2003

(1)

Value : 35 - 36 °C
Sublimation :
Method :
Year : 1992
GLP : no data
Test substance : other TS: not clearly identified, cf. Remark

Remark : The reference notes both CAS-No. 2216-51-5 and 15356-70-4 (former
CAS number for 89-78-1)

14.03.2003

(6)

2.2 BOILING POINT

Value : 212 °C at
Decomposition :
Method :
Year : 1954
GLP : no
Test substance : no data

Flag : Critical study for SIDS endpoint
14.03.2003

(4) (7) (5)

Value : ca. 216 °C at 1013 hPa
Decomposition :
Method :

2. Physico-Chemical Data

Id 2216-51-5
Date 10.06.2003

Year : 2002
GLP :
Test substance : other TS: purity > 99.7 %

18.03.2003 (2)

Value : 103 - 105 °C at 21.3 hPa
Decomposition :
Method :
Year : 1992
GLP :
Test substance : other TS: see Remark

Remark : The reference notes both CAS-No. 2216-51-5 and 15356-70-4 (former CAS number for 89-78-1)

18.03.2003 (6)

2.3 DENSITY

Type : density
Value : .89 g/cm³ at 20 °C

Flag : Critical study for SIDS endpoint

14.03.2003 (2) (4) (5)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .085 hPa at 25 °C
Decomposition :
Method :
Year : 1954
GLP : no
Test substance : no data

Remark : extrapolated to 25 °C
Flag : Critical study for SIDS endpoint

14.03.2003 (4) (7) (8)

Value : < .1 hPa at 20 °C
Decomposition :
Method :
Year : 2002
GLP : no data
Test substance : other TS: purity > 99.7 %

18.03.2003 (2)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water

2. Physico-Chemical Data

Id 2216-51-5
Date 10.06.2003

Log pow : 3.4 at °C
pH value :
Method : other (measured)
Year : 1999
GLP : no data
Test substance : other TS: purity not reported but HPLC applied

Method : Reversed-phase high-performance liquid chromatography
Flag : Critical study for SIDS endpoint
14.03.2003

(9)

Partition coefficient : octanol-water
Log pow : 3.3 at °C
pH value :
Method : other (measured)
Year :
GLP :
Test substance :

Remark : The reference "MITI (1992)" notes both CAS-No. 2216-51-5 and 15356-70-4 (former CAS-No. for 89-78-1).
The Chemical Safety Data Sheet "Menthol L H&R Cryst", Haarmann & Reimer GmbH gives no information on the method. The HSDB is secondary literature.

18.03.2003

(2) (4) (6)

Partition coefficient : octanol-water
Log pow : 3.38 at °C
pH value :
Method : other (calculated): SRC-KOWWIN v. 1.66
Year : 2002
GLP :
Test substance :

18.03.2003

(10)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 431 mg/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: flask method
Year : 1992
GLP :
Test substance :

Flag : Critical study for SIDS endpoint
06.03.2003

(11) (2)

Solubility in : Water

2. Physico-Chemical Data

Id 2216-51-5
Date 10.06.2003

Value : 456 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1992
GLP : no data
Test substance : no data

18.03.2003

(12)

Solubility in : Water
Value : 490 mg/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1992
GLP :
Test substance : other TS: see Remark

Remark : The reference notes both CAS-No. 2216-51-5 and 15356-70-4 (former CAS number for 89-78-1)

18.03.2003

(6)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : > 100 °C
Type : closed cup
Method :
Year :
GLP : no data
Test substance : other TS: purity > 99.7 %

18.03.2003

(2)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2. Physico-Chemical Data

Id	2216-51-5
Date	10.06.2003

2.10 EXPLOSIVE PROPERTIES**2.11 OXIDIZING PROPERTIES****2.12 DISSOCIATION CONSTANT****2.13 VISCOSITY****2.14 ADDITIONAL REMARKS**

Memo : Refraction index (n_D): 1.458 at 25 °C

03.06.2002 (5)

Memo : alpha D₁₈ = - 50 degree (10 % alc. solution)

03.06.2002 (5)

Memo : alpha D₂₀ = - 50.2 degree

03.06.2002 (1)

3. Environmental Fate and Pathways

Id 2216-51-5
Date 10.06.2003

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight

Method : structure estimation method
Result : Rate constant: $k = 2.4 \times 10^{-11}$ cm³/molecule/sec at 25 degrees C;
considering an atmospheric OH-radical concentration of 5×10^5 OH-radicals/cm³, the half-life is about 16 h

Reliability : (2) valid with restrictions
accepted calculation procedure

Flag : Critical study for SIDS endpoint
29.07.2002

(13) (4)

3.1.2 STABILITY IN WATER

Deg. product :
Method : other (calculated)
Year :
GLP :
Test substance :

Result : volatilization half-lives for a model river (1 m deep, flow-rate 1 m/sec, wind velocity 3 m/sec) and a model lake (1 m deep, flow-rate 0.05 m/sec, wind velocity 0.5 m/sec) are estimated to be 2 and 18 days

Reliability : (2) valid with restrictions
accepted calculation procedure

Flag : Critical study for SIDS endpoint
29.07.2002

(4)

3.1.3 STABILITY IN SOIL**3.2.1 MONITORING DATA****3.2.2 FIELD STUDIES****3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS**

Type : volatility
Media : water - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year : 2003

3. Environmental Fate and Pathways

Id 2216-51-5
Date 10.06.2003

Result : Based on a water solubility of 431 mg/l and a vapour pressure of 8.5 Pa (see chapter 2), the Henry's law constant is calculated to be 3.08 Pa x m³/mol

Reliability : (2) valid with restrictions
Generally accepted calculation method

Flag : Critical study for SIDS endpoint
14.03.2003 (14)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water

Method : Calculation according Mackay, Level I

Year : 2003

Result : Air: 43.2 %
Water: 40.6 %
Soil: 8.0 %
Sediment: 8.1 %
Biota: 0.005 %

Test condition : Base data for calculation:
temperature: 20 °C
molar mass: 156.27 g/mol
vapour pressure: 8.5 Pa
water solubility: 431 g/m³
log Kow: 3.4
environmental compartments:
- air: 6*10⁹ m³, 1.2 kg/m³
- water: 7*10⁶ m³, 1000 kg/m³
- soil: 4.5 *10⁴ m³, 1500 kg/m³, 2 % org. C
- sediment: 2.1*10⁴ m³, 1300 kg/m², 5 % org. C
- susp. sediment: 35 m³, 1500 kg/m³, 16.7 % org. C
- aerosol: 0.12 m³, 1500 kg/m³
- aquatic biota: 7 m³, 1000 kg/m³, 5 % fat

Reliability : (2) valid with restrictions
Generally accepted calculation method

Flag : Critical study for SIDS endpoint
14.03.2003 (14)

Media : water - soil

Method : other (calculation)

Year :

Result : Using the equation log Koc = 0.52 log Kow + 1.02 and based on a log Kow of 3.40 (see chapter 2) a Koc value of 614 can be calculated for the distribution between the organic phase of soil and pore water

Reliability : (2) valid with restrictions
Generally accepted calculation method

Flag : Critical study for SIDS endpoint
07.03.2003 (15)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic

3. Environmental Fate and Pathways

Id 2216-51-5
Date 10.06.2003

Inoculum	: activated sludge, domestic
Concentration	: .84 mg/l related to Test substance related to
Contact time	: 28 day(s)
Degradation	: 93 (±) % after 21 day(s)
Result	: readily biodegradable
Kinetic of testsubst.	: 0 day(s) 0 % 7 day(s) 64 % 14 day(s) 90 % 21 day(s) 93 % 28 day(s) 92 %
Control substance	: Acetic acid, sodium salt
Kinetic	: 7 day(s) 86 % 14 day(s) 100 %
Deg. product	:
Method	: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	: 2003
GLP	: yes
Test substance	: other TS: purity 99.963 %
Remark	: Measured degradation of acetic acid was 103 % after 14 d
Result	: The biodegradation in the toxic controls exceeded 25 %. According to the guideline, the test substance is not toxic to the medium
Test condition	: Two concentrations of the test substance (0.84 mg/l, 2 mg/l) a control (blank medium), an inoculum activity control (sodium acetate) and a toxicity control (sodium acetate and L-menthol) were prepared with mineral medium, saturated with oxygen, placed in approximately 300 ml BOD bottles, and incubated for 28 d in the dark at about 20 °C, except for the activity control and the toxicity control which were incubated for 14 d. To prevent leakage of gases out of the BOD bottles the bottles were incubated upside down. The O ₂ concentration was determined with an oxygen electrode after 0, 7, 14, 21, and 28 d of incubation
Reliability	: (1) valid without restriction Guideline study in accordance with the OECD principles of GLP
Flag	: Critical study for SIDS endpoint
05.03.2003	(16)
Type	: aerobic
Inoculum	: activated sludge, domestic
Concentration	: 2 mg/l related to Test substance related to
Contact time	: 28 day(s)
Degradation	: 79 (±) % after 28 day(s)
Result	: readily biodegradable
Kinetic of testsubst.	: 0 day(s) 0 % 7 day(s) 64 % 14 day(s) 76 % 21 day(s) 77 % 28 day(s) 79 %
Control substance	: Acetic acid, sodium salt
Kinetic	: 7 day(s) 86 % 14 day(s) 100 %
Deg. product	:
Method	: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	: 2003
GLP	: yes
Test substance	: other TS: purity 99.963 %
Remark	: Measured degradation of acetic acid was 103 % after 14 d

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Result	: The biodegradation in the toxic controls exceeded 25 %. According to the guideline, the test substance is not toxic to the medium	
Test condition	: Two concentrations of the test substance (0.84 mg/l, 2 mg/l) a control (blank medium), an inoculum activity control (sodium acetate) and a toxicity control (sodium acetate and L-menthol) were prepared with mineral medium, saturated with oxygen, placed in approximately 300 ml BOD bottles, and incubated for 28 d in the dark at about 20 °C, except for the activity control and the toxicity control which were incubated for 14 d. To prevent leakage of gases out of the BOD bottles the bottles were incubated upside down. The O ₂ concentration was determined with an oxygen electrode after 0, 7, 14, 21, and 28 d of incubation	
Reliability	: (1) valid without restriction	
Flag 05.03.2003	: Guideline study in accordance with the OECD principles of GLP : Critical study for SIDS endpoint	(16)
Type	: anaerobic	
Inoculum	: activated sludge	
Deg. product	:	
Method	:	
Year	: 1995	
GLP	: no	
Test substance	: other TS: (-)-menthol, analytical grade	
Method	: Determination of microbial growth under nitrate-reducing conditions	
Result	: Microbial growth was observed within 10 days to 3 weeks; no quantification of growth or degree of degradation	
Test condition	: Enrichment cultures were prepared using 2 ml sewage sludge, 400 ml mineral salt medium, and 200 mg TS with 4 ml HMN as carrier, atmosphere N ₂ /CO ₂ . Incubation at 28 degrees C in the dark.	
Reliability	: (2) valid with restrictions No standard test procedure, but in accordance with generally accepted scientific standards	
05.03.2003		(17)
Type	: aerobic	
Inoculum	: other: Rhizoctonia solani	
Contact time	:	
Degradation	: (±) % after	
Result	: other: Biotransformation	
Deg. product	: yes	
Method	: other: Growth medium	
Year	: 2001	
GLP	: no	
Test substance	: no data	
Result	: Almost all of the substrate was consumed in 3 days incubation. The major metabolite was determined to be 6-Hydroxymenthol.	
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles.	
05.03.2003		(18)
Type	: aerobic	
Inoculum	: other: activated sludge, inoculum from effluent of a laboratory facility run with municipal sewage	
Concentration	: 20 mg/l related to DOC (Dissolved Organic Carbon) related to	
Contact time	: 28 day(s)	
Degradation	: 100 (±) % after 21 day(s)	

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Result	: readily biodegradable	
Kinetic of testsubst.	: 0 hour(s) 0 %	
	: 7 day(s) 53 %	
	: 14 day(s) 93 %	
	: 21 day(s) 100 %	
	: 28 day(s) 100 %	
Control substance	: Aniline	
Kinetic	: 28 day(s) 100 %	
	: %	
Deg. product	:	
Method	: OECD Guide-line 301 E "Ready biodegradability: Modified OECD Screening Test"	
Year	: 1992	
GLP	: yes	
Test substance	: other TS: 99.9%	
Remark	: Method according to guideline: 79/831 EWG. Annex V, Part C (updated: July 1990), Method C.4-B: modified OECD Screening-Test	
Test condition	: mineral salt medium; 20-24 degrees C	
Reliability	: (3) invalid	
	Possible loss of test substance by volatilisation	
05.03.2003		(19)
Type	: aerobic	
Inoculum	: activated sludge	
Concentration	: 100 mg/l related to Test substance	
	: related to	
Contact time	: 28 day(s)	
Degradation	: 0 (±) % after	
Result	:	
Deg. product	:	
Method	: other: corresponding to OECD 301C	
Year	: 1992	
GLP	:	
Test substance	:	
Remark	: The reference notes both CAS-No. 2216-51-5 and 15356-70-4 (former Cas -No for 89-78-1)	
Test condition	: sludge concentration 30 mg/l	
Reliability	: (3) invalid	
	Biodegradation possibly affected by toxicity of the substance at the concentration tested	
05.03.2003		(6)
Deg. product	:	
Method	:	
Year	: 1999	
GLP	:	
Test substance	: other TS: (-)-menthol	
Remark	: Based on the previous literature it can be stated that the bacteria species: Thauera terpenica, strain 21Mol may degrade L-menthol.	
Reliability	: (4) not assignable	
	Review. No experimental data is given	
05.03.2003		(20)

3. Environmental Fate and Pathways

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3.6 BOD5, COD OR BOD5/COD RATIO**3.7 BIOACCUMULATION**

Species	: Cyprinus carpio (Fish, fresh water)
Exposure period	: at °C
Concentration	:
Elimination	:
Method	: other: corresponding to OECD guideline 305C
Year	: 1992
GLP	:
Test substance	: no data
Remark	: The reference notes both CAS-No. 2216-51-5 and 15356-70-4 (former CAS -No. for 89-78-1)
Result	: BCF: <0.5 - 15 l/kg at 0.2 mg/l BCF: <4.6 - 11 l/kg at 0.02 mg/l
Test condition	: flow-through system; 25 degrees C; O2 6-8 mg/l; 15-20 fish/level; exposure period 6-8 weeks
Reliability	: (2) valid with restrictions Test procedure according to guideline without detailed documentation
Flag	: Critical study for SIDS endpoint
05.03.2003	

(6)

3.8 ADDITIONAL REMARKS

4. Ecotoxicity

Id 2216-51-5
Date 10.06.2003

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	: flow through
Species	: Pimephales promelas (Fish, fresh water)
Exposure period	: 96 hour(s)
Unit	: mg/l
LC50	: 18.9
EC50	: 18.4
Limit test	: no
Analytical monitoring	: yes
Method	: other
Year	: 1985
GLP	: no
Test substance	: other TS: purity 99%
Method	: Fish (30 d old; mean length 17.6 mm; mean weight 0.079 g) exposed in Lake Superior water; 5 TS concentrations in the range of 4.39 to 24.6 mg/l tested (plus control); number of dead fish recorded every 24 h; observations of fish behaviour and body morphology at regular intervals; TS analysis by GLC
Result	: Affected fish lost schooling behaviour, were hyperactive and underreactive to external stimuli. They had increased respiration, were darkly colored and lost equilibrium prior to death. The 96-h samples were omitted due to unrealistic analytical results.
Test condition	: 24.4 degrees C; dissolved oxygen 6.8 mg/l; hardness 44.5 mg CaCO ₃ /l; alkalinity 44.5 mg CaCO ₃ /l; pH 7.7
Reliability	: (1) valid without restriction Test procedure comparable to standard method and in accordance with general accepted scientific standards; detailed documentation of test procedure and test conditions
Flag	: Critical study for SIDS endpoint
21.12.2001	(21)
Type	: Static
Species	: Brachydanio rerio (Fish, fresh water)
Exposure period	: 96 hour(s)
Unit	: mg/l
LC0	: 13.2
LC50	: 15.6
LC100	: 18.4
Limit test	: no
Analytical monitoring	: yes
Method	: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	: 1992
GLP	: yes
Test substance	: other TS: 99.9 %
Remark	: LC50-value: geom. mean of LC0 and LC100. LC50 can be calculated with probit analysis. In this test the LC50 obtained with probit analysis is identical to the geometric mean. All LC values are based on measured concentrations
Result	: RESULTS: EXPOSED - Nominal/measured concentrations: nominal (mg/l) 7.8 11 16 22 31 measured (mg/l) (0 h) 6.6 9.5 13.8 19.8 27.5 (mg/l) (24 h) 6.7 8.9 13.5 18.6 25.9 (mg/l) (48 h) 6.0 8.7 13.3 18.2 (mg/l) (72 h) 5.5 8.4 12.9 17.1

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(mg/l) (96 h) 5.2 8.0 12.5

- Effect data (Mortality):

Mortality, visible abnormalities of fishes

- Concentration / response curve:

There were no dead fishes in tanks with concentration: 7.8, 11 and 16 mg/l.

22 mg/l

hours (h) 0 2 24 48 72 96

Mortality (%) 0 0 50 50 100

31 mg/l

Mortality (%) 0 100

- Effect concentration vs. test substance solubility:

Undissolved substance particles remained on the water surface of all test media.

- Other effects:

Temperature degradation during testing process was higher than 1 °C.

RESULTS: CONTROL: No dead fish

- Number/percentage of animals showing adverse effects:

7.8 mg/l

hours (h) 2 24 48 72 96

7.8 mg/l - - - - -

11 mg/l 100%A 100%A 100%A 100%A 100%A

16 mg/l 100%A 100%A 80%A 80%A 100%A

20%B 20%B

22 mg/l 100%A 50%B 50%B 10%B 10%B

- Nature of adverse effects:

A: slow and inactive swimming behaviour

B: loss of equilibrium (uncontrolled movements)

Reliability

: (2) valid with restrictions

Guideline Study; effective concentrations decreased below 80% of the nominal during the test period

Flag

05.03.2003

: Critical study for SIDS endpoint

(22)

Type

: other: Static or semistatic

Species

: *Oryzias latipes* (Fish, fresh water)

Exposure period

: 48 hour(s)

Unit

: mg/l

LC50

: 26

Method

: other: according to JIS K 0102-1986-71 (Japanese Industrial Standard)

Year

: 1992

GLP

:

Test substance

: no data

Remark

: TS unclear. The reference notes both CAS -No. 2216-51-5 and 15356-70-4

Test condition

: 25 +/- 2 degrees C; 10 fish/level

Reliability

: (2) valid with restrictions

Test procedure according to guideline without detailed documentation

Flag

07.03.2002

: Critical study for SIDS endpoint

(6)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES**Type**

: static

Species

: *Daphnia magna* (Crustacea)

Exposure period

: 48 hour(s)

Unit

: mg/l

EC0

: 11.35

EC50

: 26.6

4. Ecotoxicity

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Date 10.06.2003

EC100	:	92.35
Analytical monitoring	:	yes
Method	:	Directive 92/69/EEC, C.2
Year	:	2002
GLP	:	yes
Test substance	:	other TS: purity 99.985 % according to H & R GmbH
Method	:	Method for chemical analysis: GC analysis Limit of quantitation: 0.5 mg/l Recovery rate: 102% ± 5% Sampling schedule of chemical analysis Stock solution: at 0 h Control: at 0 and 48 h Test concentrations: at 0, 24 and 48 h
Result	:	The results are expressed in terms of nominal concentrations (at 24 h), and in terms of mean measured concentrations (at 48 h). Measured concentrations ranged from 93.1 - 104 % of nominal values at 0 h, and from 81.3 - 91.6 % of nominal values at 48 h, respectively. After 24 h EC0 was 25 mg/l, EC50 37.7 (lower/upper 95% confidence limit: 18.8/75.6) mg/l and EC100 100 mg/l After 48 h EC0 was 11.35 mg/l, EC50 26.6 (14.7/48.2) mg/l and EC100 92.35 mg/l Highest test concentration resulting in 0 % immobilisation (EC0 48 h): 11.35 mg/l Lowest test substance concentration resulting in 100 % immobilisation (EC100 48 h): 92.35 mg/l
Test condition	:	Test species: - A population of parthenogenetic females of synchronized age structure is maintained since more than 15 years in the test facility under constant temperature conditions (20 +/- 1 °C) at a 16/8 h light-dark photoperiod (illumination: < 1000 lux) - The culture water (so-called 'M4 medium') is partly renewed once a week. The Daphnia are exclusively fed with unicellular green algae (<i>Desmodesmus subspicatus</i>) ad libitum - Mortalities of parent Daphnia during the culture period are recorded daily in a semi-quantitative way. The neonates are separated from their parent Daphnia by filtration prior to the acute test Culture and dilution water: - Reconstituted water (so-called 'M4 medium', originally described in Water Research 24 (9): 1157 - 1167), prepared according to the recommendations of Bundesgesundheitsamt Berlin. This standard dilution water is used for both, the maintenance of the test animals and the preparation of stock and test solutions of the test substance - The total hardness of the dilution water, measured at test start, was 13.8°dH (= 246.3 mg/l CaCO₃) Test substance: - The test substance was pulverized. - A stock solution was prepared to give the desired series of test concentrations. To achieve this 119.9 mg of the test substance were added to 1 litre of dilution water and treated for 1 hour in an ultrasonic bath and afterwards stirred for 24 hours on a magnetic stirrer. - Finally undissolved particles of the test substance were removed by filtration using a folded filter of pore size 7 - 12 µm. Exposure conditions: - Test vessels: holding 10 neonates in 20 ml of test medium - Experimental design: 6 test concentrations plus 1 control 10 neonates per vessel, 2 replicates per concentration/control, no feeding during the exposure period, static system - Method of initiation: neonates were placed in prepared media

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- Photoperiod: 16 h light/8 h dark
- Temperature: 20 +/- 1 °C
- Aeration: none
- Test concentration/s (nominal): 3.2, 6.3, 12.5, 25, 50 and 100 mg/l
- Method of administration: stock solution
- Medium renewal: none
- Duration of exposure: 48 h
- Criteria of effects: The criterion of adverse effects used in this study was the substance-induced alteration of the normal mobility behaviour and the loss of locomotory actions of the neonates, observed at 24 and 48 h

Reliability : (1) valid without restriction
Guideline study in accordance with the OECD principles of GLP

Flag : Critical study for SIDS endpoint

21.02.2003

(23)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus subspicatus (Algae)
Endpoint : growth rate
Exposure period : 72 hour(s)
Unit : mg/l
NOEC : 9.65
LOEC : 18.75
EC50 : 21.4
Limit test :
Analytical monitoring : yes
Method : Directive 92/69/EEC, C.3
Year : 2002
GLP : yes
Test substance : other TS: purity 99.985 % according to H & R GmbH

Method : Chemical analysis method: GC analysis
 Limit of quantitation: 0.5 mg/l
 Recovery rate: 105 % ± 5 %
 Sampling schedule of chemical analysis
 - Stock solution: at 0 h
 - Control: at 0 and 72 h
 - Test concentrations: at 0 and 72 h
 Expression of results
 · Cell density measurements in the test and control cultures are tabulated according to the concentration of test substance and the time of measurement
 · Growth curves are plotted for each test concentration and control
 · The area under the growth curve [b] is calculated for each test culture
 · The growth rate [r] is calculated for each test culture
 · The percentage inhibition of both, growth [b] and growth rate [r], is calculated for each test concentration
 · If possible, EC 50 values for both, growth [b] and growth rate [r], are calculated by probit analysis
 · If possible, NOEC and LOEC of both, growth [b] and growth rate [r], are determined by a multisample comparison (according to DUNNETT 1955, 1964)

Result : b: growth
 r: growth rate
 Results (72 h)[mg/l]:
 EbC50: 20
 ErC50: 21.4
 NOEC [b]: 9.65

4. Ecotoxicity

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	LOEC [b]: 18.75 NOEC [r]: 9.65 LOEC [r]: 18.75 All results are expressed in terms of mean measured concentrations. Measured concentrations ranged from 92.0 - 102.5 % of nominal values at 0 h, and from 88.0 - 105 % of nominal values at 72 h, respectively.
Test condition	: Test species: - Name: <i>Desmodesmus subspicatus</i>; former name: <i>Scenedesmus subspicatus</i> - Source: Non-axenic strain of the test species obtained from 'The Collection of Algal Cultures' of the Institute of Plant Physiology at the University of Göttingen (Germany) - Maintenance of stock cultures: Exponentially-growing stock cultures are maintained in the test facility under constant temperature conditions (23 +/- 2°C) at a light intensity in the range 60 - 120 µE m⁻² s⁻¹ (measured in the range 400 to 700 nm using a spherical quantum flux meter). The nutrient medium (according to BRINGMANN & KÜHN 1977) is renewed once a week. Cell density measurements are made using a microcell counter - Preparation of pre-cultures: Pre-cultures are set up three days before the start of a test. They are grown under identical exposure conditions as the stock cultures, except from the use of a different nutrient medium (annex 1) - Test cultures: The algal inocula for a test are taken from an exponentially-growing pre-culture and are mixed with the nutrient medium (annex 1) to make up to a final cell density of about 10000 cells/ml in the test medium. Pretreatment of the test substance - The test substance was pulverized - A stock solution was prepared to give the desired series of test concentrations. To achieve this 124.9 mg of the test substance were added to 1 litre of dilution water and treated for 1 h in an ultrasonic bath and afterwards stirred for 24 h on a magnetic stirrer Exposure conditions - Test vessels: 300 ml Erlenmeyer flasks with stoppers - Culturing apparatus: light chamber in which a temperature in the range 21 °C to 25 °C can be maintained at +/- 2 °C, and continuous uniform illumination is provided in the spectral range 400 to 700 nm - Light intensity: at the average of the test solutions, a light intensity in the range 60 to 120 µE m⁻² s⁻¹ - Cell density measurements: microcell counter Experimental design: - 4 Test concentrations plus 1 control, 3 replicates per concentration, 6 replicates per control, initial cell density in the test cultures approximately 10000 cells/ml additionally highest test concentration without algae - Test concentration/s (nominal): 5.0, 10, 20 and 40 mg/l - Method of administration: stock solution - Duration of exposure: 72 h - Criteria of effects: The criteria of adverse effects used in this study were the substance-induced inhibition of growth [b] and growth rate [r], respectively, of the algal population.
Reliability	: (1) valid without restriction
Flag	: Guideline study in accordance with the OECD principles of GLP
21.02.2003	: Critical study for SIDS endpoint

(24)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	: aquatic
Species	: activated sludge
Exposure period	:

4. Ecotoxicity

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Unit : mg/l
EC10 : 51
EC50 : 237
Analytical monitoring : no
Method : ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year : 1992
GLP : yes
Test substance : other TS: 99.9 %

Remark : direct weight
Reliability : (2) valid with restrictions
Guideline Study, incubation period not reported

Flag : Critical study for SIDS endpoint

07.03.2002

(25)

Type :
Species : aerobic microorganisms
Exposure period : 48 hour(s)
Unit :

Method : Test of antimicrobial activity of the essential oil of Calamintha nepeta and its main constituents against bacteria and fungi (Listeria monocytogenes, Bacillus cereus, Salmonella veneziana, Salmonella paratyphi B, Salmonella typhimulium, Fusarium moniliforme, Botrytis cinerea, Aspergillus niger, Pyricularia oryzae).
Bacteria and fungi cultures were placed on the surface of a culture medium. 20 µl menthol solution (dissolved 1:1 in Tween 80) were placed on paper disks which were placed in the petri dishes and incubated at 37 degrees C.

Result : Menthol showed no activity against any of the tested microorganisms.

Reliability : (3) invalid
Unsuitable test system. Tween 80 was present at a high concentration.

26.07.2002

(26)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

Species : other: larvae of Ostrinia nubilalis (Lepidoptera: Pyralidae; European corn borer)
Endpoint : other: survival and growth
Exposure period : 6 day(s)

4. Ecotoxicity

Id 2216-51-5
Date 10.06.2003

Unit :
Method :
Year : 1999
GLP : no
Test substance : other TS: l-Menthol, laboratory chemical

Method : Two different chemical application methods conducted: dripping the solution onto the solidified artificial diet (on-diet test) or mixing the solution in the diet before it solidified (in-diet test).
In each test cup 1 neonate larva was placed on the diet. On-diet test: 199 larvae tested at 6 doses In-diet test: 160 larvae tested at 5 doses

Result : On-diet test: LC50 = 2.35 mg per cup (1st-instar)
In-diet test: LC50 = 17.4 mg per cup (2nd-instar)

Test condition : 25 +/- 2 degrees C; photoperiod 14:10 (light:dark) hours

Reliability : (3) invalid
No conclusion from environmental concentrations to effects possible

22.12.2001

(27)

Species : other: Aspergillus flavus
Endpoint :
Exposure period : 5 day(s)
Unit :
Method :
Year : 1998
GLP : no
Test substance : other TS: menthol from Mentha piperita

Method : Maize grain protection assay against A. flavus
Result : Total inhibition of A. flavus
Test condition : Maize grains immersed in essential oil, dried, and sprayed with fungal spore suspension

Reliability : (3) invalid
No conclusion from environmental concentrations to effects possible

22.12.2001

(28)

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5. Toxicity

Id 2216-51-5
Date 10.06.2003

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Metabolism
Species : rat
Number of animals
 Males :
 Females :
Doses
 Males : 800 mg/kg bw
 Females :
Vehicle : other: 1% methyl cellulose solution
Route of administration : gavage
Exposure time : 20 day(s)
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st.
 2nd.
 3rd.
Toxic behaviour :
Deg. product : yes
Method : other: analysis of metabolites in urine
Year : 1988
GLP : no data
Test substance : other TS: L-menthol, purity not

Result : Metabolites in urine (not further quantified)
 p-menthane-3,8-diol (major),
 p-menthane-3,9-diol,
 3,8-oxo-p-menthane-7-carboxylic acid
 3,8-dihydroxy-p-menthane-7-carboxylic acid (major).

Proposed major transformation:
 hydroxylation at the C-8 position followed by oxidation of C-1 methyl group
 to a carboxylic group; the secondary carbon atoms of the ring seem to be
 resistant to hydroxylation.

Test condition : ANIMALS:
 rat strain: IISc
 No. of animals not specified.
 Control rats were given the vehicle only.

Urine was collected daily from control and treated rats and maintained at 0-
 4 °C.

Urine was acidified (pH 3-4) before analysis.

Substances were isolated by silica gel chromatography with hexane/ethyl
 acetate as solvent system. Analysis of metabolites were done by gas
 chromatographic measurements and proton NMR spectra.

Reliability : (2) valid with restrictions
 Limited documentation

Flag : Critical study for SIDS endpoint

25.02.2003

(29)

In Vitro/in vivo : In vivo
Type : Metabolism
Species : rat
Number of animals
 Males : 12

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Doses	Females	:	
	Males	:	800 mg/kg bw/d
	Females	:	
Vehicle		:	other: 1 % methyl cellulose solution
Route of administration		:	gavage
Exposure time		:	7 day(s)
Product type guidance		:	
Decision on results on acute tox. tests		:	
Adverse effects on prolonged exposure		:	
Half-lives		:	1 st ; 2 nd ; 3 rd ;
Toxic behaviour		:	
Deg. product		:	
Method		:	other: hepatic drug metabolism study
Year		:	1988
GLP		:	no data
Test substance		:	other TS: L-menthol, purity not
Result		:	Results in detail (% of control): days of treatment/cyt. P-450/cyt. b5/ NADPH-cyt. c reductase; 1/44%/+9%/+69%+ 3/82%/+14%/+80%+ 5/49%/+5%/+50%+ 7/17%/+12%/-35%+ Repeated oral administration of 800 mg/kg/day of L-menthol to rats for 3 days resulted in the increase of both liver microsomal cytochrome P-450 content and NADPH-cytochrome c reductase activity by nearly 80 %. Further treatment (for 7 days total) reduced their levels considerably, although the levels were still higher than the control values. Both cytochrome b5 and NADH-cytochrome c reductase levels were not significantly changed during the 7 days of treatment.
Test condition		:	rat strain: IISc No. of animals: 12 per group Exposure: 1, 3, 5 or 7 days Liver was removed, perfused and minced 24 hours after administration of the final dose. Cytochrome P-450 and b5 contents were determined by the method of Omura and Sato (J. Biol. Chem. 239, 1964). The NADPH-cytochrome c reductase activity was measured at 550 nm. In NADH-cytochrome c reductase assays, NADH was substituted for NADPH.
Reliability		:	(2) valid with restrictions Limited documentation
Flag		:	Critical study for SIDS endpoint
25.02.2003			(29)
In Vitro/in vivo		:	
Type		:	Metabolism
Species		:	other: rat liver microsomes
Number of animals		:	
	Males	:	
	Females	:	
Doses		:	
	Males	:	
	Females	:	
Vehicle		:	
Method		:	other

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Year	: 1988
GLP	: no data
Test substance	: other TS: L-menthol, purity not
Result	: Rat liver microsomes readily converted L-menthol to p-menthane-3,8-diol (II) in the presence of NADPH and O ₂ (reaction is NADPH dependent; NADH showed no synergistic effect and could not support the reaction alone). This activity was significantly higher in microsomes obtained from PB-induced rats than from control microsomal preparations, whereas 3-MC-induced microsomes failed to convert L-menthol to compound II in the presence of NADPH and O ₂ . PB-induced microsomal hydroxylation of L-menthol was inhibited to a significant extent by CO, SKF 525-A, metyrapone, cytochrome c, and p-chloromercuribenzoate, indicating the possible involvement of the cytochrome P-450 system in the hydroxylation reaction.
Test condition	: Rats (strain: IISc, 4-6 animals per group) were treated with phenobarbital (PB, 80 mg/kg bw) or 3-methylcholanthrene (3-MC, 25 mg/kg bw). Investigations on the hydroxylation activity of L-menthol were performed with the isolated liver microsomes. Examinations: - Co-factor specificity of L-menthol hydroxylase activity; - Effect of PB- and 3-MC treatment on the L-menthol hydroxylase activity; - Effect of inhibitors (CO, SKF 525-A, Metyrapone, Cytochrome c, p-Chloromercuribenzoate, sodium azide) on hydroxylation of L-menthol;
Reliability	: (2) valid with restrictions Limited documentation
Flag 25.02.2003	: Critical study for SIDS endpoint
In Vitro/in vivo	: In vivo
Type	: Metabolism
Species	: rat
Number of animals	
Males	: 3
Females	:
Doses	
Males	: 500 mg/kg bw
Females	:
Vehicle	: other: trioctanoin
Route of administration	: gavage
Exposure time	:
Product type guidance	:
Decision on results on acute tox. tests	:
Adverse effects on prolonged exposure	:
Half-lives	: 1 st . 2 nd . 3 rd .
Toxic behaviour	:
Deg. product	:
Method	: other
Year	: 1994
GLP	: no data
Test substance	: other TS: [3-3H]-L-Menthol, puri
Result	: In intact rats, some 71% of the dose was recovered in 48 hours with approximately equal amounts in urine and feces. 74 % of the dose was recovered from bile duct-cannulated rats, with 67% in the bile and 7% in

(29)

5. Toxicity

Id

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Date

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the urine.

Excretion of radioactivity after oral dosing of 500 mg/kg of [3H]Thymidin:

% [3H] dose excreted in intact rats:

Time period/Urine (%) / Feces (%) / Total (%)

0-24 hr/18.8/26.6/45.5

24-48 hr/19.0/7.3/26.3

Total/37.8/33.9/71.8

% [3H] dose excreted in bile duct-cannulated rats:

Time period/Bile (%) / Urine (%) / Total (%)

0-24 hr/66.9/7.3/74.2

Degradation products are mono- and di-hydroxymenthols and carboxylic acids, excreted in part as glucuroic acid conjugates

Metabolites:

Urine: p-menthane-3,8-diol (major), p-menthane-3,9-diol (minor), 3,8-

dihydroxy-p-menthane-7-carboxylic acid (major urinary),

mentholglucuronide (minor)

Bile: Mentholglucuronide (major),

Following structures of supposed metabolites are principally based on GC-MS measurements:

3-hydroxy-p-menthane-9-carboxylic acid (stereoisomers at C-8 - minor - very minor urinary),

3-hydroxy-p-menthane-7-carboxylic acid (isomers – major urinary metabolites), p-menthane-3,7,8-triol (minor urinary).

Test condition

: Animals: intact and bile duct-cannulated Fischer 344 rats (3 per group)

Excreta collection: intact rats: urine and feces 24 and 48 hrs after dosage
bile duct-cannulated rats: bile samples were collected on ice (0-2, 2-4, 4-6 and 6-24 hrs) urine samples: 0-24 hrs).

Administration: 500 mg/kg 3-Tritium-L-Menthol (128 µCi/kg) were administered as a single dose.

Metabolites in urine and bile were analyzed by TLC, solid phase extraction, GLC, and GC-MS.

Reliability

: (2) valid with restrictions
Limited documentation

Flag

25.02.2003

: Critical study for SIDS endpoint

(30)

In Vitro/in vivo

: In vivo

Type

: Excretion

Species

: Rabbit

Number of animals**Males**

:

Females

: 4

Doses**Males**

:

Females

: 1000 mg/kg bw

Vehicle

: other: warm water emulsion

Route of administration

: gavage

Exposure time

:

Product type guidance

:

Decision on results on acute tox. tests

:

Adverse effects on prolonged exposure

:

Half-lives

: 1st.

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	2 nd , 3 rd :		
Toxic behaviour	:		
Deg. product	:		
Method	:		
Year	:	1938	
GLP	:		
Test substance	:	other TS: L-Menthol, no further	
Result	:	After a single oral administration of 1000 mg/kg bw, 48% of the dose were excreted as L-menthol glucuronides	
Reliability	:	(2) valid with restrictions Limited documentation	
Flag	:	Critical study for SIDS endpoint	
25.02.2003			(31)
In Vitro/in vivo	:	In vivo	
Type	:	Distribution	
Species	:	human	
Number of animals			
Males	:		
Females	:		
Doses			
Males	:		
Females	:		
Vehicle	:		
Method	:		
Year	:	2001	
GLP	:		
Test substance	:	other TS: peppermint oil	
Result	:	Mean maximum plasma concentration of menthol were 1.2 and 1.5 mg/ml at 1.7 or 3 hours after oral administration of a immediate release formulation or an enteric coated formulation.	
Test condition	:	16 healthy male volunteers received 180 mg peppermint oil after a 10 hours fast. Menthol content was about 44 %. Plasma levels were measured by GC/MS.	
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. However, test substance was peppermint oil with only 44 % menthol content.	
25.02.2003			(32)
In Vitro/in vivo	:	In vivo	
Type	:	Metabolism	
Species	:	Human	
Number of animals			
Males	:		
Females	:		
Doses			
Males	:		
Females	:		
Vehicle	:		
Result	:	After a daily dose of 750 mg l-menthol for a total of 8 days to two human volunteers 17-38% menthol was recovered as urinary menthyl glucuronide within 24 hours. Urine was first collected 3 days after dosage with l-menthol started.	
Reliability	:	(2) valid with restrictions	

5. Toxicity

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Flag : limited documentation
05.03.2003 : Critical study for SIDS endpoint (33)

In Vitro/in vivo :
Type :
Species : other: for further data see chapter 5.11
Number of animals :
 Males :
 Females :
Doses :
 Males :
 Females :
Vehicle :
Reliability : (2) valid with restrictions
05.03.2003

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 2615 mg/kg bw
Species : rat
Strain : Wistar
Sex : female
Number of animals : 10
Vehicle : peanut oil
Doses : 1000, 2000, 2500, 3000, 3500 and 4000 mg/kg bw
Method : other: see test conditions
Year : 1974
GLP : no
Test substance : other TS: l-menthol H&R

Result : MORTALITY:
- Time of death: 1-3 days after application
- Number of deaths at each dose:
dose (mg/kg)/number of deaths
1000/0/10
2000/3/10
2500/4/10
3000/6/10
3500/7/10
4000/10/10
CLINICAL SIGNS: narcotic status (no data available on exposure level at which the clinical signs were observed)

Test condition : ADMINISTRATION:
- Volume administered or concentration: 10-20 ml/kg
- Post dose observation period: 14 days
EXAMINATIONS:
deaths, clinical signs
No information on statistical methods and confidence limits.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: No information on statistical methods and confidence limits.

Flag : Critical study for SIDS endpoint
06.08.2002 (34)

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Type : LD50
Value : = 2426 mg/kg bw
Species : rat
Strain : Wistar
Sex : female
Number of animals : 10
Vehicle : peanut oil
Doses : 1000, 2000, 2400, 2700, 3000 mg/kg bw
Method : other
Year : 1974
GLP : no
Test substance : other TS: menthol brazilian

Result : MORTALITY:
 - Time of death: 1-3 days after application
 - Number of deaths at each dose:
 dose (mg/kg)/number of deaths
 1000/0/10
 2000/2/10
 2400/4/10
 2700/7/10
 3000/9/10
 CLINICAL SIGNS: narcotic status (no data available on exposure level at which the clinical signs were observed)

Test condition : ADMINISTRATION:
 - Volume administered or concentration: 10-20 ml/kg
 - Post dose observation period: 14 days
 EXAMINATIONS:
 deaths, clinical signs
 No information on statistical methods and confidence limits.

Reliability : (2) valid with restrictions
 Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: no information on statistical methods and confidence limits.

Flag : Critical study for SIDS endpoint
 01.07.2002

(34)

Type : LD50
Value : = 3300 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other
Year : 1961
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
 Secondary literature

24.05.2002

(35) (36)

Type : LD50
Value : = 940 mg/kg bw
Species : rat
Strain : no data
Sex : male

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Number of animals	: 5
Vehicle	: other: 0.85 % saline
Doses	: 100, 250, 500, 1000, 2000, 3000 mg/kg bw
Method	: other
Year	: 1975
GLP	: no
Test substance	: other TS: menthol brazilian
Remark	: The lower LD50 (compared to other LD50 values) may be attributed to irritant effects after bolus administration in saline.
Result	: MORTALITY: - Time of death: dose (mg/kg)/time of deaths 500/day 6 1000/day 4 (2) day 5 (1) 2000/day 2 3000/day 1 (1) day 2 (3), day 4 (1) - Number of deaths at each dose: dose (mg/kg)/deaths 100/0 250/0 500/1/5 1000/3/5 2000/4/5 3000/5/5 NECROPSY FINDINGS: severe irritation of mucosal lining of the stomach and intestine. Observation period: 10 days
Test condition	: TEST ORGANISMS: - Source: no data - Age: no data - Weight at study initiation: 250 g - Controls: no data ADMINISTRATION: - Volume administered or concentration: no data - Post dose observation period: 10 days EXAMINATIONS: deaths, necropsy
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: number of animals: 5 (Mutagenic evaluation study)
Flag 10.07.2002	: Critical study for SIDS endpoint
Type	: LD50
Value	: = 3400 mg/kg bw
Species	: mouse
Strain	: no data
Sex	: no data
Number of animals	: 10
Vehicle	: other: olive oil
Doses	: 2000, 4000 mg/kg bw
Method	: other
Year	: 1932
GLP	: no
Test substance	: other TS: not further specified
Reliability	: (4) not assignable

(37)

5. Toxicity

Id 2216-51-5
Date 10.06.2003

17.07.2002 Documentation insufficient. (38)

Type : LD50
Value : = 4380 mg/kg bw
Species : mouse
Strain : no data
Sex : male
Number of animals : 6
Vehicle : other: 0.85 % saline
Doses : 2000, 2500, 3200, 4000, 5000 mg/kg bw
Method : other
Year : 1975
GLP : no
Test substance : other TS: menthol brazilian

Result : MORTALITY:
 - Time of death:
 dose (mg/kg)/time
 4000/day 4
 5000/day 2
 - Number of deaths at each dose:
 dose (mg/kg)/number of deaths
 2000/0/6
 2500/0/6
 3200/0/6
 4000/2/6
 5000/6/6
 CLINICAL SIGNS: depressed activity at day 1 (no data available on exposure levels at which the clinical signs were observed)
 NECROPSY FINDINGS: no gross abnormalities

Test condition : TEST ORGANISMS:
 - Source: no data
 - Age: no data
 - Weight at study initiation: 35 g
 - Controls: no data
 ADMINISTRATION:
 - Volume administered or concentration: no data
 - Post dose observation period: 8 days
 EXAMINATIONS:
 deaths, necropsy, clinical signs

Reliability : (2) valid with restrictions
 Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: number of animals: 6 (Mutagenic evaluation study)

Flag : Critical study for SIDS endpoint

17.07.2002 (39)

Type : other: lethal dose
Value : 800 - 1000 mg/kg bw
Species : cat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other
Year : 1926
GLP : no

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Test substance : other TS: natural menthol, not further specified

Reliability : (4) not assignable
Documentation insufficient.

25.02.2003

(40)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : = 780 mg/kg bw
Species : rat
Strain : other: white rats
Sex : no data
Number of animals : 10
Vehicle : other: olive oil
Doses : 500, 600, 700, 800, 900, 1000, 1100, 1200 mg/kg bw
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1952
GLP : no
Test substance : other TS: natural menthol

Result : MORTALITY:
- Time to death: within 12 hours after application
- Number of deaths at each dose:
dose (mg/kg bw)/number of deaths
500/1/10
600/2/10
700/5/10
800/7/10
900/6/10
1000/7/10
1100/9/10
1200/10/10
CLINICAL SIGNS: imbalance, paralysis, partial to total relaxation, deep sleep with abolition of reflexes.

Test condition : TEST ORGANISMS:
- Source: no data
- Age: no data
- Weight at study initiation: 90-120 g
- Controls: no data
ADMINISTRATION:
- Volume administered or concentration: no data
- Post dose observation period: The animals were observed until deaths or until return to normal behaviour.
EXAMINATIONS:
deaths, clinical signs

Reliability : (2) valid with restrictions
Limited documentation

25.02.2003

(41)

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Type : LD50
Value : = 710 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1961
GLP : no
Test substance : other TS: not further specified

Remark : no data on Test conditions or further results
Reliability : (4) not assignable
Secondary literature

24.05.2002

(36)

Type : LD50
Value : = 6600 mg/kg bw
Species : mouse
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1962
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
Documentation insufficient for assessment of reliability

25.02.2003

(42)

Type : LD50
Value : ca. 2000 mg/kg bw
Species : rabbit
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1961
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
Secondary literature

17.12.2001

(36)

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Type : LD50
Value : = 860 mg/kg bw
Species : guinea pig
Strain : no data
Sex : no data
Number of animals : 10
Vehicle : other: olive oil
Doses : 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 mg/kg bw
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1952
GLP : no
Test substance : other TS: natural menthol

Result : MORTALITY:
 - Time of death: 12 hours after application
 - Number of deaths at each dose:
 dose (mg/kg bw)/number of deaths
 500/2/10
 600/3/10
 700/3/10
 800/6/10
 900/7/10
 1000/8/10
 1100/7/10
 1200/6/10
 1300/8/10
 1400/10/10
 CLINICAL SIGNS: imbalance, faccid paralysis of the back, partial to total relaxation, deep sleep with abolition of reflexes.

Test condition : TEST ORGANISMS:
 - Source: no data
 - Age: no data
 - Weight at study initiation: 280-360 g
 - Controls: no data
 ADMINISTRATION:
 - Volume administered or concentration: no data
 - Post dose observation period: the animals were observed until deaths or until normal behaviour
 EXAMINATIONS:
 deaths, clinical signs

Reliability : (2) valid with restrictions
 Limited documentation

25.02.2003

(41)

Type : other: LD
Value : = 1500 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: olive oil
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1939
GLP : no

5. Toxicity

Id

2216-51-5

Date

10.06.2003

Test substance : other TS: not further specified

Reliability : (4) not assignable
Documentation insufficient for assessment

25.02.2003

(43)

Type : other: LD
Value : = 2000 mg/kg bw
Species : mouse
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: olive oil
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1939
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
Documentation insufficient for assessment

25.02.2003

(43)

Type : other: LD
Value : > 800 mg/kg bw
Species : cat
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: not specified oil
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1926
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
Documentation insufficient.

25.02.2003

(40)

Type : other: LD
Value : = 4000 mg/kg bw
Species : guinea pig
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: olive oil
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1939
GLP : no
Test substance : other TS: not further specified

5. Toxicity

Id 2216-51-5
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Reliability	:	(4) not assignable Documentation insufficient for assessment	
25.02.2003			(43)
Type	:	other: LD	
Value	:	1000 - 2500 mg/kg bw	
Species	:	rat	
Strain	:	no data	
Sex	:	no data	
Number of animals	:		
Vehicle	:	other: not specified oil	
Doses	:		
Route of admin.	:	s.c.	
Exposure time	:		
Method	:	other	
Year	:	1926	
GLP	:	no	
Test substance	:	other TS: not further specified	
Reliability	:	(4) not assignable Documentation insufficient.	
25.02.2003			(40)
Type	:	other: LD	
Value	:	5000 - 6000 mg/kg bw	
Species	:	mouse	
Strain	:	no data	
Sex	:	no data	
Number of animals	:		
Vehicle	:	other: not specified oil	
Doses	:		
Route of admin.	:	s.c.	
Exposure time	:		
Method	:	other	
Year	:	1926	
GLP	:	no	
Test substance	:	other TS: not further specified	
Reliability	:	(4) not assignable Documentation insufficient.	
25.02.2003			(40)
Type	:	other: LD	
Value	:	= 34 mg/kg bw	
Species	:	cat	
Strain	:	no data	
Sex	:	no data	
Number of animals	:		
Vehicle	:	other: alcohol with physiological saline	
Doses	:		
Route of admin.	:	i.v.	
Exposure time	:		
Method	:	other	
Year	:	1939	
GLP	:	no	
Test substance	:	other TS: not further specified	
Test condition	:	Solution or suspensions, 1:1000, were prepared by diluting 2 per cent solutions of menthol in alcohol with physiological saline and were injected	

5. Toxicity

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at one-minute intervals into the femoral vein while blood pressure was recorded from the carotid artery.
Reliability : (4) not assignable
Documentation insufficient for assessment.
25.02.2003 (43)

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : 100 %
Exposure : Semiocclusive
Exposure time : 4 hour(s)
Number of animals : 4
Vehicle : other: diethylphthalate (DEP)
PDII :
Result : moderately irritating
Classification :
Method : OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol I H&R, HR 89/620001, purity: no data

Result : AVERAGE SCORE
100%/50%/25%/5%/1 %/Vehicle
3.0/1.6/1.0/0.3/0.0/0.0 (erythema)
2.9/2.2/0.2/0.1/0.0/0.0 (oedema)
REVERSIBILITY: yes
Day 7: 100%: 4/4 - treated sites were covered with a layer of white to white-brown scales
50%: 4/4 - thin layer of white scales
Day 14: 100%: 4/4 - treated sites were covered with white to white-brown scales, underlying skin was intact
50%: 2/4 - treated sites showed scattered scale formation on intact skin.

Test condition : TEST ANIMALS:
- Strain: Chbb:HM (C.H.Boehringer/Biberach
- Sex: female
- Source: Dr. Karl Thomae GmbH, Biberach an der Riss
- Age: no data
- Weight at study initiation: 2400-3000 g
- Number of animals: 4
- Controls: internal control (one part of skin)
ADMINISTRATION/EXPOSURE
- Preparation of test substance: dilutions of substance with DEP, concentrated test substance was moistened with DEP in the ratio 6:1
- Area of exposure: six different fields on back (two anterior, two centrally located and two posterior treatment sites)
- Occlusion: substance is covered with gauze packs, gauze packs were secured with a cross of 1 cm wide adhesive tape and fixed with Scanpor tape.
- Concentration in vehicle: 100, 50, 25, 5 and 1 %, Vehicle
- Total volume applied: 0.5 ml
- Postexposure period: up to 14 days
- Removal of test substance: skin was washed with luke warm water and soap

Reliability : (2) valid with restrictions
Guideline study. Purity of TS not stated
Flag : Critical study for SIDS endpoint
25.02.2003 (44)

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Species : guinea pig
Concentration : other
Exposure : Open
Exposure time : 14 day(s)
Number of animals : 20
Vehicle : no data
PDII :
Result : not irritating
Classification : not irritating
Method : other
Year : 1974
GLP : no
Test substance : other TS: l-menthol H&R

Test condition : Substance was rubbed into the skin for 30 s once daily.
Substance was applied 2 x 5 days, results were taken after 14 days.
Reliability : (3) invalid
Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.

17.12.2001 (45)

Species : guinea pig
Concentration : no data
Exposure : Open
Exposure time : 14 day(s)
Number of animals : 20
Vehicle : no data
PDII :
Result : not irritating
Classification : not irritating
Method : other
Year : 1974
GLP : no
Test substance : other TS: menthol brazilian

Test condition : Substance was rubbed into the skin for 30 s once daily.
Substance was applied 2 x 5 days, results were taken after 14 days.
Reliability : (3) invalid
Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.

17.12.2001 (45)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : 29 %
Dose : .1 ml
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 4
Vehicle : other: diethylphthalate (DEP)
Result : slightly irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol I H&R, HR 89/620001 DEP, purity: no data

5. Toxicity

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Result : AVERAGE SCORE
- Cornea: 0.2
- Iris: 0.0
- Conjunctivae (Redness): 0.6
- Conjunctivae (Chemosis): 0.1
REVERSIBILITY: yes, only slight redness of conjunctiva observed in one rabbit after 72 hours.

Test condition : TEST ANIMALS:
- Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya)
- Sex: female
- Source: Dr. Karl Thomae GmbH, Biberach an der Riss
- Age: no data
- Weight at study initiation: 2400-2800 g
- Number of animals: 4
- Controls: internal control (right eye)
EXAMINATIONS
according guideline

Reliability : (2) valid with restrictions
Purity of TS not stated, unusual vehicle

Flag : Critical study for SIDS endpoint
25.02.2003

(46)

Species : rabbit
Concentration : 64 %
Dose : .1 ml
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 4
Vehicle : other: 29 % solution of l-menthol in DEP (HR 89/620001 DEP)
Result : slightly irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol I H&R, HR 89/620001, purity: no data

Result : AVERAGE SCORE
HR 89/620001 64%/Vehicle (29% l-menthol in DEP)
1.0/0.8 (cornea)
0.0/0.0 (iris)
2.0/1.2 (redness of conjunctivae)
0.6/0.3 (chemosis, conjunctivae)
REVERSIBILITY: yes, no reactions observed after 7 days
The test article formulation was slightly more eye-irritating compared to the vehicle control.

Test condition : TEST ANIMALS:
- Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya)
- Sex: female
- Source: Dr. Karl Thomae GmbH, Biberach an der Riss
- Age: no data
- Weight at study initiation: 2600-2800 g
- Number of animals: 4
- Controls: internal control with vehicle (right eye)
ADMINISTRATION/EXPOSURE
- Preparation of test substance: Test article was pulverized in a mortar and then diluted with vehicle (absolute concentration of substance in diethyl-phthalate (DEP) is 64%)
- Vehicle: 29% l-menthol in DEP (HR 89/620001 DEP, previously tested by

5. Toxicity

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Reliability	: Scantox, lab.no.: 11754)	
	: (2) valid with restrictions	
	: Purity of TS not stated, unusual vehicle	
Flag	: Critical study for SIDS endpoint	
25.02.2003		(47)
Species	: rabbit	
Concentration	: 60 %	
Dose	: .1 ml	
Exposure time	: 1 minute(s)	
Comment	: other: see test conditions	
Number of animals	: 8	
Vehicle	: other: olive oil	
Result	: not irritating	
Classification	:	
Method	: Draize Test	
Year	: 1974	
GLP	: no	
Test substance	: other TS: I-Menthol H&R, purity not stated	
Test condition	: Substance was initially applied in 10, 20 and 30 % solution. The eyes of 4 animals were rinsed 1 minute after application with physiological saline, substance remained in the eyes of 4 animals. In a second step animals were treated with concentration of 40, 50 and 60 %.	
Reliability	: (2) valid with restrictions	
	: Limited documentation	
25.02.2003		(45)
Species	: rabbit	
Concentration	: 60 %	
Dose	: .1 ml	
Exposure time	: 1 minute(s)	
Comment	: other: see test conditions	
Number of animals	: 8	
Vehicle	: other: olive oil	
Result	: not irritating	
Classification	:	
Method	: Draize Test	
Year	: 1974	
GLP	: no	
Test substance	: other TS: menthol brazilian, no further data	
Test condition	: Substance was initially applied in 10, 20 and 30 % solution. The eyes of 4 animals were rinsed 1 minute after application with physiologically saline, substance remained in the eyes of 4 animals. In a second step animals were treated with concentration of 40, 50 and 60 %.	
Reliability	: (2) valid with restrictions	
	: Limited documentation	
25.02.2003		(45)

5.3 SENSITIZATION

Type	: Buehler Test
Species	: guinea pig
Concentration	: 1 st . Induction 25 % occlusive epicutaneous 2 nd . Challenge 25 % occlusive epicutaneous 3 rd .
Number of animals	: 20

5. Toxicity

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Vehicle	: other: ethanol:diethylphthalate (1:1)
Result	: not sensitizing
Classification	:
Method	: other: comparable to OECD-guideline 406, see test conditions
Year	: 1991
GLP	: yes
Test substance	: other TS: menthol-I H&R, HR 90/000102
Result	: - Sensitization reaction: No irritation was noted after induction and there were no positive responses in any of the animals after challenge - Clinical signs: No clinical signs, other than skin reactions induced by treatment, were noted during study.
Test condition	: Negative control: vehicle Day 1-3: induction, 6 hrs Day 8-10: induction, 6 hrs Day 15-17: induction, 6 hrs Day 28: challenge, 6 hrs TEST ANIMALS: - Strain: Dunkin-Hartley - Sex: female - Source: David Hall Limited, Darley Oaks, Newchurch, Burton-on-Trend, Staffordshire - Age: less than one year - Weight at study initiation: 422-509 g - Number of animals: 44 (20 control, 20 test, 4 dose ranging) ADMINISTRATION/EXPOSURE - 0.5 ml were applied for induction EXAMINATIONS - Grading system: see guideline - Pilot study: 25, 10, 5 and 2 % w/v in ethanol:diethylphthalate (DEP) were investigated for 24 hrs.
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: Concentration used for induction exposure did not cause mild irritation.
Flag	: Critical study for SIDS endpoint
10.07.2002	(48)
Type	: Mouse local lymphnode assay
Species	: mouse
Number of animals	: 4
Vehicle	: other: acetone
Result	: not sensitizing
Classification	:
Method	: other: Kimber, I, Hilton, J., Weisenberger, C., The murine local lymph node assay for identification of contact allergens: A preliminary evaluation of in situ measurement of lymphocyte proliferation. Contact Dermatitis, 21, 215-220, 19
Year	: 1995
GLP	: yes
Test substance	: other TS: menthol I H&R, 99.9 %
Result	: Conc.Menthol no of lymph counts per cpm/lymph test: (%w/v) nodes minute(cpm) node ratio control assayed
	0 (vehicle) 8 1236 1.55 N/A
	1 6 1212 2.02 1.30
	10 8 2234 2.79 1.80
	30 8 1131 1.41 0.91

5. Toxicity

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Hexylcinnam-
aldehyde

0 (vehicle)	8	672	0.84	N/A	
1	8	1874	2.34		2.79
10	8	5322	6.65	7.92	
30	8	6711	8.39		9.99

The increase in isotope incorporation was less than 3-fold at all concentrations. Also the response was not consistent with a biological dose-response. Test substance has not fulfilled the criteria for a potential sensitiser under the conditions of the tests. Hexylcinnamaldehyde as control has fulfilled the criteria for a potential sensitiser.

Test condition

- : TEST ANIMALS:
- Strain: CBA/Ca/01a/Hsd strain
- Sex and number: 4 males per dose
- Source: Harlan Olac Limited, Blackthorne, Bicester, Oxon, UK
- Age: young adults
- Weight at study initiation: no data
- Controls: yes, with acetone
ADMINISTRATION/EXPOSURE
- Concentrations used were: 0 (vehicle), 1 %, 10 %, 30 %
- Positive control: Positive control study with hexylcinnamaldehyde (3 and 10% in acetone)

Reliability

- : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail.

Flag

10.07.2002

- : Critical study for SIDS endpoint

(49)

Type

- : other: Modified Draize procedure

Species

- : guinea pig

Concentration

- : 1st. Induction .25 % other: intradermal
2nd. Challenge 10 % other: intradermal in one flank and topical in the other flank
3rd.

Number of animals

- : 10

Vehicle

- : no data

Result

- : ambiguous

Classification

- :

Method

- : other: modified Draize: Draize, J.H. Dermal Toxicity, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Ass. Food and Drug Officials of the U.S., 1959, 46-59

Year

- : 1978

GLP

- : no

Test substance

- : other TS: L-menthol, not further specified, no data on purity

Remark

- : The study included 69 perfume ingredients. 9 of these were tested positive (7 of these 9 after a second induction treatment only)

Result

- : A positive reaction was obtained after a second induction treatment.

Test condition

- : TEST ANIMALS:
- Strain: Hartley strain albino
- Sex: 4 males, 6 females or vice versa / test substance
- Source: Unilever Research Laboratory, Colworth House, Sharnbrook, Beds., UK
- Age: no data
- Weight at study initiation: about 350 g
- Controls: 4 previously untreated animals of same sex
ADMINISTRATION/EXPOSURE

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Day 0: Induction (4x0.25 %) Day 14: Challenge (intradermal and topical 0.25 %), day 21: Rechallenge. In case a negative result was obtained at the first challenge, a second induction treatment was performed on day 21 (4x0.25 %) with a second challenge (intradermal and topical 2,5 or 10 % on day 35 and a re-challenge on day 42 including controls.

- Positive control: see remark

EXAMINATIONS

- Grading system: 0- +++ - System, compared to positive control reaction

- Pilot study: 4 animals were injected intradermally 0.1 ml aliquots of a range of concentrations of test material. The concentration giving slight but perceptible irritation with no oedema was selected as the injection challenge.

Reliability : (2) valid with restrictions
Limited documentation
Flag : Critical study for SIDS endpoint

25.02.2003

(50) (51)

Type : other: open repetitive dermal test
Species : guinea pig
Number of animals : 20
Vehicle : no data
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1974
GLP : no
Test substance : other TS: l-menthol H&R

Test condition : Substance was rubbed into shaved skin for 30 sec once daily for 3x5 days. After 5 days without application the test substance was rubbed into an untreated part of the skin.
Results were taken after 24 h, 2 and 3 days.

Reliability : (3) invalid
Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.

25.02.2003

(45)

Type : other: open repetitive dermal test
Species : guinea pig
Number of animals : 20
Vehicle : no data
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1974
GLP : no
Test substance : other TS: menthol brazilian

Test condition : Substance was rubbed into shaved skin for 30 sec once daily for 3x5 days. After 5 days without application the the test substance was rubbed into an untreated part of the skin.
Results were taken after 24 h, 2 and 3 days.

Reliability : (3) invalid
Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.

25.02.2003

(45)

Type : Patch-Test
Species : human
Number of animals :

5. Toxicity

Id 2216-51-5
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Vehicle :
Result :
Classification :
Method : other
Year : 1990
GLP : no data
Test substance : other TS: not further specified

Result : Identified allergens were: menthol, piperitone or pulegone.
Test condition : Three patients with allergic contact dermatitis were patch-tested against individual components of peppermint oil.
Reliability : (4) not assignable
Secondary literature

10.07.2002 (52)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1992
GLP : no data
Test substance : other TS: not further specified

Result : Case 1: positive reactions to clove oil, cinnamon oil and l-menthol
Case 2: positive reactions to clove oil and cinnamon oil.
Test condition : Case 1: dermatitis caused by Tiger Balm made in Taiwan
Case 2: dermatitis caused from two Essential balms made in China
Reliability : (4) not assignable
Documentation insufficient for assessment.

25.02.2003 (53)

5.4 REPEATED DOSE TOXICITY

Type : Sub-chronic
Species : rat
Sex : male
Strain : Wistar
Route of admin. : gavage
Exposure period : 3 m
Frequency of treatm. : daily
Post exposure period : no data
Doses : 50, 150, 450 mg/kg bw/d
Control group : yes, concurrent vehicle
Method : other
Year : 1974
GLP : no
Test substance : other TS: Menthol-JPT, melting point: 42.8-42.9°C, aD = - 49.0°

Result : NOAEL (NOEL), LOAEL (LOEL): not assignable because effects may have been caused by infection
TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death: 1 animal of control group and 1 animal of 50 mg/kg bw died in month 3 due to pneumonia.
- Clinical signs: no effect

5. Toxicity

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Date

10.06.2003

- Body weight gain: no significant effect
- Food/water consumption: 450 mg/kg bw.: Feed efficiency: 0.6 % decreased.
- Clinical chemistry: no compound related effect
- Haematology: no compound related effect
- Urinalysis: no effect
- Organ weights:
 - >= 150 mg/kg bw: absolute and relative thyroid and kidney weight increased
 - = 450 mg/kg bw: absolute and relative liver weight increased
- Testes weights showed some variations, but no dose-dependent effects: mean abs. testes weights in control animals (1.77 g right and 1.67 g left) and in high dosed animals (1.70 g and 1.73 g) no statistical analysis performed
- Gross pathology: no effect
- Histopathology:
 - no clear substance related effects, discussed to be due to temporary infection and healing:
 - >= 150 mg/kg bw: Kupffer cells in liver increased only 150 mg/kg bw: renal casts in the kidney increased

Test condition

- : TEST ORGANISMS
 - Age: six weeks
 - Weight at study initiation: no data
 - Number of animals/dose group: 12
- ADMINISTRATION / EXPOSURE
 - dosing frequency: daily except on "off days", not further specified
 - Vehicle: 10% aqueous solution of gum arabic
 - Preparation: Test substance is suspended in Vehicle
- EXAMINATIONS
 - Clinical signs: yes
 - Mortality: yes
 - Body weight: yes
 - Organ weight: yes
 - Food consumption: yes
 - Water consumption: yes
 - Feed efficiency: yes
 - Ophthalmoscopic examination: no
 - Haematology: yes
 - Biochemistry: s-GOT, s-GPT, s-AIP, glucose, protein
 - Urinalysis: yes
- ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
 - liver, kidneys, heart, lungs, spleen, suprarenal glands, thymus gland, thyroid gland, testicles, pancreas, small intestine, large intestine, stomach, thighbones
- Reliability
 - : (3) invalid
 - Due to infection of the test animals with pneumonia, effects cannot be related to TS administration.

01.08.2002

(54)

- Type : Sub-acute
- Species : rat
- Sex : male/female
- Strain : Wistar
- Route of admin. : gavage
- Exposure period : 28 days
- Frequency of treatm. : daily
- Post exposure period : no
- Doses : 200, 400, 800 mg/kg bw/d
- Control group : other: Yes, not specified

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Method	: other: mainly according to OECD TG 407
Year	: 1983
GLP	: no data
Test substance	: other TS: L-menthol, purity: 99%
Remark	: NOAEL and LOAEL: cannot be determined from the study results
Result	: TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: no data - Body weight gain: no effect - Food/water consumption: significantly increased water consumption at the highest dose level (magnitude not reported, not reported in which sex) - Clinical chemistry: no effect - Haematology: Increased number of neutrophile granulocytes at the highest dose level (magnitude not reported, not reported in which sex) - Organ weights: - m $\geq$ 200, f $\geq$ 400 mg/kg bw: absolute and relative liver weight significantly increased (no information on magnitude and incidence of this findings is given in the publication). - Histopathology: - m and f $\geq$ 200 mg/kg bw: vacuolization of hepatocytes (controls: 0/20, 200 mg/kg: 4/20; 400 mg/kg: 5/17; 800 mg/kg: 4/19, no distinction between sexes); not dose-related; is interpreted as a possible adaptation process - Other: no changes in cerebellum
Test condition	: TEST ORGANISMS - Age: 4 weeks - Weight at study initiation: no data - Number of animals/dose group: 10 males and 10 females ADMINISTRATION / EXPOSURE - Vehicle: soybean oil (food grade quality) - Total volume applied: 5 ml/kg/bw - control group: yes, not stated whether untreated or treated with vehicle EXAMINATIONS: - Clinical signs: yes (inspection twice daily) - Mortality: yes (inspection twice daily) - Body weight: yes (weekly recorded) - Organ weight: yes (kidneys, adrenals, heart, brain, liver and stomach with content) - Food consumption: yes (weekly recorded) - Water consumption: yes (weekly recorded) - Ophthalmoscopic examination: no - Haematology: yes (Hemoglobin, PCV, total erythrocyte count, total WBC, white blood cell differential count, reticulocytes, glucose) - Biochemistry: yes (creatinine, urea, activities of ASAT) - Urinalysis: urine examined for presence of blood, ketones, glucose and proteins ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): Organs examined as described in OECD guideline 407 with the exception of the full histopathology examinations of urinary bladder and prostate. OTHER EXAMINATIONS: special examination of the brain: frozen sections were prepared on selected specimens and stained with Luxol fast blue. For the light microscopic examination of organs/tissues the following staining was performed in addition to Haematoxylin-Eosin (HE) staining: Oil Red O (liver), Perl (liver and spleen), and PAS (liver). STATISTICAL METHODS: Student's t-test was performed on all parameters for males and females separately and analyses of variance were applied to organ weights. $P < 0.01$ was regarded as significant.
Reliability	: (2) valid with restrictions Study design mainly in accordance with OECD TG 407; generally sufficient

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documentation of study results, but no information was provided on the magnitude/incidence of the increase in liver weights. Therefore, and because no clearly pathological microscopic and enzymatic changes indicating an adverse effect on the liver have been reported, the relevance of this finding is questionable and a NO(A)EL cannot be deduced from this study

Flag : Critical study for SIDS endpoint
25.02.2003

(55)

Type : Sub-chronic
Species : Rat
Sex : male/female
Strain : Sherman
Route of admin. : Inhalation
Exposure period : 71, 74, 75, 79 days
Frequency of treatm. : 6.75 h daily
Post exposure period : no data
Doses : 0.087, 0.148, 0.259 ppm (according to 0.57, 0.96 and 1.68 mg/m³)
Control group : Yes
Method : other
Year : 1954
GLP : No
Test substance : other TS: not further specified

Remark : VARIABILITY of EXPOSURE CONCENTRATIONS: 0.087 ± 0.021 ppm;
0.148 ± 0.031 ppm; 0.259 ± 0.166 ppm

The VAPOUR INHALATION was performed as whole body inhalation

Result : NOAEL, LOAEL: not assignable due to invalid analytical methods

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

- Mortality and time to death: no effect
- Clinical signs: transient erythema of the conjunctiva; disappeared shortly after they were returned to their cages
- Body weight gain: no effect
- Food/water consumption: no effect
- Haematology: no effect
- Organ weights: no effect
- Gross pathology: no effect
- Histopathology:

Lung, respiratory tract (tracheitis, pneumonitis, pulmonary congestion)
3/9 (2/9 evidence of pneumonitis), 1/8, 4/8, 9/11 (severe congestion to pneumonitis)

Test condition : TEST ORGANISMS

- Age: young
 - Weight at study initiation: 125-185
 - Number of animals/dose group: 12
 - Control groups: 12 animals - 4 m, 8 f (identical conditions except menthol); one additional control group: 8 (4 m, 4 f) remained in their cages throughout the study.
- CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: yes (daily observation)
 - Mortality: yes (daily)
 - Body weight: yes (twice weekly)
 - Organ weight: yes
 - Food consumption: yes (estimated daily)
 - Water consumption: yes (estimated daily)
 - Ophthalmoscopic examination: no
 - Haematology: yes
 - Biochemistry: no

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	- Urinalysis: no
	- Histopathology: yes
	ORGANS EXAMINED AT NECROPSY (MICROSCOPIC):
	- Microscopic: eye, turbinates, nasopharynx, trachea, lungs, and skin, sections of liver, spleen, kidney, heart, testes, ovaries, intestine and skeletal muscle.
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: no valid analytical method to observe concentration of menthol in exposure chamber.
Flag 19.08.2002	: Critical study for SIDS endpoint
	(56)
Type	: Sub-chronic
Species	: rat
Sex	: male/female
Strain	: Fischer 344
Route of admin.	: inhalation
Exposure period	: 13 w
Frequency of treatm.	: 1hr/d, 5d/w
Post exposure period	: 6 w
Doses	: smoke particulate concentration: 200, 600, 1200 mg total particle matter/m ³
Control group	: other: see test conditions
Method	: other
Year	: 1997
GLP	: no data
Test substance	: other TS: synthetic l-menthol, not further specified
Result	: NOAEL (NOEL), LOAEL (LOEL): not assignable ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX - Time of death: no TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: no effect - Clinical signs: clear nasal discharge in reference nicotine and menthol smokers (higher incidence in rats exposed to reference smoke) - Body weight gain: 200, 600, 1200 mg/m ³ : decreased body weight (reference and menthol) - Food/water consumption: no effect - Clinical chemistry: 1200 mg/m ³ : decrease in glucose levels (reference and menthol) - Haematology: Dose-dependant increase in carboxyhaemoglobin level, m and f (reference and menthol) – significantly smaller in menthol- smokers - Urinalysis: no effect - Organ weights: Dose-dependent increase of lung to body weight ratio, m and f (Reference and menthol); dose-dependent heart to body weight ratio, m and f - slightly greater effect in m (Reference and menthol). - Histopathology: Dose-dependant increase in incidence of histopathological changes in respiratory tract, m and f (Reference and menthol) changes were slightly more severe in mentholated smoke females than in reference smoke females. CONCLUSION: Rats exposed to menthol cigarette smoke showed the same changes in clinical signs, clinical chemistry, organ weights and histopathology than the rats exposed to nicotine reference smoke, despite: - the cotinine level in the 200 mg total particle matter/m ³ menthol group was lower than in reference groups - carboxyhemoglobin levels in rats exposed to menthol smoke were lower

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	compared to reference smokers (observation is consistent with the reduced smoke carbon monoxide concentration for menthol smoke exposures) - dose-related increase in nasal discharge was not observed in menthol cigarette smokers
Test condition	: TEST ORGANISMS - Age: 6 weeks - Weight at study initiation: male: 200-220 g, female: 140-150 g (both estimated from graphic) - Number of animals/dose group: 15 per sex, 21 per sex (reference) ADMINISTRATION / EXPOSURE - Examined groups: Menthol: 3 dosed groups (mentholated cigarette smoke), Reference: 3 reference dosed group (non-mentholated cigarette smoke), Control: 1 control group (no smoke) - Duration of test/exposure: 30 min smoke, 15 min filtered air, 30 min smoke - Post exposure period: a portion of each group was autopsied immediately, remaining rats: 6 wk non-exposure recovery period - Vehicle: nicotin smoke - Concentration in vehicle: 5000 ppm menthol - Particle size: 0.47-0.90 µm - Type or preparation of particles: 30-port AMESA Mark IIIA smoking machine (individual nose-only) using machine vacuum to puff the cigarettes according to Federal Trade Commission standards of a 35 ml, 2-sec puff taken once per minute. CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: yes (weekly examination) - Mortality: yes (twice daily) - Body weight: yes (weekly) - Organ weight: Adrenal glands, heart, right kidney, lungs, liver, spleen, and right testis were recorded on animals terminated after 13 wk exposure. Lungs and heart weights were recorded at autopsy of all recovery group animals. - Food consumption: yes - Water consumption: yes - Ophthalmoscopic examination: no - Haematology: yes (hemoglobin, hematocrit, leucocyte count and differentials, erythrocyte count and indices) - Clinical chemistry: yes (aspartate and alanine aminotransferase, blood urea, nitrogen, alkaline phosphatase, bilirubin, cholesterol, creatinine, glucose, g-glutamyltransferase, total protein, albumin, globulin, sodium, potassium, calcium and chloride) ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): Organs examined as described in OECD guideline 408. STATISTICAL METHODS: One-way analysis of variance (ANOVA) followed by a Tukey's HSD test for body weights, organ weights, calculated organ weight/body weight ratios, quantitative clinical pathology data.
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles. However, since the study compares toxic effects of cigarette smoke with those of mentholated cigarette smoke no assessment can be made for the effects of menthol alone.
12.11.2002	
Type	: Sub-acute
Species	: rat
Sex	: male/female
Strain	: Sprague-Dawley
Route of admin.	: i.p.

(57)

5. Toxicity

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Date 10.06.2003

Exposure period : 3 d
Frequency of treatm. : daily
Post exposure period : no
Doses : 40 mg/kg bw/d in 10 % ethanol and 90 % corn oil
Control group : yes, concurrent vehicle
Method : other
Year : 1988
GLP : no data
Test substance : other TS: 97-99 %

Result : No significant change in the total cytochrome P-450 content of liver microsomal membranes, no visible change in the pattern of liver microsomal membrane proteins, no effect on the amount of hepatic cytochrome b5, small but significant inductions of PB P-450 in liver microsomal membrane vesicles.

Test condition : Rats were given three consecutive daily intraperitoneal injections of menthol. Test was conducted to study the effect of terpenoid compounds on cytochrome P-450 Levels in rat liver.

Reliability : (3) invalid
Unsuitable test system (see test conditions).

18.01.2002

(58)

Type : Sub-acute
Species : rat
Sex : no data
Strain : no data
Route of admin. : other: oral by stomach tube
Exposure period : 7 d
Frequency of treatm. : daily
Post exposure period : no data
Doses : 800 mg/kg bw/d
Control group : no data specified
Method : other
Year : 1988
GLP : no data
Test substance : other TS: not further specified

Result : Biochemical changes in the liver.

Reliability : (4) not assignable
Secondary literature

17.12.2001

(59)

Type :
Species : rat
Sex : male/female
Strain : no data
Route of admin. : other: diet
Exposure period : 5.5 weeks
Frequency of treatm. : daily
Post exposure period : no data
Doses : 0, 100 or 200 mg/kg bw/d
Control group : other: Yes, not specified
NOAEL : 200 mg/kg bw
Method : other: no data
Year : 1961
GLP : no
Test substance : other TS: L-menthol, purity not stated

Remark : Type: other: Repeated dose study with L-menthol and D/L-menthol

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Result	: No adverse effects on weight gain, excretion of glucuronides, water, or electrolytes, or interference with central nervous system reactions to stimulants were observed	
Test condition	: NUMBER OF ANIMALS: 40 rats of each sex/dose	
Reliability	: (4) not assignable secondary citation from peer-reviewed document (FAO/WHO report 1999)	
Flag 25.02.2003	: Critical study for SIDS endpoint	(60)
Type	: Sub-chronic	
Species	: mouse	
Sex	: male	
Strain	: other: white mice	
Route of admin.	: inhalation	
Exposure period	: 3 m	
Frequency of treatm.	: 5h/d	
Post exposure period	: no data	
Doses	: 50, 100 mg/m ³	
Control group	: yes	
NOAEL	: 5 ppm	
Method	: other	
Year	: 1962	
GLP	: no	
Test substance	: other TS: menthol oil vapors, not further specified	
Result	: NOAEL (NOEL), LOAEL (LOEL): Concentration of 5 ppm is assumed by the authors as safe from the toxicological point of view. No further data. TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - clinical signs: fatigue, lower mobility - histopathology: regressive changes in the liver and kidney (no detailed data given)	
Test condition	: TEST ORGANISMS: - weight at study initiation: ca. 20g - number of animals: 10 SATELLITE GROUPS AND REASONS THEY WERE ADDED: A group of 10 mice was exposed to menthol oil vapors in the production site for 76 days. No data on exposure concentrations are given. CLINICAL OBSERVATIONS AND FREQUENCY: - clinical signs: yes, no data on frequency - mortality: no data - body weight: no data - organ weight: no data - food consumption: no data - water consumption: no data - ophthalmoscopic examination: no data - haematology: no data - biochemistry: no data - urinalysis: no data ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC) No detailed data on macroscopic or microscopic examinations are given. STATISTICAL METHODS: no data	
Reliability	: (3) invalid Significant methodological deficiencies: no detailed data on observed changes. No correlation to doses. The method of menthol concentration measurement is doubtful (colour reaction with vaniline).	
10.07.2002		(61)
Type	: Sub-acute	

5. Toxicity

Id 2216-51-5
Date 10.06.2003

Species : mouse
Sex : male
Strain : other: white mice
Route of admin. : inhalation
Exposure period : 6 days
Frequency of treatm. : 6h/day
Post exposure period : 21 days
Doses : 1 mg/l
Control group :
Method : other
Year : 1962
GLP : no
Test substance : other TS: menthol oil vapors, not further specified

Result : NOAEL (NOEL), LOAEL (LOEL): No NOAEL assigned.
 TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
 - clinical signs: no signs of intoxication
 - histopathology: erythraemia, small hematoma in brain, heart, lungs, kidneys and regressive changes in the liver and kidney

Test condition : TEST ORGANISMS:
 - weight at study initiation: ca.20g
 - number of animals: 10
 CLINICAL OBSERVATIONS AND FREQUENCY:
 - clinical signs: yes, no data on frequency
 - mortality: no data
 - body weight: no data
 - organ weight: no data
 - food consumption: no data
 - water consumption: no data
 - ophthalmoscopic examination: no data
 - haematology: no data
 - biochemistry: no data
 - urinalysis: no data
 ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC)
 No detailed data on macroscopic or microscopic examinations are given.
 STATISTICAL METHODS: no data

Reliability : (3) invalid
 Significant methodological deficiencies: no detailed data on observed changes. No correlation to doses. The method of menthol concentration measurement is doubtful (colour reaction with vaniline).

10.07.2002

(61)

Type : Sub-acute
Species : mouse
Sex : male
Strain : ICR
Route of admin. : oral unspecified
Exposure period : 5 d
Frequency of treatm. : daily
Post exposure period : 9 d
Doses : 2000, 2500, 3200, 4000 and 5000 mg/kg bw/d
Control group : no
Method : other
Year : 1975
GLP : no
Test substance : other TS: menthol, natural, brazilian

Result : ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX

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MORTALITY:

- Time and number of deaths at each dose:

dose (mg/kg)/deaths/time of deaths

2000/2(6)/day 2 (2)

2500/2(6)/day 1 (1), day 2 (2)

3200/3(6)/day 1 (1), day 2 (1)

4000/6(6)/day 2 (6)

5000/6(6)/day 2 (6)

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:

- Mortality and time to death: 14-day subacute oral LD50 in male mice is

2652 mg/kg with 95% confidence limits of 1951 to 3218 mg/kg

- Clinical signs: Signs of toxicity and abnormal behavior included

depression, excitability, rapid respiration and unthrifty appearance

- Histopathology: No abnormal gross findings in gross necropsy were observed.

Test condition

: TEST ORGANISMS

- Age: no data

- Weight at study initiation: average bw. 35 g

- Number of animals/dose group: 6

ADMINISTRATION / EXPOSURE

- Vehicle: 0.85% saline

CLINICAL OBSERVATIONS AND FREQUENCY:

- Clinical signs: yes

- Mortality: yes

- Body weight: no

- Organ weight: no

- Food consumption: no

- Water consumption: no

- Ophthalmoscopic examination: no

- Haematology: no

- Biochemistry: no

- Urinalysis: no

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):

Animals were subjected to gross necropsy; no further data.

Reliability

: (3) invalid

Significant methodological deficiencies: e.g. no control group, relevant parameters were not studied (Mutagenic evaluation study)

17.12.2001

(39)

5.5 GENETIC TOXICITY 'IN VITRO'

Type

: Ames test

System of testing

: S. typhimurium (TA 98, 100, 2637)

Test concentration

: 0.02, 0.05, 0.1, 0.2, 0.5 mg/plate

Cycotoxic concentr.

: 0.1 mg/plate (lethal dose in TA 100; 0.5 mg/plate lethal for TA 98 and 2637)

Metabolic activation

: with and without

Result

: negative

Method

: other: according to Ames et al. (1975)

Year

: 1985

GLP

: no data

Test substance

: other TS: purity not stated

Result

: No increases in mutant frequency were seen in any strain both in the absence and in the presence of metabolic activation. The positive control compounds induced strongly enhanced number of revertants in all strains.

Test condition

: Metabolic activation system: S9-mix from PCB induced BALB/c mice

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	Vehicle: DMSO Negative control: DMSO Positive controls: AF-2 0.02 µg/plate (for TA 100 and TA 98 without S9-mix), 2-aminoacridine 0.2 mg/plate (for TA 1537 without S9-mix), 2-aminoanthracene 0.05 mg/plate (for all used strains with S9-mix)	
Reliability	: (2) valid with restrictions Limited documentation	
Flag 25.02.2003	: Critical study for SIDS endpoint	(62)
Type	: Ames test	
System of testing	: S. typhimurium TA 1537, TA 1535, TA 100, TA 98	
Test concentration	: 6.4, 32, 160, 800 µg/plate	
Cycotoxic concentr.	: 800 µg/plate (lethal dose)	
Metabolic activation	: with and without	
Result	: negative	
Method	: other: according to Ames et al. (1975)	
Year	: 1984	
GLP	: no data	
Test substance	: other TS: menthol, purity: 99%	
Result	: No increases in mutant frequency were seen in any strain both in the absence and in the presence of metabolic activation. At a concentration of 800 µg/plate strong cytotoxicity was induced. The positive control compounds induced strongly enhanced number of revertants in all strains.	
Test condition	: Metabolic activation system: S9-mix from Aroclor-1254 induced male Wistar rats Vehicle: DMSO Negative control: DMSO Positive controls: sodium azide (for TA 1535 and TA 100 without S9-mix), 2-nitrofluorene (for TA 1537 and TA 98 without S9-mix), 2-anthramine (for all used strains with S9-mix)	
Reliability	: (2) valid with restrictions Only 3 concentrations evaluated for mutagenicity	
Flag 25.02.2003	: Critical study for SIDS endpoint	(63)
Type	: Ames test	
System of testing	: S. typhimurium TA100, TA98, TA97a and TA102	
Test concentration	: 5 - 800 µg/plate	
Cycotoxic concentr.	: Tested up to and including cytotoxic concentration	
Metabolic activation	: with and without	
Result	: negative	
Method	: other: according to Maron and Ames, Mutat. Res. 113 (1983) 173-215	
Year	: 1997	
GLP	: no data	
Test substance	: other TS: commercial grade	
Result	: No increases in mutant frequency were seen in any strain both in the absence and in the presence of metabolic activation. The positive control compounds induced strongly enhanced numbers of revertants in all strains.	
Test condition	: Metabolic activation system: S9-mix from Aroclor-1254 induced rats Vehicle: ethanol Negative control: ethanol Positive controls: sodium azide, nitro-o-phenylene-diamine, 4-nitroquinolineoxid, 2-aminoanthracene, mitomycin C, 2-aminofluorene, benzo-a-pyrene Tested concentrations: TA 100: 100 - 700 µg/plate	

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	TA 98: 100 - 800 µg/plate (cytotoxic at 800 µg) TA 97a: 100 - 800 µg/plate (cytotoxic at 800 µg) TA 102: 5 - 500 µg/plate (cytotoxic at 500 µg)	
Reliability	: (1) valid without restriction	
Flag	: Critical study for SIDS endpoint	
25.02.2003		(64) (65)
Type	: Bacterial gene mutation assay	
System of testing	: Bacillus subtilis	
Test concentration	: up to 20 mg/plate	
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	: negative	
Method	: other	
Year	: 1978	
GLP	: no	
Test substance	: other TS: not further specified	
Reliability	: (4) not assignable Secondary literature.	
14.02.2002		(66)
Type	: other: Antimutagenicity	
System of testing	: E. coli WP2 uvrA (trp-), pre-treated with AF-2	
Test concentration	: 0.5 - 2 mg/ml	
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	: negative	
Method	: other	
Year	: 1986	
GLP	: no data	
Test substance	: other TS: analytical grade	
Result	: In the present study an antimutation test in E.coli WP2 uvrA (trp-) was performed. Bacteria were pretreated with AF-2 and MNNG and the mutation of AF-2 and MNNG induced trp+ revertants was investigated. L-Menthol had no antimutagenic effect.	
Test condition	: Ratio (maximal revertants/spontaneous revertants x 100) >> 2.0 is regarded as a positive result.	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(67)
Type	: Escherichia coli reverse mutation assay	
System of testing	: E.coli WP 2 uvrA	
Test concentration	: 0.1 0.8 mg/plate	
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	: negative	
Method	: other	
Year	: 1986	
GLP	: no data	
Test substance	: other TS: analytical grade	
Remark	: The positive control AF -2 exhibited clear mutagenic activity in this test	
Test condition	: Ratio (minimal revertants/AF -2-induced revertants x 100) less than 50 % is regarded as a positive result.	
Reliability	: (2) valid with restrictions Limited documentation	

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25.02.2003 (67)

Type : other: DNA repair assay
System of testing : Bacillus subtilis M 45 (rec-) and H 17 (rec+)
Test concentration : up to 10 mg/disk
Cycotoxic concentr. :
Metabolic activation :
Result : positive
Method : other: rec-assay
Year : 1986
GLP : no data
Test substance : other TS: analytical grade

Remark : The DNA damaging activity was measured by the difference in growth inhibition zones between strains M45 and H17; a test substance was considered negative if the difference was < 4 mm.

Result : Inhibition zones:
M45: 42 cm
H17: 23 cm
Difference: 19 cm

Reliability : (2) valid with restrictions
Limited documentation; no information on dose response.

Flag : Critical study for SIDS endpoint

25.02.2003 (67)

Type : Chromosomal aberration test
System of testing : Human peripheral blood lymphocytes
Test concentration : 0.1, 1, 10 mM
Cycotoxic concentr. : > 10mM
Metabolic activation : with and without
Result : negative
Method : other
Year : 1991
GLP : no data
Test substance : other TS: purity: > 98 %

Result : Combined percentage structural aberration rate for males and female)
Results/without S-9/with S-9
Solvent controls: 1.76/2.00
10mM menthol: 2.11/2.25
MMC-positive control: 9.13
Results with the test material were not statistically significantly different from the solvent controls.
No changes in polyploid cells.

Test condition : SYSTEM OF TESTING
- Species/cell type: 12 male and 12 female donors
- Culturing: RPME 1640 medium, 2mM-L-glutamine, 100 U penicillin/ml, 100 µg streptomycin/ml, 10% foetal calf serum, 1% phytohaemagglutinin, test substance (from beginning of incubation)
- Metabolic activation system: S9
- No. of metaphases analyzed: 100
ADMINISTRATION:
- Positive and negative control groups and treatment:
negative control: solvent DMSO
positive Control: 5 male donors with mitomycin C in distilled water (1 x 10E - 7 M)

Reliability : (2) valid with restrictions
Limited documentation

Flag : Critical study for SIDS endpoint

5. Toxicity

Id 2216-51-5
Date 10.06.2003

25.02.2003 (68)

Type : Sister chromatid exchange assay
System of testing : Human peripheral blood lymphocytes
Test concentration : 0.1, 1, 10 mM
Cycotoxic concentr. : > 10mM
Metabolic activation : with and without
Result : negative
Method : other
Year : 1991
GLP : no data
Test substance : other TS: purity: > 98 %

Test condition : SYSTEM OF TESTING
 - Species/cell type: 12 male and 12 female donors
 - Culturing: RPME 1640 medium, 2mM-L-glutamine, 100 U penicillin/ml, 100 µg streptomycin/ml, 10% foetal calf serum, 1% phytohaemagglutinin, 10 µM 5-bromodeoxyuridine, test substance (from beginning of incubation)
 - Metabolic activation system: S9
 - No. of metaphases analyzed: >= 25 second-division cells/each culture
 - No. of cells in which chromosomal aberrations were investigated: >= 100
 ADMINISTRATION:
 - Positive and negative control groups and treatment:
 negative control: solvent DMSO
 positive Control: 5 male donors with mitomycin C in distilled water (1 x 10E - 8 M)

Reliability : (2) valid with restrictions
 Limitd documentation

Flag : Critical study for SIDS endpoint

25.02.2003 (68)

Type : Chromosomal aberration test
System of testing : Chinese hamster lung cells
Test concentration : 0.0313, 0.0625, 0.125 mg/ml
Cycotoxic concentr. : no data
Metabolic activation : without
Result : negative
Method : other
Year : 1982
GLP : no data
Test substance : other TS: not further specified

Result : Test was negative after 24 / 48 hours of treatment (- S9):
 - Vehicle control: 1 / 0 % aberrations
 - 0.0313 mg/ml: 1 / 1 % aberrations
 - 0.0625 mg/ml: 3 / 1 % aberrations
 - 0.125 mg/ml: 3 / 0 % aberrations

Test condition : Solvent: DMSO
Reliability : (2) valid with restrictions
 Limited documentation

Flag : Critical study for SIDS endpoint

25.02.2003 (69)

Type : Chromosomal aberration test
System of testing : Chinese hamster cells (direct method)
Test concentration : 0.1 - 0.3 mg/ml
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : negative

5. Toxicity

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Method	: other	
Year	: 1982	
GLP	: no data	
Test substance	: other TS: not further specified	
Result	: No TS related effects on polyploidy. Gaps were included in the frequency of aberrations. Test was negative (- / + S9): - Vehicle control: 1 / 1 % aberrations - 0.1 mg/ml: 0 / 0 % aberrations - 0.2 mg/ml: 0 / 1 % aberrations - 0.3 mg/ml: - / 1 % aberrations Overall conclusion: negative	
Test condition	: Method: Direct method Solvent: DMSO Application: 24 and 48 hours. chromosomal effects evaluated: chromatid and isochromatid gaps, chromatid breaks, chromatid exchanges, chromosome breaks, chromosome exchanges including dicentric and ring chromosomes. Duration of treatment: 24 and 48 hours In this study 25 chemicals have been tested. Acrylamide and acrylonitrile gave positive results (up to 15% and 25% aberrations, respectively). No further positive controls tested. Statistics: not performed	
Reliability	: (2) valid with restrictions Limited documentation	
Flag 25.02.2003	: Critical study for SIDS endpoint	(69)
Type	: other: Anaphase chromosome aberration	
System of testing	: Human tissue culture cells (fibroblasts)	
Test concentration	: 0.1, 1.0, 10.0 µg/ml	
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	: negative	
Method	: other	
Year	: 1975	
GLP	: no	
Test substance	: other TS: Menthol natural brazilian, FDA 71-57	
Result	: negative control: two cells with bridges positive control: within normal limits 1.0 and 10 µg/ml: each a cell with an acentric fragment % cells with aberrations (0.1, 1.0, 10.0 %, negative control, positive control): 0, 1, 1, 2, 30	
Test condition	: Negative control: saline Positive control: Triethylene Melamine (TEM) No. of cells: 100	
Reliability	: (2) valid with restrictions Limited documentation	
Flag 25.02.2003	: Critical study for SIDS endpoint	(39)
Type	: Chromosomal aberration test	
System of testing	: Chinese hamster cells	
Test concentration	: 0.1, 0.2, 0.3 mg/ml	
Cycotoxic concentr.	: up to cytotoxic concentrations	
Metabolic activation	: with and without	
Result	: negative	

5. Toxicity

Id 2216-51-5
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Method : other
Year : 1998
GLP : no data
Test substance : other TS: 99.9% purity

Test condition : Metabolic activation with mouse liver S9 mix
No. of cells with chromosomal aberrations was counted on 100 well-spread metaphases.
Judgement of clastogenicity (based on historical database):
- less than 4%: negative
- between 5.0 and 9.9%: equivocal
- more than 10.0%: positive

Reliability : (2) valid with restrictions
Limited documentation

Flag : Critical study for SIDS endpoint
25.02.2003

(70)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : rat
Sex : male
Strain : other: albino rats
Route of admin. : gavage
Exposure period : acute
Doses : 1.45, 14.5, 145.0 mg/kg and 500, 3000 mg/kg (second test)
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : other TS: Menthol natural brazilian, FDA 71-57

Result : Treated animals showed no increased number of aberrations (max. 0.8 %) compared to control animals (max. 0.66 %).
Positive control animals showed 22.8 - 37 % aberrant metaphases.

Test condition : Analysis of chromosome aberrations in bone marrow
Age: 10-12 weeks
Groups: Animals were killed 6 hours, 24 hours and 48 hours after treatment respectively
Number of animals/group: 5
number of negative control animals/group: 3
Number of positive control animals: 5 animals killed after 48 hours
No. of metaphases investigated/animal: 50
Negative control: Vehicle (saline)
Positive control: 0.30 mg/kg Triethylene melamine injected intraperitoneally
Colcemid injection: 4 mg/kg administered 2 hrs prior killing

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: only 50 metaphases per animal investigated.

Flag : Critical study for SIDS endpoint
10.07.2002

(39)

Type : Cytogenetic assay
Species : Rat
Sex : Male
Strain : other: albino rats
Route of admin. : Gavage

5. Toxicity

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Exposure period : 5 days
Doses : 1.45, 14.5, 145.0 mg/kg and 1150 mg/kg (second test)
Result : Negative
Method : other
Year : 1975
GLP : No
Test substance : other TS: Menthol natural brazilian, FDA 71-57

Result : Treated animals showed a comparable number of aberrations (max. 0.8 %) to that of control animals (0 %).

Test condition : Analysis of chromosome aberrations in bone marrow
Age: 10-12 weeks
Number of animals/dose group: 5
Number of negative control animals: 3
No. of metaphases investigated/animal: 50
Negative control: vehicle (saline)
Exposure: Five doses 24 hours apart, animals killed 6 hours after last dose
Colcemid injection: 4 mg/kg administered 2 hrs prior killing
No positive control animals.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: only 50 metaphases per animal investigated.

Flag : Critical study for SIDS endpoint
10.07.2002

(39)

Type : Dominant lethal assay
Species : rat
Sex : male
Strain : no data
Route of admin. : gavage
Exposure period : acute
Doses : 1.45, 14.5, 145.0 mg/kg and 500, 3000 mg/kg (second test)
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : other TS: Menthol natural brazilian, FDA 71-57

Result : Positive control showed strong effects on implantation, fertility, number of dead implants etc. The values calculated for menthol did not significantly vary from those of the negative control.

Test condition : Following treatment 10 males/dose were mated with 2 females/week for 8 weeks - resulting in 14 to 20 pregnant females per dose, negative and positive control per mating interval.
females were killed 14 days after separation from males
Positive control: triethylene melamine (TEM 0.3 mg/kg, i.p.)
Negative control: saline
Fertility index, preimplantation loss and lethal effects on the embryos were determined and compared to those calculated from negative (saline dosed) and positive (TEM-dosed) control animals.

Statistics:
fertility index: chi-square test
number of implants: t-test
number of corpora lutea: t-test
preimplantation loss: Freeman-Tukey transformation and t-test

Reliability : (2) valid with restrictions
Limited documentation

Flag : Critical study for SIDS endpoint

5. Toxicity

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25.02.2003 (39)

Type : Dominant lethal assay
Species : rat
Sex : male
Strain : no data
Route of admin. : gavage
Exposure period : 5 days
Doses : 1.45, 14.5, 145.0 mg/kg and 1150 mg/kg (second test)
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : other TS: Menthol natural brazilian, FDA 71-57

Result : The values calculated for menthol did not significantly vary from those of the negative control.

Test condition : Subacute study
 Following treatment 10 males/dose were mated with 2 females/week for 7 weeks - resulting in 13 to 19 pregnant females per dose, negative and positive control per mating interval.
 females were killed 14 days after separation from males
 Positive control: triethylene melamine (TEM 0.3 mg/kg, i.p.)
 Negative control: saline
 Fertility index, preimplantation loss and lethal effects on the embryos were determined and compared to those calculated from negative (saline dosed) and positive (TEM-dosed) control animals.
 Statistics:
 fertility index: chi-square test
 number of implants: t-test
 number of corpora lutea: t-test
 preimplantation loss: Freeman-Tukey transformation and t-test

Reliability : (2) valid with restrictions
 Limited documentation

Flag : Critical study for SIDS endpoint

25.02.2003 (39)

Type : other: Host mediated Assay
Species : other: mouse (indicator organism: Salmonella typhimurium G-46)
Sex : male
Strain : ICR
Route of admin. : gavage
Exposure period : acute
Doses : 1.45, 14.5, 145.0 (first test) and 500, 5000 mg/kg bw (second test)
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : other TS: Menthol natural brazilian, FDA 71-57

Result : The corresponding in vitro tests gave negative results.

Test condition : Acute Ex vivo study:
 Number of animals/dose level: 10
 Indicator Organisms (reverse mutation): Salmonella typhimurium (his G-46)
 Negative control: solvent
 Positive control: dimethyl nitrosamine (only at 100 mg/kg)
 Injection of indicator organisms in mice: 2.0 ml (each ml contained 3.0 x 10E8 cells) intraperitoneally
 Three hours later mice were killed, Salmonella species were collected and

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	the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(39)
Type	: other: Host mediated assay	
Species	: other: mouse (indicator organism: Salmonella typhimurium TA 1530)	
Sex	: Male	
Strain	: ICR	
Route of admin.	: Gavage	
Exposure period	: Acute	
Doses	: 1.45, 14.5, 145.0 (first test) and 500, 5000 mg/kg bw (second test)	
Result	: Ambiguous	
Method	: other	
Year	: 1975	
GLP	: No	
Test substance	: other TS: Menthol natural brazilian, FDA 71-57	
Result	: Positive effects occurred only at intermediate dose levels with no dose response (significant increased mutant frequency at 145.0 mg/kg bw; 500 and 5000 mg/kg were negative). Effects can therefore be considered as non-relevant. The corresponding in vitro test gave negative results.	
Test condition	: Acute Exvivo study: Number of animals/dose level: 10 Indicator Organisms (reverse mutation): Salmonella typhimurium (TA-1530) Negative control: solvent Positive control: dimethyl nitrosamine (only at 100 mg/kg) Injection of indicator organisms in mice: 2.0 ml (each ml contained 3.0 x 10E8 cells) intraperitoneally Three hours later mice were killed, Salmonella species were collected and the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(39)
Type	: other: Host mediated assay	
Species	: other: mouse (indicator organism: Salmonella typhimurium G-46)	
Sex	: Male	
Strain	: ICR	
Route of admin.	: Gavage	
Exposure period	: 5 days	
Doses	: 1.45, 14.5, 145.0, mg/kg (first test) and 1150 mg/kg (second test)	
Result	: Negative	
Method	: other	
Year	: 1975	
GLP	: No	
Test substance	: other TS: Menthol natural brazilian, FDA 71-57	
Result	: The in vitro test results were negative as well.	
Test condition	: Subacute Ex-vivo study: Number of animals/dose level: 10 Dosages: Aute dosage was given once a day. Indicator Organisms (reverse mutation): Salmonella typhimurium (his G-46) Negative control: solvent Positive control: dimethyl nitrosamine (only at 100 mg/kg) Injection of indicator organisms in mice: Within 30 minutes after the last	

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	dosage each animal was given 2.0 ml (each ml contained 3.0 x 10E8 cells) intraperitoneally Three hours later mice were killed, Salmonella species were collected and the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(39)
Type	: other: Host mediated assay	
Species	: other: mouse (indicator organism: Saccharomyces cerevisiae (D-3))	
Sex	: Male	
Strain	: ICR	
Route of admin.	: Gavage	
Exposure period	: Acute	
Doses	: 1.45, 14.5, 145.0 mg/kg (first test) 500, 5000 mg/kg (second test)	
Result	: Negative	
Method	: other	
Year	: 1975	
GLP	: No	
Test substance	: other TS: Menthol natural brazilian, FDA 71-57	
Result	: The recombinant frequency in the in vitro test with D3 was slightly elevated.	
Test condition	: Acute Exvivo study: Number of animals/dose level: 10 Indicator Organisms (mitotic recombination): Saccharomyces cerevisiae (D-3) Negative control: solvent Positive control: ethyl methane sulfonate (intramuscularly injected at a dose of 350 mg/kg) Injection of indicator organisms in mice: 2.0 ml (each ml contained 5.0 x 10E8 cells) intraperitoneally Three hours later mice were killed, Yeast species were collected and the number of recombinants was counted. Recombinant frequency (RF): total recombinants counted/total number colonies screened	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(39)
Type	: other: Host mediated assay	
Species	: other: mouse (indicator organism: Saccharomyces cerevisiae (D-3))	
Sex	: Male	
Strain	: ICR	
Route of admin.	: Gavage	
Exposure period	: 5 days	
Doses	: 1.45, 14.5, 145 (first test), 1150 mg/kg (second test)	
Result	: Ambiguous	
Method	: other	
Year	: 1975	
GLP	: No	
Test substance	: other TS: Menthol natural brazilian, FDA 71-57	
Result	: Slightly enhanced recombinant frequencies in all subacute dose levels. In a second test with a single subacute dose level of 1150 mg/kg the test result was negative. The recombinant frequency in the in vitro test with D3 was slightly elevated too.	
Test condition	: Subacute Ex-vivo study:	

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	Number of animals/dose level: 10 Indicator Organisms (mitotic recombination): <i>Saccharomyces cerevisiae</i> (D-3) Negative control: solvent Positive control: ethyl methane sulfonate (intramuscularly injected at a dose of 350 mg/kg) Injection of indicator organisms in mice: Within 30 minutes after last dosage mice were given 2.0 ml (each ml contained 5.0×10^8 cells) intraperitoneally Three hours later mice were killed, Yeast species were collected and the number of recombinants was counted. Recombinant frequency (RF): total recombinants counted/total number colonies screened
Reliability	: (2) valid with restrictions Limited documentation
25.02.2003	(39)
Type	: other: Host mediated assay
Species	: other: mouse (indicator organism: <i>Salmonella typhimurium</i> TA 1530)
Sex	: Male
Strain	: ICR
Route of admin.	: Gavage
Exposure period	: 5 days
Doses	: 1.45, 14.5, 145.0, mg/kg (first test) and 1150 mg/kg (second test)
Result	: Negative
Method	: other
Year	: 1975
GLP	: No
Test substance	: other TS: Menthol natural brazilian, FDA 71-57
Result	: The in vitro test results were negative as well.
Test condition	: Subacute Ex-vivo study: Number of animals/dose level: 10 Dosages: Auto dosage was given once a day. Indicator Organisms (reverse mutation): <i>Salmonella typhimurium</i> (TA 1530) Negative control: solvent Positive control: dimethyl nitrosamine (only at 100 mg/kg) Injection of indicator organisms in mice: Within 30 minutes after the last dosage each animal was given 2.0 ml (each ml contained 3.0×10^8 cells) intraperitoneally Three hours later mice were killed, <i>Salmonella</i> species were collected and the number of mutants was counted. Mutation frequency (MF): MF of experimental sample/MF of control sample
Reliability	: (2) valid with restrictions Limited documentation
25.02.2003	(39)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type	: other: Sub-chronic
Species	: Rat
Sex	:
Strain	: Wistar
Route of admin.	: Gavage
Exposure period	: 3 m

5. Toxicity

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Frequency of treatm. : Daily
Premating exposure period :
Male :
Female :
Duration of test :
No. of generation :
studies :
Doses : 50, 150, 450 mg/kg bw/d
Control group : yes, concurrent vehicle
Result : 150 mg/kg bw/d: decrease of absolute testis weight, no changes in histopathological examinations of testes
Method : other
Year : 1974
GLP : No
Test substance : other TS: menthol-JPT

Remark : There are discrepancies between the full text of the present study and the attached table concerning testes weights.
Given information are obtained from table analysis.

Test condition : The testis were weighed and histopathologically examined.
Details of the study design see chapter 5.4 Repeated dose toxicity.

Reliability : (3) invalid
Significant methodological deficiencies, see remark.

12.11.2002

(71)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : Gavage
Exposure period : gestation day 6-15
Frequency of treatm. : Daily
Duration of test : 10 consecutive days
Doses : 2.18, 10.15, 47.05 or 218.0 mg/kg bw/d
Control group : other: sham treated with corn oil
other: NOEL Maternal : = 218 mg/kg bw
Toxicity :
other: NOEL : = 218 mg/kg bw
Teratogenicity :
Result : Negative
Method : other
Year : 1973
GLP : No
Test substance : other TS: white needle-like crystalline material

Result : Survival of dams: no deaths
Body weight of dams: no compound-related changes compared to control (only positive control treated mice showed decreased body weight gain)
Fetotoxicity: no dead fetuses in dosage groups (3 deaths in positive control)
Average fetus weight: no change in treated groups compared to controls
Abnormalities/malfunctions (no. of fetuses affected/no. of litters affected) (sham control, pos.control, 2.18, 10.15, 47.05, 218 mg/kg bw)
Skeletal findings:
sternbrae (incomplete oss.): 80/22, 94/18, 92/20, 93/22, 101/19, 92/19
sternbrae (missing): 14/6, 11/19, 11/8, 17/5, 11/4, 0/22

5. Toxicity

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skull (incomplete closure): 41/16, 114/19, 46/15, 63/16,
67/20, 49/17

Soft tissue abnormalities:

- pos. control: 7 pups with meningoencephalocele and spina bifida
- 10.15 mg/kg: 1 pup: petechiae, 1 pup: anophthalmia
- 47.05 mg/kg: 2 pups anophthalmia, 2 pups: gastroschisis
1 pup hydrocephalus

All other findings were completely in the range of spontaneous abnormalities found in negative controls.

Test condition

- : TEST ORGANISMS
No of animals/dose group: 25
No of pregnant animals (2.18, 10.15, 47.05, 218 mg/kg bw):
22, 23, 23, 22
ADMINISTRATION / EXPOSURE
- Vehicle: corn oil
- sacrifice: Day 20
NEGATIVE CONTROL: 25 pregnant
POSITIVE CONTROL: aspirin - 250 mg/kg (23 pregnant), no data on expected historical range
MATING PROCEDURES: Virgin adult were mated with young adult males (observation of the vaginal sperm plug was considered Day 0 of gestation)
PARAMETERS ASSESSED DURING STUDY P AND F1:
- Clinical observations : appearance (daily), food consumption (daily), body weight (day 0, 6, 11, 15, 20)
- Estrous cycle: no data
- Other: number of live and dead fetuses, body weights of live pups
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Organ weights P and F1: no
- Histopathology P and F1:
P: urogenital tract, number of implantation and resorption sites
F1: All fetuses were examined grossly, one-third of fetuses of each litter underwent detailed visceral examinations employing 10x magnification, two-third were examined for skeletal defects.

Reliability

- : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: no full macroscopic examination of dams; no data on statistical evaluation, not tested at maternally toxic doses.

Flag

25.02.2003

- : Critical study for SIDS endpoint

(72)

Species

- : mouse

Sex

- : female

Strain

- : CD-1

Route of admin.

- : gavage

Exposure period

- : gestation days 6-15

Frequency of treatm.

- : daily

Duration of test

- : 10 consecutive days

Doses

- : 1.85, 8.59, 39.9 or 185.0 mg/kg bw/d

Control group

- : other: sham treated with corn oil

other: NOEL Maternal

- : = 185 mg/kg bw

Toxicity**other: NOEL**

- : = 185 mg/kg bw

Teratogenicity**Result**

- : negative

Method

- : other

Year

- : 1973

GLP

- : no

5. Toxicity

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Test substance	: other TS: Menthol natural brazilian, FDA 71-57
Result	: Survival of dams: no deaths Body weight of dams: no compound-related changes compared to control (only positive control treated mice showed decreased body weight gain) Fetotoxicity: dead fetuses (sham control/pos. control/1.85/8.59/39.9/185.5 mg/kg bw): 3/0/0/5/1/3 Average fetus weight: no change in treated groups compared to controls (pos. control: fetus weight reduced) Abnormalities/malfunctions (no. of fetuses affected/no. of litters affected) (sham control, pos. control, 1.85, 8.59, 39.9, 185.5 mg/kg bw) Skeletal findings: sternebrae (incomplete oss.): 53/16, 101/21, 55/15, 69/17, 58/15, 50/17 extremities (incomplete oss.): 1/1, 12/5, 7/5, 8/4, 6/3, 4/2 miscellaneous (hyoid missing): 24/11, 60/13, 31/12, 43/15, 31/13, 45/15 Soft tissue abnormalities: no compound related changes All other findings were completely in the range of spontaneous abnormalities found in negative controls.
Test condition	: TEST ORGANISMS No of animals/dose group: 25 No of pregnant animals (1.85, 8.59, 39.9, 185.0 mg/kg bw): 19 (28 animals were mated), 22, 22 (2 animals were not mated), 22 ADMINISTRATION / EXPOSURE - Vehicle: corn oil - Total volume applied: 10 ml/kg bw - sacrifice: Day 17 NEGATIVE CONTROL: 23 pregnant POSITIVE CONTROL: aspirin - 150 mg/kg (23 pregnant), no data on expected historical range MATING PROCEDURES: Virgin adult were mated with young adult males (observation of the vaginal sperm plug was considered Day 0 of gestation) PARAMETERS ASSESSED DURING STUDY P AND F1: - Clinical observations: appearance (daily), food consumption (daily), body weight (day 0, 6, 11, 15, 17) - Estrous cycle: no data - Other: number of live and dead fetuses, body weights of live pups ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Organ weights P and F1: no - Histopathology P and F1: P: urogenital tract, number of implantation and resorption sites F1: All fetuses were examined grossly, one-third of fetuses of each litter underwent detailed visceral examinations employing 10x magnification, two-third were examined for skeletal defects.
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: no full macroscopic examination of dams; no data on statistical evaluation, not tested at maternally toxic doses.
Flag 25.02.2003	: Critical study for SIDS endpoint (72)
Species	: rabbit
Sex	: female
Strain	: no data

5. Toxicity

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Route of admin. : gavage
Exposure period : gestation days 6-18
Frequency of treatm. : daily
Duration of test : 13 consecutive days
Doses : 4.25, 19.75, 91.7 or 425.0 mg/kg bw/d
Control group : other: sham treated with corn oil
other: NOEL Maternal : = 425 mg/kg bw
Toxicity
other: NOEL : = 425 mg/kg bw
Teratogenicity
Result : negative
Method : other
Year : 1973
GLP : no
Test substance : other TS: Menthol natural brazilian, FDA 71-57

Result : Survival of dams:
 Died or aborted before day 29 (sham control/pos. control/4.25/19.75/91.7/425 mg/kg bw): 1, 1, 2, 3, 1, 4
 Body weight of dams: no compound-related changes compared to control
 Fetotoxicity:
 dead fetuses (sham control/pos. control/4.25/19.75/91.7/425 mg/kg bw): 0, 2, 2, 0, 0, 0
 Average fetus weight: weight of fetuses from treated groups (19.75, 91.7 and 425 mg/kg) is slightly higher compared to controls
 Abnormalities/malfunctions
 Skeletal findings and soft tissue abnormalities: All findings were completely in the range of spontaneous abnormalities found in negative controls.
 Pos. controls: scrambled vertebrae in 23, Scoliosis in 14, tail defects in 41 fetuses (in neg. controls and treated animals max. seen in 2 fetuses); enhanced numbers of anopia

Test condition : TEST ORGANISMS
 No of mated animals/dose group: 17/4.25 mg/kg bw, 19/19.75 mg/kg bw, 15/91.7 mg/kg bw, 19/425 mg/kg bw
 No of pregnant animals (4.25, 19.75, 91.7, 425.0 mg/kg bw): 13, 12, 11, 14
 ADMINISTRATION / EXPOSURE
 - Vehicle: corn oil
 - Time of death: Day 29
 NEGATIVE CONTROL: 16 mated, 12 pregnant animals
 POSITIVE CONTROL: 6-aminonicotinamide dosed on day 9 - 2.5 mg/kg (17 mated, 12 pregnant animals), no data on expected historical range
 MATING PROCEDURES: Day 0 each virgin adult - 0.4 ml human chorionic gonadotropin (injection via ear vein); three hours later - insemination with 0.3 ml of diluted semen with appr. 20 x 10E6 motile sperm (Vogin et al.: Pharmacologist 11, 282, 1969)
 PARAMETERS ASSESSED DURING STUDY P AND F1:
 - Clinical observations: appearance (daily), food consumption (daily), body weight (day 0, 6, 12, 18, 29)
 - Estrous cycle: no data
 - Other: number of live and dead fetuses, body weights of live pups
 ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
 - Organ weights P and F1: no
 - Histopathology P and F1:
 P: urogenital tract, numbers of corpora lutea, number of implantation and resorption sites
 F1: All fetuses- detailed gross examination, neonatal survival of live

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	fetuses of each litter was observed (incubator for 24 hrs), all surviving pups were sacrificed and all pups examined for visceral abnormalities (dissection); all fetuses were examined for skeletal defects.	
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: no full macroscopic examination of dams; no data on statistical evaluation, not tested at maternally toxic doses.
Flag 25.02.2003	:	Critical study for SIDS endpoint (72)
Species	:	Syrian hamster
Sex	:	female
Strain	:	no data
Route of admin.	:	gavage
Exposure period	:	gestation days 6-10
Frequency of treatm.	:	daily
Duration of test	:	5 consecutive days
Doses	:	4.05, 21.15, 98.2 or 405.0 mg/kg bw/d
Control group	:	other: sham treated with corn oil
other: NOEL Maternal	:	= 405 mg/kg bw
Toxicity	:	
other: NOEL	:	= 405 mg/kg bw
Teratogenicity	:	
Result	:	negative
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	other TS: Menthol natural brazilian, FDA 71-57
Result	:	Survival of dams: no deaths Died or aborted before day 14 (sham, pos. control, 4.05, 21.15, 98.2, 405.0 mg/kg bw) 0, 0, 2, 1, 1, 1, Body weight of dams: average body weights of all treated groups is increased compared to control Fetotoxicity: dead fetuses (sham, pos. control, 4.05, 21.15, 98.2, 405.0 mg/kg bw) 1, 18, 1, 1, 0, 0 Average fetus weight: no change in treated groups compared to controls Abnormalities/malfunctions (no. of fetuses affected/no. of litters affected)(sham, pos. control, 4.05, 21.15, 98.2, 405.0 mg/kg bw) - Skeletal findings: - sternebrae (incomplete oss.): 84/21, 79/21, 56/18, 112/21, 74/18, 62/18 - ribs (more than 13): 40/18, 62/18, 74/17, 56/16, 38/13, 44/15 - vertebrae (incomplete oss.): 1/1, 0, 0, 7/5, 5/5, 0 - extremities (incomplete oss.): 0, 0, 2/2, 5/3, 3/3, 1/1 - miscellaneous (hyoid reduced): 3/1, 1/1, 2/2, 8/6, 9/5, 2/2 Soft tissue abnormalities: - sham: 1 pup: cardiomegaly, apulmonism - pos. control: 1 pups: moderate hydrocephalus - 4.05 mg/kg bw: 1 pup: abdominal hernia, 1 pup: gastroschisis - 21.15 mg/kg bw: 2 pups: cardiomegaly, apulmonism, 2 pups atelocardia All other findings were completely in the range of spontaneous abnormalities found in negative controls. Positive control enhanced lethality of fetuses but did not induce teratogenic effects.
Test condition	:	TEST ORGANISMS No of animals/dose group: 25

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No of pregnant animals (4.05, 21.15, 98.2, 405.0 mg/kg bw):
22 (one animal was not mated), 23, 21, 21
ADMINISTRATION / EXPOSURE
- Vehicle: corn oil
- Time of death: Day 14
NEGATIVE CONTROL: 22 pregnant
POSITIVE CONTROL: aspirin - 250 mg/kg (22 pregnant), no data on expected historical range
MATING PROCEDURES: Virgin adult were mated (1:1) with mature males (appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation)
PARAMETERS ASSESSED DURING STUDY P AND F1:
- Clinical observations: appearance (daily), food consumption (daily), body weight (day 0, 8, 10, 14)
- Estrous cycle: no data
- Other: number of live and dead fetuses, body weights of live pups
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Organ weights P and F1: no
- Histopathology P and F1:
P: genital tract, number of implantation and resorption sites
F1: All fetuses were examined grossly, one-third of fetuses of each litter underwent detailed visceral examinations employing 10x magnification, two-third were examined for skeletal defects.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: no full macroscopic examination of dams; no data on statistical evaluation, not tested at maternally toxic doses.

Flag : Critical study for SIDS endpoint
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(72)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Type of experience : Human - Medical Data

Result : Concentration of menthol oil vapor in the production site ranged within limits of 0.005 to 0.378 mg/l. Reported complaints were: pain in the area around the liver and kidney. Signs of intoxication: Enlargement of the liver, protein in urine (50 % of subjects), dysfunction in the detoxification capacity of the liver (14 % of subjects).

Test condition : In order to determine the value of the maximum allowable concentration of vapors of menthol medical examinations (twice in a period of six months) of employers working in the essential oil manufacturing plant were carried out. 104 subjects were examined (30 % females). 25 subjects were aged between 36-46 years, 12 subjects were older than 56 years. Duration of employment was 3-12 years with an average value of 6 years. Environmental investigations were done as well: Method of determination of menthol vapor was based on the colour reaction with vaniline.

Reliability : (4) not assignable

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25.02.2003	Documentation insufficient for assessment. No detailed data on study population. Nodata on dose relation. The method of menthol concentration measurement is doubtful.	(61)
Type of experience	: Human	
Result	: Results: Self-desensitization for both chemicals Cross-desensitization of menthol by capsaicin Revealed cross-sensitization of capsaicin by menthol	
Test condition	: 15 persons (9f, 6m, aged 24-34 years) 3.5 ppm capsaicin and 0.30% l-menthol was given to the tongue tip.	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(73) (74)
Type of experience	: Direct observation, poisoning incidents	
Result	: Ingestion causes severe abdominal pain, nausea, vomiting, vertigo, ataxia, drowsiness, and coma.	
Test substance	: obtained principally from peppermint oil	
Reliability	: (4) not assignable Data from handbook or collection of data.	
25.02.2003		(75)

5.11 ADDITIONAL REMARKS

Type	: Biochemical or cellular interactions	
Result	: L-Menthol enhances transdermal drug penetration.	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(76) (77)
Type	: Excretion	
Result	: In the urine of sheep fed with l-menthol, the glucuronide and the ammonium salt of l-menthol was obtained in nearly quantitative recovery.	
Test condition	: The detoxification mechanism of sheep was examined.	
Reliability	: (4) not assignable Documentation insufficient for assessment.	
03.05.2002		(78)
Type	: Metabolism	
Result	: L-Menthol was not a potent in vitro inhibitor of MROD (CYP1A2) activity (IC ₅₀ : >300 µM) and of EROD (CYP1A1) activity (IC ₅₀ µM: >400 µM); it did inhibit PROD (CYP2B1) activity (IC ₅₀ : 10.6 µM), but was much less potent than beta-ionon which had an IC ₅₀ of 0.03 µM.	
Test condition	: The inhibitory effects of (-)-menthol on liver microsomal enzymes involved in biotransformation of xenobiotic substances were studied in vitro.	
Reliability	: (2) valid with restrictions non-standard in vitro test system	
18.01.2002		(79)
Type	: Metabolism	

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- Result** : (-)-/(+)-menthol glucuronidation ratio: 2.6/1
Vmax (-)/(+)-menthol glucuronidation ratio: 2.8/1
Data suggest, that monkey UGT2B9 and human UGT2B7 are functionally similar (89 % identity in cDNA library).
- Test condition** : Enantioselective glucuronidation for (+)- and (-)-menthol was studied using expressed monkey UGT2B9 (UDP-glucuronosyltransferase)
- Reliability** : (2) valid with restrictions
non-standard in vitro test system
- 22.01.2002 (80)
- Type** : Metabolism
- Result** : Addition of 1% l-menthol increased the total skin permeability coefficient (cm/h) to about 2.5 times. The metabolic index was decreased, compared to the controls.
- Test condition** : The effect of l-menthol as an enhancer on the simultaneous transport and metabolism of ehtyl nicotinate through excised hairless rat skin were measured.
- Reliability** : (2) valid with restrictions
non-standard in vitro test system
- 22.01.2002 (81)
- Type** : Metabolism
- Result** : After a daily dose of 750 mg l-menthol for a total of 8 days to two human volunteers 17-38% menthol was recovered as urinary menthyl glucuronide within 24 hours. Urine was first collected 3 days after dosage with l-menthol started.
- Reliability** : (2) valid with restrictions
Limited documentation
- Flag** : Critical study for SIDS endpoint
- 03.05.2002 (33)
- Type** : other: carcinogenicity inhibition
- Result** : Dietary additions of (-)-menthol resulted in a significant inhibition of mammary carcinogenesis.
- Test condition** : Rats administered 7,12-dimethyl benz[a]anthracene were given (-)-menthol in diet and the inhibition effect of mammary carcinogenesis was observed.
- Reliability** : (4) not assignable
Documentation insufficient
- 05.03.2003 (82)
- Type** : other: typical flavor
- Result** : Only l-menthol shows the peppermint typical flavor and odour, associated with the cooling effect.
- 22.05.2002 (83)
- Type** : other: ventilation depression
- Result** : The addition of l-menthol to a warm airflow depresses ventilation to a similar extent as compared to cold airflows.
Menthol influences the activity of the sensory and cold receptors of the larynx.
- Test condition** : The respiration rate of 8 anesthetized 7-14-days old dogs was investigated.
Constant flows of warm air (37 °C) with and without addition of l-menthol

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Reliability

and cold air (25 °C) were delivered through the upper airway.
: (2) valid with restrictions
limited documentation

05.03.2003

(84)

6. Analyt.Meth. for Detection and Identification

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6.1 ANALYTICAL METHODS**6.2 DETECTION AND IDENTIFICATION**

7. Eff. Against Target Org. and Intended Uses

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7.1 FUNCTION**7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED****7.3 ORGANISMS TO BE PROTECTED****7.4 USER****7.5 RESISTANCE**

8. Meas. Nec. to Prot. Man, Animals, Environment

Id

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8.1 METHODS HANDLING AND STORING**8.2 FIRE GUIDANCE****8.3 EMERGENCY MEASURES****8.4 POSSIB. OF RENDERING SUBST. HARMLESS****8.5 WASTE MANAGEMENT****8.6 SIDE-EFFECTS DETECTION****8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER****8.8 REACTIVITY TOWARDS CONTAINER MATERIAL**

9. References

Id	2216-51-5
Date	10.06.2003

- (1) Hopp, R., Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties, Recent Advances Tobacco Sci 19, 3 -46 (1993)
- (2) Haarmann & Reimer GmbH: Chemical Safety Data Sheet "Menthol L H&R Cryst", revision 17.4.2002
- (3) Haarmann & Reimer GmbH: Product Specification, Determination of the Melting Point, internal method
- (4) Hazardous Substances Data Bank, print from 09/05/2001
- (5) The Merck Index, 12th Edition, Ed. Budavari et al., Whitehouse Station, NJ (1996)
- (6) MITI (1992): Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Compiled under the Supervision of Chemical Products Safety Division, Basic Industries Bureau MITI, Ed. by CITI, October 1992
- (7) Jordan TE (1954) Vapor Pressure of Organic Compounds. Interscience Publishers, Inc., New York, pp. 72, 79, 82, 83
- (8) Perry et al., Perry's Chemical Engineers' Handbook, Sixth Edition, McGraw-Hill Book Company
- (9) Griffin S, Wyllie SG, Markham J (1999) Determination of octanol-water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography. J Chromatography A 864: 221- 228
- (10) Bayer AG 2002, Calculation of log Pow with SRC-KOWWIN v. 1.66 (2000)
- (11) Bayer AG, Internal Report, Determination of the Water Solubility, 15.05.1992
- (12) Hazardous Substances Data Bank, print from 09/05/2001, original literature: Yalkowsky SH, Dannenfelser RM; The AQUASOL dATABASE of Aqueous Solubility. Fifth Ed, Tucson, AZ: Univ Az, College of Pharmacy (1992)]
- (13) Calculation of OH Rate Constant with SRC-AOP v 1.90
- (14) Bayer AG (2003): Calculation of Mackay Distribution Level I
- (15) EC, Technical guidance document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 1488/94 on risk assessment for existing substances. European Chemicals Bureau, Ispra, Italy (1996)
- (16) TNO Chemistry (2003) Unpublished study V4106/01 Determination of the ready biodegradability of Menthol L Dist. in a Closed Bottle Test (OECD Guideline No. 301D, EU C.4-E)
- (17) Harder, J. & Probian, C.: Appl. Environ. Microbiol. 61, 2804-3808 (1995)
- (18) Miyazawa, M. et al., Journal of Chemical Technology and Biotechnology 77: 21-24 (online:2001)
- (19) Bayer AG: Biodegradation of menthol in the Modified OECD Screening Test according to OECD Guideline 301 E. Report No. 342 A/92 (1992)
- (20) Hylemon, P.B. et al., Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems, FEMS Microbiology Reviews 22, 475-488 (1999).

9. References

Id 2216-51-5
Date 10.06.2003

- (21) Geiger, D.L. et al.: Acute Toxicities of Organic Chemicals to Fathead Minnows (*Pimephales Promelas*), Vol. IV. Center for Lake Superior Environmental Studies, University of Wisconsin-Superior (1988)
- (22) Bayer AG: Toxicity of menthol on *Brachydanio rerio* in the acute fish test according to OECD Guideline 203. Report No. 370 A/92 (1992)
- (23) Bayer AG (2002) Menthol L H & R, Acute *Daphnia* Toxicity. Unpublished study 1242 A/02 D
- (24) Bayer AG (2002) Menthol L H & R, Alga, Growth Inhibition Test. Unpublished study 1242 A/02 AI
- (25) Bayer AG: Toxicity of menthol in the respiration inhibition test according to ISO 8192. Report No. 370 A/92 B (1992)
- (26) Flamini, G. et al.: *Phytother. Res.* 13(4), 349-351 (1999)
- (27) Lee, S. et al.: *J. Econ. Entomol.* 92(1), 56-67 (1999)
- (28) Montes-Belmont, R. & Carvajal, M.: *J. Food Prot.* 61(5), 616-619 (1998)
- (29) Madhava Madyastha, K. and Srivatsan, V. (1988), *Drug Metab. Dispos.* 16 (5), 765-772
- (30) Yamaguchi, T. et al. (1994), Metabolic Fate of [³H]-Menthol in the Rat, *Drug Metab. Dispos.* 22 (4), 616-624
- (31) Williams, R.T. (1938), The Conjugation of Isomeric 3-Menthanols with Glucuronic Acid and the Asymmetric Conjugation of dl-Menthol and dl-isoMenthol in the Rabbit, *Biochem. J.* 32, 1849-1855
- (32) Mascher, H. et al (2001), Pharmacokinetics of Menthol and Carvone after Administration of an Enteric Coated Formulation Containing Peppermint Oil and Caraway Oil, *Arzneimittelforschung* 51(6),
- (33) Eisenberg, F. et al (1955), Studies on Glucuronide Conjugation in Man, *Arch. Biochem. Biophys.* 59, 297-299.
- (34) Haarmann & Reimer GmbH (1974), short report, menthol - examination of acute oral toxicity, Bayer AG, Steinhoff, D., 17.05.1974
- (35) FAO/WHO, Menthol, WHO food additives series: 42: Safety evaluation of certain food additives, 57-76 (1999)
- (36) Herken, H. (1961): Pharmacological report about the tolerance of naturally (l-) and synthetically (d,l-) menthol (Original title: *Pharmakologisches Gutachten ueber die Vertraeglichkeit von natuerlichem (l-) und synthetischem (d,l-) Menthol*). Unpublished report from the Director of the pharmacological institute of "Freie Universitaet, Berlin-Dahlem", submitted to the World Health Organization by Schering, A.G.: cited in: WHO Food Additives Series No. 10, 64-69 (1976)
- (37) Food and Drug Administration (1975), Mutagenetic evaluation of compound FDA 71-57, menthol. US National Technical Information Service report No. PB-245444. Litton Bionetics, Inc. Submitted to WHO by International Federation of Chewing Gum Associations, Keller and Hechman LLP, Washington DC, United States, 14 January 1975
- (38) Wokes, F. (1932), The Antiseptic Value and Toxicity of Menthol Isomers, *Quarterly Journal of Pharmacy & Pharmacology* 5, 233-244

9. References

Id 2216-51-5
Date 10.06.2003

- (39) Food and Drug Administration (1975), Mutagenetic evaluation of compound FDA 71-57, menthol. US National Technical Information Service report No. PB-245444. Litton Bionetics, Inc. Submitted to WHO by International Federation of Chewing Gum Associations, Keller and Hechman LLP, Washington DC, United States, 14 January 1975
- (40) Flury, F. and Seel, H. (1926), Synthetisches Menthol, Muenchener Medizinische Wochenschrift 48, 2011-2012
- (41) Hazard, R. and Lechat, P. (1952), Toxicité comparée du menthol naturel et du menthol synthétique racémique, Ann. Pharm. Fr. 10, 481-487
- (42) Dmitrieva, N. et al. (1962), Farm. Zh. 17(3), 53-57
- (43) Macht, D. (1939), Comparative Pharmacology of Menthol and its Isomers, Arch. Int. Pharmacodyn. 63, 43-58
- (44) Haarmann & Reimer GmbH (1989), Assessment of the skin irritant effect of HR 89/620001 in rabbits, Scantox - biological laboratory ltd lab no. 11874, 16.08.1989
- (45) Haarmann & Reimer GmbH (1974), menthol- medical report ("Aerztliches Gutachten"), Prof. Hopf, 26.4.1974
- (46) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/620001 in rabbits, Scantox – biological laboratory ltd lab no. 11754, 02.05.1989
- (47) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/620001 in rabbits, Scantox – biological laboratory ltd lab no. 11870, 16.08.1989
- (48) Haarmann & Reimer GmbH (1991), Menthol L H&R, Buehler Sensitisation test in Guinea pigs, Cuthbert J.A., Jackson D., Inveresk Research International, IRI Project No. 550726, report no.: 6870, 24.02.1991
- (49) Haarmann & Reimer GmbH (1995), Menthol L H&R Local Lymph Node Assay, Lees, D., Leah, A.M., Zeneca Central Toxicology Laboratory, Report No.: CTL/E/160, 20.12.1995
- (50) Hostynek, J. and Magee, P. (1997), Fragrance Allergens: Classification and Ranking by QSAR, Toxicol. in Vitro 11, 377-384
- (51) Sharp, D.W. (1978), The Sensitization Potential of Some Perfume Ingredients Tested Using a Modified Draize Procedure, Toxicology 9, 261-271
- (52) Saito and Oka (1990), Allergic contact dermatitis due to peppermint oil, Skin Res. 32: 161-167, cited in Nair, B. (2001), Final Report on the Safety Assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water, Int. J. Toxicol. 20, 61-73
- (53) Kubo, Y. (1992), Two Cases of Allergic Contact Dermatitis Caused by Tiger Balm and Essential Balm, Skin Res. 34, 295-300 (suppl)
- (54) Haarmann & Reimer GmbH (1974c), Menthol - "Subacute toxicity of synthetic l-menthol", Dr. A. Komatsu, Takasago Perfumery Co.LTD, December 20, 1974
- (55) Thorup, I. et al. (1983a), Short Term Toxicity Study in Rats Dosed with Pulegone and Menthol, Toxicol. Lett. 19, 207-210
- (56) Rakieten, N. et al. (1954), Effects of Menthol Vapor on the Intact Animal with Special Reference to the Upper Respiratory Tract, J. Am. Pharm. Ass. 43, 390-392

9. References

Id 2216-51-5
Date 10.06.2003

- (57) Gaworski, C. et al. (1997), 13-Week Inhalation Toxicity Study of Menthol Cigarette Smoke, *Food Chem. Toxicol.* 35(7), 683-692
- (58) Austin, C.A. et al. (1988), The Effect of Terpenoid Compounds on Cytochrome P-450 Levels in Rat Liver, *Biochemical Pharmacology* 37, 2223-2229
- (59) Madyastha, K.M. and Srivatsan V. (1988), Studies on the Metabolism of l-Menthol in Rats, *Drug Metab. Dispos.* 16, 765-772, cited in *Bibra Toxicity Profile* (1990), The British Industrial Biological Research Association, Carshalton Surrey, 1-9
- (60) Herken, H (1961) : Pharmacological expertise on tolerance to natural and synthetic menthol. Unpublished report from Pharmakologischen Institut der Freien Universität, Berlin, Dahlem. Submitted to WHO by Schering AG, Berlin (in German). As cited in: FAO/WHO (1999) Menthol. In: WHO food additives series: 42: Safety evaluation of certain food additives. Geneva, World Health Organization 57 - 76
- (61) Kowalski, Z. et al. (1962), Investigations on Value of the Maximum Allowable Concentration of Natural Essential Oils in the Air, *Med. Pr.* 13(2), 69-84
- (62) Nohmi, T. et al. (1985), Mutagenicity Tests on Organic Chemical Contaminants in City Water and Related Compounds I. Bacterial Mutagenicity Tests, *Bull. Nat. Inst. Hyg. Sci.* 0(103), 60-64
- (63) Andersen, P.H. and Jensen, N.J. (1984), Mutagenic investigation of peppermint oil in the Salmonella/mammalian-microsome test, *Mutat. Res.* 138, 17-20
- (64) Gomes -Carneiro, M. (1998), Mutagenicity testing of (+-)-camphor, 1,8-cineole, citral, citronella, (-)-menthol and terpineol with the Salmonella/microsome assay, *Mut. Res.* 416, 129-136
- (65) Gomes -Carneiro, M. et al. (1997), Evaluation of mutagenic potential of monoterpenoid compounds, *Mutat. Res.* 379 suppl., S.111
- (66) Oda, Y. et al. (1978), Mutagenicity of food flavors in bacteria (1st report), *Shodunin Eisei Hen*, 9, 177-181, cited in: WHO Food additives series (1999), 42: Safety evaluation of certain food additives, 57-76
- (67) Yoo, Y. (1986), Mutagenic and Antimutagenic Activities of Flavoring Agents used in Foodstuffs, *J. Osaka City Med. Cent.* 34, 267-288
- (68) Murthy, P.B.K. (1991), Lack of Genotoxicity of Menthol in Chromosome Aberration and Sister Chromatid Exchange Assays Using Human Lymphocytes in Vitro, *Toxic. in vitro* 5(4), 337-340
- (69) Sofuni, T. et al. (1985), Mutagenicity Tests on Organic Chemical Contaminants in City Water and Related Compounds II. Chromosome Abberation Tests in Cultured Mammalian Cells, *Bull. Natl. Inst. Hyg. Sci. (Tokyo)* 103, 64-75
- (70) Matsuoka, A. et al. (1998) In vitro clastogenicity of 19 organic chemicals found in contaminated water and 7 structurally related chemicals, *Environ.Mutagen. Res.* 20, 159-165,
- (71) Haarmann & Reimer GmbH (1974c), Menthol - "Subacute toxicity
- (72) Food and Drug Administration (1973), Teratologic evaluation of compound FDA 71-57 (menthol natural, brazilian). US National Technical Information Service report No. PB-223815; Food and Drug Research Laboratories, Inc., New Jersey, June 1, 1973

9. References

Id 2216-51-5
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- (73) Cliff, M.A. and Green, B.G. (1996), Sensory Irritation and Coolness Produced by Menthol: Evidence for Selective Desensitization of Irritation, *Physiol. Behav.* 59(3), 487-494
- (74) Green, B. (1996), Regional and Individual Differences in Cutaneous Sensitivity to Chemical Irritants: Capsaicin and Menthol, *J. Toxicol.* 15(3), 277-295
- (75) Gleason, M. et al. (1969), *Clinical toxicology of commercial products: acute poisoning*; 3rd ed. The Williams&Wilkins Co., Baltimore
- (76) Kitagawa, S. (1998), Permeability of benzoic acid derivatives in excised guinea pig dorsal skin and effects of l-menthol, *Int.J.Pharm.* 161 (9), 115-122
- (77) Kitagawa, S. et al. (1997), Skin Permeation of Parabens in Excised Guinea Dorsal Skin, its Modification by Penetration Enhancers and their Relationship with n-Octanol/Water Partition Coefficients, *Chem. Pharm. Bull.* 45(8), 1354-1357,
- (78) Wright, S.E. (1945), Detoxication Mechanisms in the Sheep, *Univ. Queensl. Papers* 1(25), 1-10
- (79) De-Oliveira, A.C.A.X. et al. (1999), In vitro inhibition of liver monooxygenase by β -ionone, 1,8-cineole, (-)-menthol and terpineol, *Toxicology* 135(1), 33-41
- (80) Green, M. et al. (1997), Glucuronidation of Opioids, Carboxylic Acid-Containing Drugs, and Hydroxylated Xenobiotics Catalyzed by Expressed Monkey UDP-Glucuronosyltransferase 2B9 Protein, *Drug Metab. Dispos.* 25(12), 1389-1394
- (81) Hayashi, T. (1997), The effects of several penetration-enhancers on the simultaneous transport and metabolism of ethyl nicotinate in hairless rat skin, *Int.J.Pharm.* 154, 141-148
- (82) Russin, W. et al. (1989), Inhibition of rat mammary carcinogenesis by monoterpenoids, *Carcinogenesis* 10(11), 2161-2164
- (83) Hartke, K. and Mutschler, E. (eds.) (1986), *Deutsches Arzneibuch*, 9th issue; Vol 3, 2261-2266
- (84) Sant' Ambrogio, F.B. et al. (1992), Menthol in the upper airway depresses ventilation in newborn dogs, *Respir. Physiol.* 89(3), 299-307

10. Summary and Evaluation

Id
Date2216-51-5
10.06.2003**10.1 END POINT SUMMARY****10.2 HAZARD SUMMARY****10.3 RISK ASSESSMENT**

I U C L I D Data Set

Existing Chemical : ID: 1490-04-6
CAS No. : 1490-04-6
EINECS Name : Menthol
EC No. : 216-074-4
TSCA Name : Cyclohexanol, 5-methyl-2-(1-methylethyl)-
Molecular Formula : C₁₀H₂₀O

Producer related part

Company : Bayer AG
Creation date : 09.11.2001

Substance related part

Company : Bayer AG
Creation date : 09.11.2001

Status :
Memo : ICCA - Category Menthole

Printing date : 18.03.2003
Revision date :
Date of last update : 18.03.2003

Number of pages : 1

Chapter (profile) : Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile) : Reliability: without reliability, 1, 2, 3, 4
Flags (profile) : Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE),
Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information

Id	1490-04-6
Date	18.03.2003

1.0.1 APPLICANT AND COMPANY INFORMATION**1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR****1.0.3 IDENTITY OF RECIPIENTS****1.0.4 DETAILS ON CATEGORY/TEMPLATE****1.1.0 SUBSTANCE IDENTIFICATION****1.1.1 GENERAL SUBSTANCE INFORMATION**

Purity type	:	
Substance type	:	organic
Physical status	:	
Purity	:	>= 99 % w/w
Colour	:	colourless to white
Odour	:	minty
Remark	:	"Unspecified mixture of menthol isomers". The CAS-No. 1490-04-6 is correctly assigned to "5-Methyl-2-(1-methylethyl)cyclohexane" in the English EINECS. In the German EINECS it is incorrectly assigned to "DL-Menthol" (GESTIS). Thus the English EINECS does not exclude the neo-, iso- and neoisomenthols from the mixture!
Flag 07.08.2002	:	Critical study for SIDS endpoint (1)
Purity type	:	
Substance type	:	organic
Physical status	:	
Purity	:	
Colour	:	colourless
Odour	:	like peppermint smelling
Flag 14.03.2003	:	Critical study for SIDS endpoint (2)
Purity type	:	
Substance type	:	organic
Physical status	:	solid
Purity	:	
Colour	:	white
Odour	:	
Remark	:	White crystals (cf. 2.1 Melting Point)
Flag 14.03.2003	:	Critical study for SIDS endpoint (3)

1. General Information

Id	1490-04-6
Date	18.03.2003

1.1.2 SPECTRA**1.2 SYNONYMS AND TRADENAMES****5-METYL-2-(1-METHYLETHYL)-CYCLOHEXANOL**

Flag : Critical study for SIDS endpoint
29.05.2002

5-METYL-2-(1-METHYLETHYL)-CYCLOHEXANOL ISOMERS

Flag : Critical study for SIDS endpoint
03.06.2002

CYCLOHEXANOL, 5-METYL-2-(1-METHYLETHYL)-

Flag : Critical study for SIDS endpoint
29.05.2002

MENTHOL, UNSPECIFIED MIXTURE OF MENTHOL ISOMERS

Flag : Critical study for SIDS endpoint
29.05.2002

1.3 IMPURITIES**1.4 ADDITIVES****1.5 TOTAL QUANTITY****1.6.1 LABELLING**

Labelling : provisionally by manufacturer/importer
Specific limits :
Symbols : Xi, , ,
Nota : , ,
R-Phrases : (38) Irritating to skin
S-Phrases : (25) Avoid contact with eyes

Flag : Critical study for SIDS endpoint
29.05.2002

1.6.2 CLASSIFICATION

Classified : provisionally by manufacturer/importer
Class of danger : irritating
R-Phrases : (38) Irritating to skin
Specific limits :

1. General Information

Id 1490-04-6
Date 18.03.2003

Flag : Critical study for SIDS endpoint
29.05.2002

1.6.3 PACKAGING**1.7 USE PATTERN**

Type of use : type
Category : Use in closed system

Remark : used in chemical industry as an intermediate in synthesis
Flag : Critical study for SIDS endpoint
18.03.2003

Type of use : type
Category : Wide dispersive use

Remark : use in veterinary activities and pharmaceutical industry for production of pharmaceuticals
Flag : Critical study for SIDS endpoint
25.07.2002

1.7.1 DETAILED USE PATTERN**1.7.2 METHODS OF MANUFACTURE**

Origin of substance : Synthesis
Type : Production

Remark : Menthol is produced via reaction of m-cresol with propen to thymol, and hydrogenation of thymol, resulting in 4 isomers: D/L-neomenthol, D/L-neoisomenthol, D/L-menthol and D/L-isomenthol. D/L-menthol is isolated by fractional distillation. To produce L-menthol, D/L-menthol is transesterificated with methylbenzoate and further manufactured. Resulting products are L- and D-menthol. Plant materials from some Mentha and other species also contain various menthols in the essential oils. These oils may vary in composition.
03.06.2002 (4)

Origin of substance : Natural origin
Type : Plant extract

03.06.2002 (4)

Origin of substance : Synthesis
Type : Plant extract

Remark : Various synthetic methods start from plant extracts
30.07.2002 (4)

1. General Information

Id 1490-04-6
Date 18.03.2003

1.8 REGULATORY MEASURES**1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES****1.8.2 ACCEPTABLE RESIDUES LEVELS****1.8.3 WATER POLLUTION**

Classified by :
Labelled by :
Class of danger : 1 (weakly water polluting)

29.05.2002 (1)

1.8.4 MAJOR ACCIDENT HAZARDS

Legislation : Störfallverordnung (DE)
Substance listed : no
No. in Seveso directive :

29.05.2002 (1)

1.8.5 AIR POLLUTION**1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES**

Type : EINECS
Additional information :

29.05.2002

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS**1.9.2 COMPONENTS****1.10 SOURCE OF EXPOSURE****1.11 ADDITIONAL REMARKS**

1. General Information

Id	1490-04-6
Date	18.03.2003

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered : 5
Date of search : 01.09.2001

Remark : Human Health: last literature search September 2001: CAS number search in external and internal databases, e.g. Biosis, Embase, Toxline, Scisearch

Flag : Critical study for SIDS endpoint
10.07.2002

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 14.01.2002

Remark : Physico-chemical properties / Environment / Ecotoxicology :
last literature search January 2002: CAS number search in external and internal databases, e.g. HSDB, Aquire.

Flag : Critical study for SIDS endpoint
29.07.2002

1.13 REVIEWS

Memo : Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties

Flag : Critical study for SIDS endpoint
03.06.2002

(4)

2. Physico-Chemical Data

Id 1490-04-6
Date 18.03.2003

2.1 MELTING POINT

Value : 5 °C
Sublimation :
Method : other: no data
Year : 1996
GLP : no data
Test substance : no data

Reliability : (3) invalid
The melting point of 5 °C is in contrast to the reported "white crystals". The vapour pressure is reported twice (with small differences) in mm Hg and hPa. The solubility is reported to be "508,000 mg/l" (= 508 g/l) which is interpreted to be a typographical error. A wrong structural formula and a low boiling point are also reported in the reference.

11.03.2003

(3)

2.2 BOILING POINT

Value : ca. 215.5 °C at 1013 hPa
Decomposition :
Method :
Year : 2002
GLP : no data
Test substance : no data

Flag : Critical study for SIDS endpoint

18.03.2003

(2)

Value : 212 °C at
Decomposition :
Method : other: no data
Year :
GLP : no data
Test substance : no data

18.03.2003

(5)

Value : 175 °C at
Decomposition :
Method : other: no data
Year : 1996
GLP : no data
Test substance : no data

Reliability : (3) invalid
The boiling point reported by Verschueren is very low compared to other sources. This is in line with other information on this substance: Verschueren reports the solubility to be "508,000 mg/l" (= 508 g/l) which was interpreted to be a typographical error. Verschueren also reports a wrong structural formula. The melting point of 5 °C is in contrast to the reported "white crystals" (expected: colourless liquid when melting point is correct). The vapour pressure is reported twice (with small differences) in mm Hg and hPa.

11.03.2003

(3)

2. Physico-Chemical Data

Id 1490-04-6
Date 18.03.2003

2.3 DENSITY

Type : density
Value : .898 g/cm³ at 25 °C
Method :
Year : 2002
GLP : no data
Test substance : no data

Flag : Critical study for SIDS endpoint
18.03.2003

(2)

Type : density
Value : .89 at °C
Method :
Year : 1996
GLP : no data
Test substance : no data

18.03.2003

(5)

2.3.1 GRANULOMETRY**2.4 VAPOUR PRESSURE**

Value : .085 hPa at 25 °C
Decomposition :
Method :
Year : 1996
GLP : no data
Test substance : no data

Remark : Extrapolated to 25 °C. The vapour pressure is reported twice (with small differences) in mm Hg and hPa. The mm Hg data are identical with data of Jordan (1954) reported for L-menthol. These data were extrapolated to 25 °C.
The handbook also contains a wrong structural formula and a wrong solubility. Both were assumed to be typographical errors. The melting point of 5 °C is in contrast to the reported "white crystals". The boiling point is lower than reported by others.

Flag : Critical study for SIDS endpoint
14.03.2003

(3)

Value : 1.3 hPa at 55 °C
Decomposition :
Method :
Year : 1996
GLP : no data
Test substance : no data

Remark : The vapour pressure is reported twice (with small differences) in mm Hg and hPa. The mm Hg data are identical with data of Jordan (1954) reported for L-menthol.
The handbook also contains a wrong structural formula and a wrong solubility. Both were assumed to be typographical errors. The melting point

2. Physico-Chemical Data

Id 1490-04-6
Date 18.03.2003

14.03.2003	of 5 °C is in contrast to the reported "white crystals". The boiling point is lower than reported by others.	(3)
Value	: 13 hPa at 95 °C	
Decomposition	:	
Method	:	
Year	: 1996	
GLP	: no data	
Test substance	: no data	
Remark	: The vapour pressure is reported twice (with small differences) in mm Hg and hPa. The mm Hg data are identical with data of Jordan (1954) reported for L-menthol. The handbook also contains a wrong structural formula and a wrong solubility. Both were assumed to be typographical errors. The melting point of 5 °C is in contrast to the reported "white crystals". The boiling point is lower than reported by others.	(3)
14.03.2003		

2.5 PARTITION COEFFICIENT

Partition coefficient	: octanol-water	
Log pow	: 3.4 at °C	
pH value	:	
Method	: other (calculated)	
Year	: 1999	
GLP	: no data	
Test substance	: other TS: L-menthol and D/L-menthol	
Method	: The log kow was determined for L-menthol and D/L-menthol by reversed-phase high-performance liquid chromatography. The log kow was 3.40 for both L-menthol and D/L-menthol and is thus valid for any mixture of D-menthol and L-menthol	
Flag	: Critical study for SIDS endpoint	
14.03.2003		(6)
Partition coefficient	: octanol-water	
Log pow	: 3.38 at °C	
pH value	:	
Method	: other (calculated): SRC-KOWWIN v. 1.66	
Year	: 2002	
GLP	:	
Test substance	:	
Flag	: Critical study for SIDS endpoint	
14.03.2003		(7)
Partition coefficient	: octanol-water	
Log pow	: 3.25 at °C	
pH value	:	
Method	: other (calculated)	
Year	: 1996	
GLP	:	
Test substance	:	
Remark	: The vapour pressure is reported twice (with small differences) in mm Hg and	

2. Physico-Chemical Data

Id 1490-04-6
Date 18.03.2003

hPa. The mm Hg data are identical with data of Jordan (1954) reported for L-menthol.

The handbook also contains a wrong structural formula and a wrong solubility. Both were assumed to be typographical errors. The melting point of 5 °C is in contrast to the reported "white crystals". The boiling point is lower than reported by others.

18.03.2003

(3)

Partition coefficient : octanol-water
Log pow : 3.3 at °C
pH value :
Method :
Year : 2002
GLP : no data
Test substance : no data

18.03.2003

(8)

Partition coefficient : octanol-water
Log pow : 3.23 at °C
pH value :
Method : other (calculated)
Year : 1991
GLP :
Test substance :

18.03.2003

(9)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 420 mg/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: stalogrametric method
Year : 1922
GLP : no
Test substance : no data

Remark : Seidell refers to a report of Rhode (1922) and briefly describes method. Measuring temperature is room temperature. Wakita K, Yoshimoto M, Miyamoto S, Watanabe H (1986) [Chem Pharm Bull 34: 4663 - 4681] cite Yalkowsky and Morzowich [(1980) in Drug Design Vol 9, ed. by Ariens EJ, Academic Press New York, p. 121] as log 1/S (S = solubility, mol/l) = 2.57, which equals to 420 mg/l.

Flag : Critical study for SIDS endpoint

14.03.2003

(10)

Solubility in : Water
Value : 431 mg/l at 20 °C
pH value :

2. Physico-Chemical Data

Id 1490-04-6
Date 18.03.2003

concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 2002
GLP : no data
Test substance : no data

18.03.2003

(2)

Solubility in : Water
Value : 456 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 2002
GLP :
Test substance :

18.03.2003

(8)

Solubility in : Water
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

Remark : "slightly soluble"
25.01.2002

(5)

Solubility in : Water
Value : 508 mg/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

The handbook reports the solubility to be "508,000 mg/l" (= 508 g/l) which was interpreted to be a typographical error.
The handbook also contains a wrong structural formula and reports low melting and boiling points. The melting point of 5 °C is in contrast to the reported "white crystals" (expected: colourless liquid when melting point is

2. Physico-Chemical Data

Id 1490-04-6
Date 18.03.2003

10.03.2003	correct). The vapour pressure is reported twice (with small differences) in mm Hg and hPa.	(3)
Solubility in	: Water	
Value	: 593 mg/l at °C	
pH value	:	
concentration	: at °C	
Temperature effects	:	
Examine different pol.	:	
pKa	: at 25 °C	
Description	:	
Stable	:	
Deg. product	:	
Method	: other: calculated with fragment solubility constants	
Year	: 1986	
GLP	:	
Test substance	: other TS: not indicated which menthol was used for calculation, but method is applicable for all menthol isomers	
Remark	: Wakita et al. (1986) cite Yalkowsky and Morzowich [(1980) in Drug Design Vol 9, ed. by Ariens EJ, Academic Press New York, p. 121] as log 1/S (S = solubility, mol/l) = 2.57, which equals to 420 mg/l. Their calculated solubility is log 1/S (S = solubility, mol/l) = 2.42, which equals to 593 mg/l.	
14.03.2003		(11)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value	: 100 °C
Type	: closed cup
Method	:
Year	: 2002
GLP	: no data
Test substance	: no data

18.03.2003 (2)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2. Physico-Chemical Data

Id	1490-04-6
Date	18.03.2003

2.13 VISCOSITY**2.14 ADDITIONAL REMARKS**

Memo : Threshold odour concentration

Result : Threshold odour concentration, detection 0.9 mg/m³
Threshold odour concentration, recognition 2.1 mg/m³

11.03.2003

(3)

3. Environmental Fate and Pathways

Id 1490-04-6
Date 18.03.2003

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight

Method : structure estimation method
Result : Rate constant: $k = 2.4 \times 10^{-11}$ cm³/molecule/sec at 25 degrees C;
considering an atmospheric OH-radical concentration of 5×10^5 OH-radicals/cm³, the half-life is about 16 h

Reliability : (2) valid with restrictions
accepted calculation procedure

Flag : Critical study for SIDS endpoint
29.07.2002

(12)

3.1.2 STABILITY IN WATER

Deg. product :
Method : other: calculated
Year : 2001
GLP :
Test substance :

Result : volatilization half-lives for a model river (1 m deep, flow-rate 1 m/sec, wind velocity 3 m/sec) and a model lake (1 m deep, flow-rate 0.05 m/sec, wind velocity 0.5 m/sec) are estimated to be 2 and 18 days

Reliability : (2) valid with restrictions
accepted calculation procedure derived from L-Menthol cause of structural similarities

Flag : Critical study for SIDS endpoint
18.03.2003

(13)

3.1.3 STABILITY IN SOIL**3.2.1 MONITORING DATA**

Type of measurement : background concentration
Media : surface water
Concentration :
Method : GC/MS

Remark : Measurements in the river Neckar (Germany) at various seasons gave menthol concentrations between 0.0093 and 0.139 µg/l.

Reliability : (2) valid with restrictions
Acceptable procedure and publication.

26.07.2002

(14)

Result : In an investigation of eight small rivers and brooks which flow into Lake Constance (SW Germany) menthol was detected qualitatively.

Reliability : (2) valid with restrictions
Acceptable procedure and publication.

3. Environmental Fate and Pathways

Id 1490-04-6
Date 18.03.2003

30.07.2002

(15)

3.2.2 FIELD STUDIES**3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS**

Type : volatility
Media : water - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: estimation method
Year : 2003

Result : Based on a water solubility of 420 mg/l and a vapour pressure of 8.5 Pa (see chapter 2), the Henry's law constant is calculated to be 3.16 Pa x m³/mol

Reliability : (2) valid with restrictions
 Generally accepted calculation method

Flag : Critical study for SIDS endpoint

14.03.2003

(16)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 2003

Result : Distribution
 air: 44.2 %
 water: 40.4 %
 soil: 8.0 %
 sediment: 7.3 %
 biota: 0.005 %

Test condition : Base data for calculation:
 temperature: 20 °C
 molar mass: 156.27 g/mol
 vapour pressure: 8.5 Pa
 water solubility: 420 g/m³
 log Kow: 3.4
 environmental compartments:
 - air: 6*10⁹ m³, 1.2 kg/m³
 - water: 7*10⁶ m³, 1000 kg/m³
 - soil: 4.5 *10⁴ m³, 1500 kg/m³, 2 % org. C
 - sediment: 2.1*10⁴ m³, 1300 kg/m², 5 % org. C
 - susp. sediment: 35 m³, 1500 kg/m³, 16.7 % org. C
 - aerosol: 0.12 m³, 1500 kg/m³
 - aquatic biota: 7 m³, 1000 kg/m³, 5 % fat

Reliability : (2) valid with restrictions
 Generally accepted calculation method

Flag : Critical study for SIDS endpoint

14.03.2003

(16)

3. Environmental Fate and Pathways

Id 1490-04-6
Date 18.03.2003

Media : water - soil
Method : other (calculation)
Year :
Result : Using the equation $\log K_{oc} = 0.52 \log K_{ow} + 1.02$ and based on a $\log K_{ow}$ of 3.4 a K_{oc} value of 614 can be calculated for the distribution between the organic phase of soil and pore water
Reliability : (2) valid with restrictions
 Generally accepted calculation method
Flag : Critical study for SIDS endpoint
 11.03.2003

(17)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, adapted
Concentration : 200 mg/l related to COD (Chemical Oxygen Demand) related to
Contact time :
Degradation : 95.1 (±) % after 5 day(s)
Result : inherently biodegradable
Deg. product :
Method : other: batch system (comparable to Zahn-Wellens Test)
Year : 1976
GLP : no
Test substance : other TS: menthol, not further specified
Method : Test compound as sole source of carbon; inoculum density: 100 mg dry matter/l; gradual increase of TS concentration during 20 days adaptation period; losses due to volatilization were monitored
Result : Pitter reported the biodegradation rate (for menthol 17.7 mg COD/g/h) and the total removal after 5 days (for menthol 95.1 %).
 The total removal is due to biodegradation and other removal mechanisms e.g. volatilization. When testing volatile substances he incubated blanks (test assay without inoculum) and measured removals after short periods to distinguish between volatilization and biodegradation, and to derive a kinetic for biodegradation. Using this normalized biodegradation rate (in mg COD/g/h) he considered substances with a biodegradation rate larger than 15 mg COD/g/h "to be biologically readily decomposable".
Test condition : 20 +/- 3 °C; pH 7.2; mineral salts medium; dark; continuously stirred
Reliability : (2) valid with restrictions
 Basic data given
Flag : Critical study for SIDS endpoint
 12.03.2003

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Contact time :
Degradation : (±) % after
Result : other: Readily degradable
Deg. product :
Method :
Year : 1985
GLP : no data
Test substance : no data

3. Environmental Fate and Pathways

Id	1490-04-6
Date	18.03.2003

Method : Method not described but reported to be recommended by the Department of Environment, Standing Committee of Analysts (1981) and by King (1981)

Reliability : (4) not assignable
Documentation insufficient for assessment. Missing details.

11.03.2003

(19)

3.6 BOD5, COD OR BOD5/COD RATIO**3.7 BIOACCUMULATION****3.8 ADDITIONAL REMARKS**

4. Ecotoxicity

Id 1490-04-6
Date 18.03.2003

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : other: QSAR estimation
Species : Pimephales promelas (Fish, fresh water)
Exposure period :
Unit : mg/l
LC50 : 19
Method :
Year : 1999
GLP :
Test substance :

Method : QSAR estimation for non-polar narcotics
Reliability : (3) invalid
QSAR not usable for risk assessment

18.03.2003

(20)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : other: Hydractinia echinata
Exposure period : 3 hour(s)
Unit : mg/l
EC50 : 78.3
Limit Test : no
Analytical monitoring : no data
Method : other: as summarised
Year : 2000
GLP : no
Test substance : other TS: 1-Isopropyl -5-methylcyclohexanol CAS Nr. 1490-04-6

Remark : Missing information, namely, details of the method, whether or not an analytical monitoring was performed and tested concentrations.
Result : Result is given as $\log (1/EC50)=3.3$ where EC50 is the concentration in mol/l at which the frequency of induction (methamorphosis) was reduced by 50 % with respect to a control.
Test condition : The culture medium was artificial seawater (980 mosmol, pH 8.2, 18 degree C).
Observed was the percentage of animals that underwent methamorphosis (development into polyps).
For 3 hours the larvae were exposed to seawater containing 20 mM CsCl and simultaneously the test substance. On the day after the effect (metamorphosis) was measured.
Experiments were performed in triplicate for each concentration and were repeated at least twice.
Reliability : (2) valid with restrictions

18.03.2003

(21)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE**4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA**

Type : other: potato-dextrose-agar

4. Ecotoxicity

Id 1490-04-6
Date 18.03.2003

Species : other fungi: Colletotrichum gloeosporioides
Exposure period :
Unit : mg/l
EC50 : 452
Analytical monitoring : no data
Method : other: as described
Year : 1998
GLP : no data
Test substance : other TS: Several monoterpenoids, including menthol

Remark : Assessment of the effect under real environmental conditions not possible
Result : EC50 for the mycelial growth inhibition of C. gloeosporioides on potato-dextrose-agar medium was determined by the poisoned food technique. The percentages of inhibition observed at the tested concentrations (250, 400, 500, 600 and 700 mg/l) were converted to probits and EC50 was computed from a linear relationship between logarithms of concentrations and probits.

Test condition : The oils of peppermint were obtained by hydrodistillation of the leaves. Appropriate quantity of test substance was dissolved in 0.25 ml acetone and were added to 30 ml of sterilized medium to get the medium of required concentration, the same amount of acetone being added in the control also. Mycelial discs of C. gloeosporioides were transferred aseptically to the Petri dishes and these were incubated at 27 +/- 2 degrees C for 5 days.

Reliability : (2) valid with restrictions
 Unsuitable test system namely the tested medium, not relevant for environmental hazard assessment

07.08.2002

(22)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

Species : other: Apis mellifera (honey bee)
Endpoint : other: colony population, growth, foraging activity, honey production
Exposure period :
Unit :
Method :
Year : 1999
GLP : no
Test substance : no data

4. Ecotoxicity

Id 1490-04-6
Date 18.03.2003

Method : Corrugated cardboard dipped in a menthol - vegetable oil mixture placed in bee colony. Board replaced after 8 days.
Result : Brood survival, adult population, foraging activity, and honey production did not differ from control.
Brood area was lower than in control colonies.
Reliability : (3) invalid
No conclusion from environmental concentrations to effects possible, not relevant for environmental hazard assessment

07.08.2002

(23)

Species : other: Apis mellifera
Endpoint : mortality
Exposure period : 48 hour(s)
Unit : other: microg/Bee
LC6 : 100
Method : other: as summarised
Year : 1999
GLP : no data
Test substance : other TS: no purity given

Method : Bees were fed with liquid sandwiches with syrup drawn into micropipette, followed by menthol in ethanol followed again by syrup.

Remark : No mortality in honeybees attributable to menthol.

Reliability : (2) valid with restrictions
No conclusion from environmental concentrations to effects possible. The same effect was observed in the control sample. Not relevant for environmental hazard assessment.

07.08.2002

(24)

4.7 BIOLOGICAL EFFECTS MONITORING**4.8 BIOTRANSFORMATION AND KINETICS****4.9 ADDITIONAL REMARKS**

5. Toxicity

Id

1490-04-6

Date

18.03.2003

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo	:	In vivo
Type	:	Distribution
Species	:	rat
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	470 mg/kg bw
Females	:	
Vehicle	:	other: olive oil
Route of administration	:	gavage
Exposure time	:	
Product type guidance	:	
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	1 st ; 2 nd ; 3 rd ;
Toxic behaviour	:	
Deg. product	:	
Method	:	
Year	:	1982
GLP	:	no
Test substance	:	other TS: [3-3H]-Menthol, analyt
Result	:	Tissue or fluid distribution of menthol after 17 hours (in % of administered radioactivity): Urine: 52 % Feces: 4.5 % Ileum: 3.5 % Liver: 0.8 % Fat: 2.1 % Kidney: 0.2 % Brain and Testes: < 0.1 %- Serum: 0.31 % After 17 hours after treatment HMG-CoA reductase activity was inhibited by up to 70%. The transient nature of this effect (no inhibition 41 h after dosing) was compatible with the rapid metabolism and excretion of menthol.
Test condition	:	3.0 mmol/kg (470 mg/kg bw) menthol were administered to 3 male rats (strain: Wistar) in a single dose. Controls were given olive oil alone. After 17 hours the animals were killed. Urine and faeces were collected over the 17 h period. After killing, samples of a variety of tissues were removed, digested with tissue solubilizer, and counted for radioactivity. Body fluids were counted directly without prior solubilization. Liver 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (HMG-CoA) inhibition was measured.
Reliability	:	(2) valid with restrictions menthol isomer not specified, short sampling period
Flag	:	Critical study for SIDS endpoint
24.02.2003		
In Vitro/in vivo	:	In vivo
Type	:	Excretion
Species	:	human

(25)

5. Toxicity

Id 1490-04-6
Date 18.03.2003

Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Method	:	other
Year	:	1972
GLP	:	
Test substance	:	
Result	:	Individual data is shown for only one volunteer. In this person 77.5 % of the dose could be recovered in the urine within 11 hours. The excretion of menthol after dermal application was slower than after oral administration. No data on the applied amount of menthol are given. Menthol was found in the urine of an untreated person, who was living in the same room as a patient rubbed with a menthol-containing unguent. It is concluded that a great amount of menthol (among other etheral oils) even after dermal application is inhaled.
Test condition	:	10-20 mg menthol was administered orally to humans (number not specified) and urine samples were collected after 3, 6, 9, 12, 24 and 36 hours. Additionally urine-samples of persons were analyzed after dermal application of a menthol containing unguent.
Reliability	:	(2) valid with restrictions limited documentation
Flag 24.02.2003	:	Critical study for SIDS endpoint
In Vitro/in vivo	:	In vivo
Type	:	Excretion
Species	:	other: human and dog
Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Method	:	
Year	:	1928
GLP	:	
Test substance	:	
Result	:	Human: 79 % of a 1 g oral dose of menthol is excreted in the urine via glucuronide conjugate within 6 h after administration. Number of persons not given Dog: 5 % of a 5 g oral dose of menthol is excreted in the urine via glucuronide conjugate. Sampling time is not given.
Reliability	:	(2) valid with restrictions limited documentation
Flag	:	Critical study for SIDS endpoint

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5. Toxicity

Id 1490-04-6
Date 18.03.2003

24.02.2003 (27)

In Vitro/in vivo : In vivo
Type : Excretion
Species : human
Number of animals
 Males :
 Females :
Doses
 Males :
 Females :
Vehicle :

Result : 35-40 % of menthol was recovered in the urine in 24 hours.
Test condition : Pharmacokinetic studies were performed in six volunteers (four m, two f, 17-37 years). Peppermint oil was ingested in two soft gelatine capsules. Each capsule contained 91-97 mg menthol (total dose: 180-190 mg). Urine was collected for 24 hours (2 hours aliquots up to 14 h).
Reliability : (2) valid with restrictions
 limited documentation
Flag : Critical study for SIDS endpoint

24.02.2003 (28)

In Vitro/in vivo : In vivo
Type : Excretion
Species : Human
Number of animals
 Males :
 Females :
Doses
 Males :
 Females :
Vehicle :
Method :
Year : 1990
GLP :
Test substance : other TS: commercial grade

Result : Human investigation with 4 male volunteers: An average of 40 % of menthol was recovered in urine after ingestion of 180 mg of peppermint oil (= 72 mg menthol) in an enteric-coated capsule following a 16 h fast. Total urine output was collected every 2 hours for up to 14 hours after dosing.
Reliability : (2) valid with restrictions
 limited documentation; isomer not specified
Flag : Critical study for SIDS endpoint

24.02.2003 (29)

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Human
Number of animals
 Males :
 Females :
Doses
 Males :
 Females :
Vehicle :
Method :
Year : 1967

5. Toxicity

Id 1490-04-6
Date 18.03.2003

GLP	:	
Test substance	:	other TS: unspecified isomer
Result	:	Between 40.1 % and 98.7 % of the oral dose of menthol was excreted as glucuronide in urine in 24 hours. Most individuals (11/19) excreted between 70 and 89% of the administered dose.
Test condition	:	Within the first few hours after the intake of menthol (dose was approx. 20 mg menthol/kg bw) a mild abdominal discomfort was nearly always felt, sometimes with nausea. In 19 healthy men, aged between 19 and 24 years, urine was collected up to 24 hours after ingestion of a 1.59 g oral dose of menthol, administered as an oil-in-water emulsion.
Reliability	:	(2) valid with restrictions limited documentation; isomer not specified
Flag 24.02.2003	:	Critical study for SIDS endpoint
In Vitro/in vivo	:	
Type	:	
Species	:	other: further data see chapter 5.11
Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Reliability	:	(2) valid with restrictions
Flag 04.03.2003	:	Critical study for SIDS endpoint

(30)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 8100 mg/kg bw
Species	:	mouse
Strain	:	NMRI
Sex	:	male
Number of animals	:	2
Vehicle	:	physiol. saline
Doses	:	4.64, 6.81, 8.25, 10.00, 12.10 ml/kg
Method	:	other: orientating study
Year	:	1980
GLP	:	no data
Test substance	:	other TS: menthol liquid
Remark	:	LD50 value is calculated, given value is LD 50: ca. 9 ml/kg bw., estimated density factor: 0.9 g/ml
Reliability	:	(2) valid with restrictions With regard to the screening purposes of this study the restrictions e.g. number of animals/per dose do not affect the suitability for assessment in principle.
Flag 21.08.2002	:	Critical study for SIDS endpoint

(31)

5. Toxicity

Id 1490-04-6
Date 18.03.2003

Type : LD50
Value : = 3180 mg/kg bw
Species : rat
Strain : Osborne-Mendel
Sex : no data
Number of animals :
Vehicle : other: corn oil
Doses : no data
Method : other
Year : 1964
GLP : no
Test substance : other TS: not specified isomer

Result : MORTALITY:
- Time of death: 4 hrs to 3 days after application
CLINICAL SIGNS: ataxia, scrawny appearance
Test condition : - Post dose observation period: 14 days
EXAMINATIONS:
time of deaths, clinical signs
Reliability : (4) not assignable
Documentation insufficient.

17.07.2002

(32)

Type : other: LD
Value : = 2000 mg/kg bw
Species : rabbit
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other
Year : 1883
GLP : no
Test substance : other TS: not specified isomer

Remark : Weight of rabbit is supposed to be 2.0 kg ("4 g of menthol were lethal to a rabbit")
Reliability : (4) not assignable
Documentation insufficient.

24.02.2003

(33)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : other: LD
Value : = 34500 mg/kg bw
Species : mouse
Strain : no data
Sex : no data
Number of animals : 1
Vehicle : other: no
Doses :
Method : other
Year : 1939

5. Toxicity

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GLP : no
Test substance : other TS: pure menthol liquid (density 0.897)

Result : The mouse showed depression 15 min after administration, unconsciousness after 20 min and died 105 min after administration.

Test condition : one mouse of 26 g bw
1 ccm of the TS was applied to skin of back and abdomen.

Reliability : (2) valid with restrictions
With regard to the screening purposes of this study the restrictions (e.g. number of animals) do not affect the suitability for assessment in principle.

Flag : Critical study for SIDS endpoint
24.02.2003 (34)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : = 700 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: 50/50 v/v
Doses : no data
Route of admin. : i.p.
Exposure time : unspecified
Method : other
Year : 1982
GLP : no data
Test substance : other TS: not further specified

Reliability : (4) not assignable
Insufficient documentation
24.02.2003 (35)

Type : other: LD
Value : = 1500 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: olive oil
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1939
GLP : no
Test substance : other TS: menthol liquid

Reliability : (4) not assignable
Documentation insufficient for assessment
24.02.2003 (34)

Type : other: LD
Value : = 1800 mg/kg bw
Species : mouse
Strain : no data

5. Toxicity

Id 1490-04-6
Date 18.03.2003

Sex : no data
Number of animals :
Vehicle : other: olive oil
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1939
GLP : no
Test substance : other TS: menthol liquid

Reliability : (4) not assignable
Documentation insufficient for assessment

24.02.2003

(34)

Type : other: LD
Value : = 4000 mg/kg bw
Species : guinea pig
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: no
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1939
GLP : no
Test substance : other TS: menthol liquid

Reliability : (4) not assignable
Documentation insufficient for assessment

24.02.2003

(34)

Type : LDLo
Value : = 2600 mg/kg bw
Species : rabbit
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: olive oil
Doses :
Route of admin. : s.c.
Exposure time :
Method : other
Year : 1922
GLP : no
Test substance : other TS: not specified isomer

Result : Immediate cause of death was paralysis of the respiratory center.
Reliability : (4) not assignable
Documentation insufficient for assessment

24.02.2003

(36)

Type : LD50
Value : = 10000 mg/kg bw
Species : rat
Strain : no data
Sex : no data

5. Toxicity		Id	1490-04-6
		Date	18.03.2003
Number of animals	:		
Vehicle	:	no data	
Doses	:		
Route of admin.	:	i.m.	
Exposure time	:		
Method	:	other	
Year	:	1954	
GLP	:	no	
Test substance	:	other TS: not specified isomer	
Reliability	:	(4) not assignable Documentation insufficient for assessment	
24.02.2003			(37)
Type	:	other: LD	
Value	:	= 37 mg/kg bw	
Species	:	cat	
Strain	:	no data	
Sex	:	no data	
Number of animals	:		
Vehicle	:	other: alcohol with physiological saline	
Doses	:		
Route of admin.	:	i.v.	
Exposure time	:		
Method	:	other	
Year	:	1939	
GLP	:	no	
Test substance	:	other TS: menthol liquid	
Test condition	:	Solution or suspensions, 1:1000, were prepared by diluting 2 per cent solutions of menthol in alcohol with physiological saline and were injected at one-minute intervals into the femoral vein while blood pressure was recorded from the carotid artery.	
Reliability	:	(4) not assignable Documentation insufficient for assessment.	
24.02.2003			(34)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	
Exposure time	:	4 hour(s)
Number of animals	:	4
Vehicle	:	other: 100% was applied pure, other concentrations in diethylphthalate (DEP)
PDII	:	
Result	:	moderately irritating
Classification	:	
Method	:	OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year	:	1989
GLP	:	yes
Test substance	:	other TS: menthol fl, HR 89/620006, purity: no data
Result	:	AVERAGE SCORE 100%/50%/25%/5%/1%/Vehicle 2.8/1.8/0.8/0.0/0.0/0.0 (erythema)

5. Toxicity

Id 1490-04-6
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2.4/1.0/0.0/0.0/0.0/0.0 (oedema)
REVERSIBILITY: yes
Day 7: 100%: 4/4 - treated sites were covered with a massive layer of white scales
50%: 4/4 - thin layer of white scales
25%: 2/4 - very thin layer of white scales
Day 14: 100%: 4/4 - treated sites were covered with white to white-brown scales, underlying skin was intact
50%: 3/4 - treated sites showed scattered scale formation on intact skin.

Test condition : TEST ANIMALS:
- Strain: Chbb:HM (C.H.Boehringer/Biberach)
- Sex: female
- Source: Dr. Karl Thomae GmbH, Biberach an der Riss
- Age: no data
- Weight at study initiation: 2400-2700 g
- Number of animals: 4
- Controls: internal control (one part of skin)
ADMINISTRATION/EXPOSURE
- Area of exposure: six different fields on back (two anterior, two centrally located and two posterior treatment sites)
- Total volume applied: 0.5 ml
- Postexposure period: up to 14 days
- Removal of test substance: skin was washed with luke warm water and soap

Reliability : (2) valid with restrictions
purity of TS not stated

Flag : Critical study for SIDS endpoint

24.02.2003

(38)

Species : guinea pig
Concentration : undiluted
Exposure : Open
Exposure time : 7 day(s)
Number of animals : 12
Vehicle : other: no
PDII :
Result : not irritating
Classification : not irritating
Method : other
Year : 1980
GLP : no data
Test substance : other TS: menthol fluid, 620006

Test condition : Guinea pig (6 female, 6 male), Pirlbright white, weight: 400-700 g
The test substance was rubbed into the animals' skin (flank) for ca. 30 sec./d, the exposure (open) being repeated once daily on 7 days. Results were taken immediately

Reliability : (3) invalid
Significant methodological deficiencies. e.g. no standardized amount of applied substance.

17.12.2001

(39)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : .1 ml

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Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 4
Vehicle : none
Result : slightly irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol fl, HR 89/620006, purity: no data

Result : AVERAGE SCORE
 - Cornea: 1.0
 - Iris: 0.0
 - Conjunctivae (Redness): 2.2
 - Conjunctivae (Chemosis): 0.7
 REVERSIBILITY: yes, slight reactions of cornea and conjunctiva were seen in one rabbit on day 7, no reactions were seen on day 14.

Test condition : TEST ANIMALS:
 - Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya)
 - Sex: female
 - Source: Dr. Karl Thomae GmbH, Biberach an der Riss
 - Age: no data
 - Weight at study initiation: 2300-2900 g
 - Number of animals: 4
 - Controls: internal control (right eye)

Reliability : (2) valid with restrictions
 purity of TS not stated

Flag : Critical study for SIDS endpoint
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(40)

Species : rabbit
Concentration : 71 %
Dose : .1 ml
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 4
Vehicle : other: diethylphthalate (DEP)
Result : slightly irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol fl, HR 89/620006 DEP, purity: no data

Result : AVERAGE SCORE
 - Cornea: 1.0
 - Iris: 0.0
 - Conjunctivae (Redness): 2.2
 - Conjunctivae (Chemosis): 0.7
 REVERSIBILITY: slight reactions of conjunctiva were seen in two rabbits on day 7.

Test condition : TEST ANIMALS:
 - Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya)
 - Sex: female
 - Source: Dr. Karl Thomae GmbH, Biberach an der Riss
 - Age: no data
 - Weight at study initiation: 2600-2800
 - Number of animals: 4

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Reliability	:	- Controls: internal control (right eye) (2) valid with restrictions purity of TS not stated, short duration of test (not up to reversibility of effects)	
Flag 24.02.2003	:	Critical study for SIDS endpoint	(41)
Species	:	rabbit	
Concentration	:	25 %	
Dose	:	.1 ml	
Exposure time	:	unspecified	
Comment	:	other: see test conditions	
Number of animals	:	8	
Vehicle	:	other: olive oil	
Result	:	not irritating	
Classification	:	not irritating	
Method	:	Draize Test	
Year	:	1980	
GLP	:	no data	
Test substance	:	other TS: menthol fluid, 620006	
Test condition	:	After application of 25 % test substance in one eye and closing of lids for 1 minute, the eyes of four rabbits were rinsed with physiologically sodium salt solution for one minute. Substance remained in the eyes of the other 4 rabbits.	
Reliability 24.02.2003	:	(2) valid with restrictions limited documentation	(39)
Species	:	rabbit	
Concentration	:		
Dose	:		
Exposure time	:		
Comment	:	not rinsed	
Number of animals	:		
Vehicle	:		
Result	:	irritating	
Classification	:		
Method	:	other	
Year	:	1946	
GLP	:	no	
Test substance	:	other TS: not specified isomer, purity: no data	
Result	:	0.005 ml undiluted testmaterial and 5 % solution yield scores of over 5.0, 1 % solution not over 5.0. (A level of 5.0 is representative of severe injury, corresponding to necrosis, visible only after staining and covering about three fourths of the surface of the cornea, or a more severe necrosis covering a smaller area.)	
Test condition	:	Exposure time: 18 to 24 hours. No. of animals: no data Concentration of TS: undiluted, 5 and 1 % 0.005 ml of the undiluted TS was applied to the center of the cornea of normal albino rabbit eyes while the lids were retracted. About one minute later, the lids were released. 18 to 24 h later, the eye was examined in strong diffuse daylight, stained with fluorescein, and the injury scored. The individual numerical scores of each eye (total of 20 at maximum) treated with a given volume or concentration of a TS are added together and then divided by the number of eyes (usually 5) to obtain the score of the injury caused by the treatment. Where dilution of a TS was necessary, the	

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	preferred solvent was propylene glycol. Preference is next given to water, and in some cases a deodorized kerosene has been used. The applied volume for the solutions is not mentioned The observation period is not known.	
Reliability	: (2) valid with restrictions Restrictions: no precise information on: e.g. number of animals; reading time; scores for each individual animal; observation period, reversibility of lesions, purity of test substance not stated	
Flag 24.02.2003	: Critical study for SIDS endpoint	(42)
Species	: other: in vitro	
Concentration	: 100 %	
Dose	:	
Exposure time	: 24 hour(s)	
Comment	:	
Number of animals	:	
Vehicle	: none	
Result	: irritating	
Classification	:	
Method	: other: EYETEX in vitro test	
Year	: 1992	
GLP	: no data	
Test substance	: other TS: menthol, not specified isomer	
Result	: Result of the biomacromolecular test method (EYETEX): mild/moderate irritation	
Test condition	: Study was conducted to investigate a target biomacromolecular test method, in order to predict in vivo ocular irritancy potentials of chemicals and formulations. The test results were compared with data from in vivo tests (Draize test).	
Reliability 24.02.2003	: (4) not assignable non-validated test system	(43)

5.3 SENSITIZATION

Type	: other: open repetitive dermal test	
Species	: guinea pig	
Number of animals	: 12	
Vehicle	: other: no	
Result	: not sensitizing	
Classification	:	
Method	: other	
Year	: 1980	
GLP	: no data	
Test substance	: other TS: menthol liquid	
Test condition	: Guinea pig (6 female, 6 male), Pirlbright white, weight: 400-700 g. The test substance was rubbed into the animals' skin (flank) for ca. 30 sec./d, the exposure (open) being repeated once daily on 7 days. Results were taken immediately. After 5 days without treating, test substance was applied and rubbed into not-pretreated part of shaved skin for 3 days once daily. Skin was observed 24 hours, 2 days and 3 days after termination of challenge.	
Reliability	: (3) invalid Significant methodological deficiencies. e.g. no standardized amount of	

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18.01.2002 applied substance and procedure of application. (39)

Type : Patch-Test
Species : Human
Number of animals :
Vehicle : Petrolatum
Result :
Classification :
Method : other
Year : 1955
GLP : No
Test substance : other TS: not specified isomer, 1 %

Result : Incidence of pronounced sensitization: 1.3 % (reaction 2+ or stronger)
Test condition : Allergic hypersensitivity was investigated in a group of 228 selected patients with dermatoses.
Reliability : (2) valid with restrictions
limited documentation
Flag : Critical study for SIDS endpoint

24.02.2003 (44)

Type : Patch-Test
Species : Human
Number of animals :
Vehicle : other: white petrolatum
Result :
Classification :
Method : other: Fregert et al, Epidemiology of contact dermatitis. Transactions of the St. John's Hospital Dermatological Society 55, 17-35, 1969.
Year : 1978
GLP : No
Test substance : other TS: not specified isomer, 1 %

Result : The percentage of allergic reactions (positive patch tests) was 6.1 %.
Test condition : 330 patients with eczematous lesions (88 patients (57 female and 31 male) with leg ulcers and 242 patients (141 female and 101 male) with eczematous dermatitis) were tested with menthol 1 % in white petrolatum. Patch tests are placed on the back and removed after 48 hours. Results were read at 48 and 72 (or 96) h after application.
Reliability : (2) valid with restrictions
limited documentation
Flag : Critical study for SIDS endpoint

24.02.2003 (45)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1978
GLP : no
Test substance : other TS: not specified isomer, 1 %

Result : 6/1385 (= ca. 0.4 %) reacted positive, the incidences of sensitization were as follows: 2/131 in 1972; 0/205 in 1973;

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Test condition	: 2/252 in 1974; 0/408 in 1975 and 2/389 in 1976. 1385 patients (824 female, 561 male, average age: 34.1 and 41.1) with varying dermatologic complaints were tested against menthol in the years 1972-1976.	
Reliability	: (2) valid with restrictions limited documentation	
Flag 24.02.2003	: Critical study for SIDS endpoint	(46)
Type	: Patch-Test	
Species	: Human	
Number of animals	:	
Vehicle	: other: Vaseline flav.	
Result	:	
Classification	:	
Method	: other: Magnusson, B. et al.: Routine patch testing IV, Acta derm.-vener. 48: 110-114 1968	
Year	: 1971	
GLP	: No	
Test substance	: other TS: not specified isomer	
Result	: 0.9 % were positive with 0 % strong positive reactions.	
Test condition	: 1070 patients with atopic eczema or dermatitis were patch tested against menthol.	
Reliability	: (2) valid with restrictions limited documentation	
Flag 24.02.2003	: Critical study for SIDS endpoint	(47)
Type	: Patch-Test	
Species	: Human	
Number of animals	:	
Vehicle	: Petrolatum	
Result	:	
Classification	:	
Method	: other	
Year	: 1987	
GLP	: no data	
Test substance	: other TS: not specified isomer, 5 %	
Result	: 1 % reacted positive.	
Test condition	: 1200 persons (750 female, 450 male, average age 40.7 years) with contact dermatitis were patch tested against menthol. Patch tests were performed on 2 sides of the upper back using Finn Chambers on Scanpor. Tests were read at 48, 72, 96 hours according to the ICDRG scale; the last reading was taken as definitive.	
Reliability	: (2) valid with restrictions limited documentation	
Flag 24.02.2003	: Critical study for SIDS endpoint	(48)
Type	: Patch-Test	
Species	: human	
Number of animals	:	
Vehicle	: other: yellow paraffin	
Result	:	
Classification	:	
Method	: other	
Year	: 1970	

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GLP : no
Test substance : other TS: not specified isomer, 5 %

Remark : These patients are presumably included in the study reported by Rudzki (1971)

Result : 1% (0.9 % male and 1.1 % female) reacted positive.

Test condition : 877 persons with primary contact, atopic, nummular and stasis dermatitis, and unclassified eczema were patch-tested against menthol (among other substances).

Reliability : (2) valid with restrictions
limited documentation

04.03.2003

(49)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1996
GLP : no data
Test substance : other TS: not specified isomer, 1 %

Result : Number of patients with positive reaction to menthol:
21/1077 (1.9 %).

Test condition : Among 1077 patients with crural ulceration and eczema contact allergy to externally applied drugs and its basic vehicles was confirmed in 491 persons (45.6 %) using the method of patch tests.

Reliability : (2) valid with restrictions
limited documentation

Flag : Critical study for SIDS endpoint

24.02.2003

(50)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other: Andersen, K.G., Contact Dermatitis 4, 195-198 (1978)
Year : 1995
GLP : no data
Test substance : other TS: not specified isomer, 5 %

Method : Standard protocol: Patches are applied to the patient's back and removed after 2 days. Readings were made 15 min after patch removal and again 2 days later. Reactions were graded according to the International Contact Dermatitis Research Group protocol.
(Andersen, K.G., Contact Dermatitis 4, 195-198 (1978)).

Result : 4/5 patients with burning mouth syndrome reacted positive to menthol.
4/4 patients with recurrent intra-oral ulceration were sensitive to menthol.
3/3 patients with an oral lichenoid reaction were positive to menthol.
After a mean follow-up of 32.7 months (range 9-48 months), of the 9 patients that could be contacted, 6 patients described clearance or improvement of their symptoms as a consequence of avoidance of menthol/peppermint. 7/11 menthol-positive patients also reacted positively with peppermint oil.

Test condition : 512 patients with intraoral complaints (burning mouth syndrome, recurrent

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	oral ulceration, lichenoid reaction) were tested to menthol over a 4-year period for assessment of the possible contribution of contact sensitivity to their complaints.	
Reliability	: (1) valid without restriction	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(51)
Type	: Patch-Test	
Species	: human	
Number of animals	:	
Vehicle	: other: vaseline	
Result	:	
Classification	:	
Method	: other	
Year	: 1995	
GLP	: no data	
Test substance	: other TS: not specified isomer, 1%	
Result	: 1/350 patients (0.3%) reacted positive.	
Test condition	: From 1990 to 1993 351 patients (188 m, 163 f, 28.5% younger than 40 years, 38.5% between 40 and 60 years and 33.0% older than 60 years; 20.2% suffered from atopic dermatitis actually or historically) were patch tested with the test series for anal eczema; among them menthol (350 patients).	
Reliability	: (2) valid with restrictions	
	limited documentation	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(52)
Type	: Patch-Test	
Species	: human	
Number of animals	:	
Vehicle	:	
Result	:	
Classification	:	
Method	: other	
Year	: 2001	
GLP	: no data	
Test substance	: other TS: not specified isomer	
Result	: Menthol provoked neither allergic nor irritant patch test reactions.	
Test condition	: Retrospective study: patch test data were collected from 7 patch test clinics in Finland. Patch tests were performed between 1994 and 1998. A total of 75 patients were tested against menthol.	
Reliability	: (2) valid with restrictions	
	limited documentation	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(53)
Type	: Patch-Test	
Species	: human	
Number of animals	:	
Vehicle	: petrolatum	
Result	:	
Classification	:	
Method	: other	
Year	: 1978	
GLP	: no	
Test substance	: other TS: not specified isomer, 1 %	

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Result	: A woman showed a positive result. Since she stopped smoking mentholated cigarettes, the patient is totally free of symptoms.	
Test condition	: A female patient who was suffering from a mild scaly erythema with irregular edges was tested with menthol. She regularly smoked menthol cigarettes.	
Reliability	: (2) valid with restrictions case-report	
Flag 24.02.2003	: Critical study for SIDS endpoint	(54)
Type	: Patch-Test	
Species	: human	
Number of animals	:	
Vehicle	:	
Result	:	
Classification	:	
Method	: other	
Year	: 1963	
GLP	: no	
Test substance	: other TS: not specified isomer	
Result	: Both patients reacted positive to menthol.	
Test condition	: Case reports: After using "la pommade Vick", a 69 years old man suffered from an eczema on the nose and the upper lip. Patient was patch-tested against menthol. A 43 years old man suffered from an contact eczema on his hands. After treating his hands with "Baume tranquille" his eczema got worse and reached up to the arms. He was patch-tested against menthol.	
Reliability 24.02.2003	: (4) not assignable Documentation insufficient for assessment.	(55)
Type	: Patch-Test	
Species	: human	
Number of animals	:	
Vehicle	: petrolatum	
Result	:	
Classification	:	
Method	: other	
Year	: 1977	
GLP	: no	
Test substance	: other TS: not specified isomer, 1 %	
Result	: No sensitization reaction to menthol observed. Hypersensitivity to peppermint oil was observed and referred to the sensitizing properties of the three ingredients alpha-pinene, limonene, and phellandrene.	
Test condition	: Case report: a patient suffering from swelling of the tongue, lips and gingival mucosa was tested against menthol.	
Reliability	: (2) valid with restrictions case-report	
Flag 24.02.2003	: Critical study for SIDS endpoint	(56)
Type	: Patch-Test	
Species	: Human	
Number of animals	:	
Vehicle	: other: glycerine	

5. Toxicity

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Result :
Classification :
Method : other
Year : 1939
GLP : No
Test substance : other TS: not specified isomer, 1%, 2% and 5%

Result : Positive result with all concentrations; symptoms of sensitization: erythema and pruritus.

Test condition : Case report: a patient who was suffering from an anal eczema was tested with mentholated glycerin ointments containing 1%, 2 % or 5 % menthol.

Reliability : (4) not assignable
Case report. Documentation insufficient for assessment.

24.02.2003

(57)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : other: as recommended in literature
Result :
Classification :
Method : other
Year : 1991
GLP : no data
Test substance : other TS: not specified isomer

Result : There was no positive allergic skin reaction found.

Test condition : 31 males, average age 30.8 years, range 20 to 49 years, were patch tested. None had any history of allergy, including to dental materials. Finn Chambers on Scanpor tape were used, following ICDRG guidelines. Test results were read at 2 and 3 days and scored according to the Japanese standard method.

Reliability : (4) not assignable
Documentation insufficient for assessment.

02.05.2002

(58)

Type : Patch-Test
Species : human
Number of animals :
Vehicle :
Result :
Classification :
Method : other
Year : 1984
GLP : no data
Test substance : other TS: not specified isomer, not further specified

Result : One of them showed sensitization (menthol, among other substances, used in production).

Test condition : 11 industrial workers suffering from disorders with a probable immune pathogenesis were tested against menthol.

Reliability : (4) not assignable
Documentation insufficient for assessment

24.02.2003

(59)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum

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Result :
Classification :
Method : other
Year : 1996
GLP : no data
Test substance : other TS: not specified isomer 1 %

Result : No reaction.
Test condition : Case report: A 46-year-old man with an acute eczema of the genitals due to benzyl alcohol was patch-tested.
Reliability : (4) not assignable
Documentation insufficient for assessment.

24.02.2003

(60)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1995
GLP : no data
Test substance : other TS: not specified isomer, 2 %

Result : A woman had a positive reaction to menthol on patch testing. On appropriate avoidance her symptoms resolved rapidly.

A man was allergic to menthol on patch testing. When excluding these substances from the diet definite reduction in lip swelling was noted. On re-exposure to menthol, further episodes of lip swelling occurred.
Test condition : Case Reports:
A 26-year-old woman presented with a 12-month history of recurrent oral ulceration. This occurred weekly and had not responded to corticosteroid mouthwashes.

A 43-year-old man with Down's syndrome had histologically proven orofacial granulomatosis mainly affecting the lower lips.

Patch tests were performed on both persons.
Reliability : (4) not assignable
Documentation insufficient for assessment.

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(61)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : other: vaseline
Result :
Classification :
Method : other
Year : 1974
GLP : no data
Test substance : other TS: not specified isomer, 2 %

Result : There were no reactions observed
Test condition : 3/4 patients with allergic reaction to toothpastes were tested epicutaneously against 2 % menthol in vaseline
Reliability : (4) not assignable

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24.02.2003 Insufficient documentation for assessment (62)

Type : Patch-Test
Species : human
Number of animals :
Vehicle :
Result :
Classification :
Method : other
Year : 1962
GLP : no
Test substance : other TS: not specified isomer

Result : An allergy to oil of turpentine can cause a group sensitization to some related substances, among them menthol.

Reliability : (4) not assignable
Insufficient documentation for assessment

24.02.2003 (63)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1998
GLP : no data
Test substance : other TS: not specified isomer, 5%

Result : Positive reaction.
Test condition : One patient suffering from oral and lip dermatitis was patch tested against menthol (5% in pet.).

Reliability : (4) not assignable
Case report. Insufficient documentation for assessment.

24.02.2003 (64)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1998
GLP : no data
Test substance : other TS: not specified isomer, 5%

Result : Day 5: itch, erythema and swelling at sites of application of menthol
Day 7: ++ reaction

Earlier readings were negative.

Prick tests were negative.

Test condition : Case report: 34 year old woman, 9-year history of oral burning and discomfort.

Reliability : (4) not assignable
Case report. Insufficient documentation for assessment.

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24.02.2003 (65)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 2000
GLP : no data
Test substance : other TS: not specified isomer, 1%

Result : 13/54 patients reacted positive with TCS. 1/13 patients reacted positive with menthol
Test condition : 54 patients (33f, 21 m, aged 15-74 years) with exzematous lesions on the lips were tested (among other substances) against specially-targeted toothpaste cheilitis series (TCS) containing menthol.
The persons reacting positive against the TCS were patch tested against the single allergens.
Reliability : (2) valid with restrictions
limited documentation

24.02.2003 (66)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method : other
Year : 1991
GLP : no data
Test substance : other TS: not specified isomer

Result : positive reaction
Test condition : Case report: 66 year old man with chronic eczematous eruption 3 years ago and actual contact dermatitis was patch-tested against menthol (among other substances).
Reliability : (4) not assignable
Insufficient documentation for assessment.

24.02.2003 (67)

Type : Patch-Test
Species : Human
Number of animals :
Vehicle : Petrolatum
Result :
Classification :
Method : other
Year : 1996
GLP : no data
Test substance : other TS: not further specified

Result : In 13 patients showing mouth or lip swelling 5 patients were considered to have positive relevant allergic reactions on patch testing. 3 patients were allergic to food additives or flavourings (butylhydroxyanisole, dodecyl gallate, peppermint oil, and menthol). All 3 patients were put on an appropriate exclusion diet, resulting in rapid improvement or clearing of

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Test condition	: symptoms. Patches were removed after 2 days, and readings were taken at 2 and 4 days according to standard practice. Menthol concentration tested: 2%	
Reliability	: (2) valid with restrictions Limited documentation.	
Flag 24.02.2003	: Critical study for SIDS endpoint	(68)
Type	: other: Provocative systemic test/provocative skin test	
Species	: Human	
Number of animals	:	
Vehicle	: other: ethanol	
Result	:	
Classification	:	
Method	: other	
Year	: 1964	
GLP	: No	
Test substance	: other TS: not specified isomer	
Result	: Oral provocation: after 30 minutes a flushing reaction evolved and at 40 minutes she complained of a headache; there was a marked decrease in circulating basophils. Skin provocation: within 20 minutes the patient experienced a severe burning sensation and there was a fiery erythematous reaction about the treated area. Basophil degranulation tests were negative to menthol.	
Test condition	: The case of a woman suffering from generalized urticaria is reported: she had been exposed to menthol in various forms (peppermint-flavored toothpaste, peppermint candies, mentholated cigarettes, mentholated facial cream) partly for years. Oral provocative test: the patient ingested 10 mg of menthol in 5 cc of 50 % ethanol. Skin provocative test: one-tenth of a milliliter of 75 % menthol in ethanol was applied to the forearm. Additional provocative tests with thymol and terpin hydrate were performed.	
Reliability 24.02.2003	: (4) not assignable Case report. Insufficient documentation for assessment.	(69)
Type	: other: challenge test	
Species	: Human	
Number of animals	:	
Vehicle	:	
Result	:	
Classification	:	
Method	: other	
Year	: 1978	
GLP	: No	
Test substance	: other TS: not specified isomer	
Result	: Immediately after challenge the dermatitis recurred. Since discontinuing the offending cigarettes again, her condition has remained completely clear.	
Test condition	: Case report: A 25-year-old woman suffered from a chronic dermatitis of the upper lip. When she discontinued her menthol cigarettes temporarily, her dermatitis immediately cleared. After several weeks, she performed a challenge test by resuming the menthol cigarettes.	
Reliability	: (4) not assignable Case report. Insufficient documentation for assessment.	

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24.02.2003 (70)

Type : other: challenge test
Species : Human
Number of animals :
Vehicle :
Result :
Classification :
Method : other
Year : 1992
GLP : no data
Test substance : other TS: not further specified

Result : Menthol contained in toothpastes may act as asthma-inducing agents.
Test condition : Case reports are given on aspirin-sensitive patients and patients with aspirin-induced asthma, whose asthma was exacerbated by the mint flavour contained in their toothpaste. Challenge tests were performed with menthol.

Reliability : (4) not assignable
Case report. Insufficient documentation for assessment.

24.02.2003 (71) (72)

Type : other: challenge test
Species : Human
Number of animals :
Vehicle :
Result :
Classification :
Method : other
Year : 2001
GLP : no data
Test substance : other TS: not further specified

Result : She showed positive patch test reaction and was positive after challenge (FEV1 decreased).
Test condition : Case-report: 40 year old nonsmoking woman experienced attacks of sneezing, nasal obstruction, rhinorrhea, dyspnea, and wheezing whenever she brushed her teeth and/or ingested mint candies. Patch test was performed and a challenge test: the patient rinsed her mouth with a 0.02 % menthol solution.

Reliability : (4) not assignable
Case report. Insufficient documentation for assessment.

24.02.2003 (73)

Type : other: provocative test by oral challenge
Species : Human
Number of animals :
Vehicle :
Result :
Classification :
Method : other
Year : 1966
GLP : No
Test substance : other TS: not specified isomer

Result : An urticarial reaction and a fall in the total number of circulating basophils following menthol challenge were observed.
Epicutan and Scratch Tests with menthol were negative.

Test condition : The case of a young girl suffering from generalized urticaria is reported:

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she had been exposed to menthol in various forms for many years, the initial episode of urticaria came coincidentally with an increased exposure to cough drops, aerosol room spray and a medicated petrolatum.

Provocative test by oral challenge: menthol was administered to the patient at a dose of 10 mg menthol in 5 cc of 50 % ethanol.

Reliability : (4) not assignable
Case report. Insufficient documentation for assessment.

24.02.2003

(74)

5.4 REPEATED DOSE TOXICITY

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Wistar
Route of admin. : oral feed
Exposure period : 2 w
Frequency of treatm. : Continuously
Post exposure period : no data
Doses : 0.5%, 1 %
Control group : Yes
Method : other
Year : 1985
GLP : no data
Test substance : other TS: not specified isomer

Result : Increased serum cholesterol and serum triglycerides were observed in the high-dose group, no effect on apo A-1 lipids, an indicator of high-density lipoprotein status.
Body weight was unaffected. Liver weight was slightly increased

Test condition : Rats weight: 240-300 g
Reliability : (4) not assignable
Documentation insufficient for assessment.

02.08.2002

(75)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : other: FDA-Osborne Mendel or Rockland Wistar
Route of admin. : Gavage
Exposure period : 3 d
Frequency of treatm. : Daily
Post exposure period : No
Doses : 20 mg/kg bw/d
Control group : Yes
Method : other
Year : 1972
GLP : No
Test substance : other TS: not specified isomer

Result : Decrease in hepatic aminopyrine demethylation and in aniline hydroxylation, increase in hepatic hexobarbital hydroxylation.

Test condition : Menthol was melted and suspended in either water or 0.5% methylcellulose, each warmed to 50 °C;
doses administered was equivalent to 1ml/100 g bw;
6 rats were treated with menthol,
no further examinations.
Test was conducted to identify whether menthol changes parathion toxicity.

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Reliability	: (3) invalid Unsuitable test system (see test conditions)	
30.11.2001		(76)
Type	: Sub-acute	
Species	: Mouse	
Sex	: male/female	
Strain	: no data	
Route of admin.	: oral unspecified	
Exposure period	: 5 d	
Frequency of treatm.	: see test conditions	
Post exposure period	: No	
Doses	: 40 or 60 mg/animal/d (= ca. 2000 or 3000 mg/kg bw/d)	
Control group	: Yes	
Method	: other	
Year	: 1940	
GLP	: No	
Test substance	: other TS: not specified isomer	
Result	: increased activity of beta-glucuronidase in the liver, kidney and spleen, no increase in enzymic activity in the testis, ovary, uterus and vagina	
Test condition	: Experimental design: the animals received 20 mg of menthol, 3 doses/d, for 4 d and 2 doses of 20 mg of menthol on the 5th d. Study was conducted to investigate the increase in β -Glucuronidase activity of mammalian tissues induced by feeding glucuronidogenic substances.	
Reliability	: (3) invalid Unsuitable test system (see test condition)	
17.12.2001		(77)
Type	: Chronic	
Species	: Rabbit	
Sex	: no data	
Strain	: no data	
Route of admin.	: Inhalation	
Exposure period	: 9 m	
Frequency of treatm.	: Daily	
Post exposure period	: No	
Doses	: 1% and 5 % in liquid petrolatum	
Control group	: yes, concurrent vehicle	
NOAEL	: < 1 %	
Method	: other	
Year	: 1929	
GLP	: No	
Test substance	: other TS: not specified isomer	
Result	: When applied to the nasal mucous membrane of a rabbit for nine months, menthol (1% solution) cause some degenerative changes, menthol (5 % solution) cause definite destructive changes throughout all layers of the nasal membrane.	
Test condition	: Rabbits used were healthy and about 1 year old. Daily history was kept of each animal, with observations as to the amount and type of nasal discharge, general state of activity of the animal and the animal's weight. Paraffin sections of the nasal mucosa was performed. The tissues selected were usually from the posterior or ethmoturbinate of the animal.	
Reliability	: (3) invalid Unsuitable test system: is only regarding a specific topic.	
17.12.2001		(78)

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Type : Sub-acute
Species : Monkey
Sex : no data
Strain : no data
Route of admin. : Inhalation
Exposure period : 14 d
Frequency of treatm. : 8 h, daily
Post exposure period : no data
Doses : 40 mg/kg bw/d
Control group : no data specified
Method : other
Year : 1976
GLP : no data
Test substance : other TS: not specified isomer

Result : no overt toxicity
Reliability : (4) not assignable
Secondary literature

17.12.2001

(79)

5.5 GENETIC TOXICITY 'IN VITRO'

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

Species : Mouse
Sex : Female
Strain : Strain A
Route of admin. : i.p.
Exposure period : 7 w
Frequency of treatm. : 3 doses/w
Post exposure period : 17 w
Doses : 25 or 100 mg/kg bw (= single dose)
Result : Negative
Control group : other: two series of base-line controls were maintaining during the experimental period, one consisting of untreated mice killed along with the treated animals and the other control receiving injections of tricapyrylin (vehicle)
Method : other
Year : 1973
GLP : No
Test substance : other TS: not specified isomer

Remark : It became apparent, that the used tricapyrylin 2097 was an unsuitable vehicle (mice lost weight after a single injection of tricapyrylin, appr. 20 % of the animals died after 12 injections of the vehicle, mean tumor value of 0.59/mouse was considerably higher than expected from earlier reports)
Result : Both dose groups: no increase in incidence of lung tumors compared to the controls.
Test condition : Total number of i.p. injections: 20 the animals were killed at 24 w after the first injection.
Reliability : (3) invalid
Unsuitable test system.

30.11.2001

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5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Type of experience	: Human - Medical Data	
Result	: In vivo: The methaemoglobin concentration of the erythrocytes was raised (within physiological limits). In vitro: Methemoglobin reductase and glucose-6-phosphate-dehydrogenase activity were reduced.	
Test condition	: Clinical tests: mentholated ointments were applied to 2 groups of 4 patients (2-11 months old) and the methaemoglobin values obtained, before treatment and 24 h afterwards respectively, were compared. The haemoglobin-reductase-activity and the glucose-6-phosphate-dehydrogenase-activity were measured. In vitro investigations in human erythrocyte homogenisates were performed: methemoglobin reductase and glucose-6-phosphate-dehydrogenase were analyzed.	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions Limited documentation.	
24.02.2003		(81)
Type of experience	: Human - Medical Data	
Result	: In young children (younger than 1 year), application of menthol to the nostrils can result in reflex apnoea (reflectory reaction of the nervus trigeminus – Kratschmer reflex), laryngospasm, spasm of the glottis or in instant collapse (even reported after local application). Further clinical signs are: dyspnea, unconsciousness, irregular and decreased respiration rate, apnea, bradycardia, cyanosis, hyperextensive extremities.	
Reliability	: (1) valid without restriction	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(82) (83) (84) (85) (86) (87) (88) (89) (90) (91)
Type of experience	: Human - Medical Data	
Result	: The consumption of menthol lozenges or the smoke of mentholated cigarettes may have played role in acquired essential cold urticaria.	

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Test condition	: A few cases of acquired essential cold urticaria (a kind of cold dermatological hypersensitivity) are reported.	
Reliability	: (4) not assignable Documentation insufficient for assessment.	
04.03.2003		(92)
Type of experience	: Human - Medical Data	
Result	: 1. The laryngeal examination itself has no mechanical pathological effect on the laryngeal mucosa. 2. The laryngoscopic results showed no difference between the group of ill children and the group who had received additional menthol rub treatment. 3. The menthol contained in the ointment had no adverse effect on the laryngeal mucous membrane.	
Test condition	: A controlled clinical study on a mentholated preparation (Vicks VapoRub) which is mostly employed at home in the United States was carried out in order to determine whether rubbing with mentholated ointments in normal dosages can provoke toxic symptoms.	
	3 groups of patients: - Healthy children who had not been treated (control group), - Children with acute respiratory affections receiving standard treatment - Children with acute respiratory affections being treated with mentholated ointment.	
	Laryngoscopy took place within 48 hours after hospitalization, during treatment 48 hours later and also 96 hours after the treatment with mentholated rub. Dosage: 1 teaspoon ful of ointment per 10 kg of body weight twice a day).	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions Limited documentation	
24.02.2003		(93)
Type of experience	: Human - Medical Data	
Result	: After drinking 2500-3500 mg menthol (corresponding 200-250 mg/kg bw) a 3 year old child became drowsy, somnolent, felt pain in the stomach and vomited. The symptoms were fully reversible within 4 days.	
Reliability	: (2) valid with restrictions Limited documentation	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(94)
Type of experience	: Direct observation, clinical cases	
Result	: Ingestion of menthol causes severe abdominal pain, nausea, vomiting, vertigo, ataxia, drowsiness, and coma.	
Reliability	: (2) valid with restrictions Limited documentation	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(95)
Type of experience	: Direct observation, clinical cases	
Remark	: Chronic inhalation of menthol in cigarettes can cause ataxia.	
Result	: Case report: 13 year old boy with history of bronchial asthma had nasal catarrh for which he started to use olbas oil by inhalation. Examination: weakness of the left arm and leg and ataxia; he was euphoric.	

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Content of olbas oil: 4.1 % menthol, 18.5 % oil of cajuput, 0.1 % clove 35.5 % eucalyptus, 2.7 % juniper berry, 35.5 % peppermint, 3.7 % wintergreen oil (methyl salicylate). The amount of menthol inhaled was approximately 200 mg.

Reliability : Patient fully recovered 12 h later.
: (2) valid with restrictions
: Limited documentation
24.02.2003 (96)

Type of experience : Direct observation, clinical cases

Result : An allergic reaction to menthol may be exhibited in active patients who use topical analgesics. The major symptom is slight redness the day after exposure.

Test condition : The occurrence of allergic contact dermatitis in active patients after exposure to certain types of sports equipment and topical analgesics is studied.

Reliability : (4) not assignable
: Documentation insufficient for assessment.
24.02.2003 (97)

Type of experience : Direct observation, clinical cases

Result : Topical menthol is a rare sensitizer, but such reactions have occurred, including allergic contact cheilitis, contact urticaria, and shaking chills when used over a wide area of the body, especially in elderly persons.

Test substance : unspecified isomer

Reliability : (4) not assignable
: Documentation insufficient.
23.05.2002 (98) (99) (100)

Type of experience : Direct observation, clinical cases

Result : A case of non-thrombocytopenic purpura caused by mentholated cigarettes was described.

Test substance : unspecified isomer

Reliability : (4) not assignable
: Documentation insufficient for assessment
24.02.2003 (101)

Type of experience : Direct observation, clinical cases

Result : Case reports:
A woman became addicted to mentholated cigarettes and developed toxic exhaustive psychosis.

A woman who smoked 80 mentholated cigarettes daily for 3 months developed insomnia, unsteady and ataxic gait, thick speech, tremor of the hands, vomiting, and cramp in the legs. Her heart-rate was 44 per minute. Mentally she became oversensitive, irritable, confused, depressed and quarrelsome.

The latter woman was given 1 g menthol three times daily for one week. On the third day she was apathetic and tired; and on the seventh day she complained of nausea, anorexia, and exhaustion and had difficulty in concentrating. She looked pale and drawn. The pulse-rate slowed down (from 76 at the beginning of the menthol application to 60 at the seventh day).

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Test substance	: Symptoms disappeared when menthol was withheld.	
Reliability	: unspecified isomer	
	: (2) valid with restrictions	
	Limited documentation	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(102)
Type of experience	: Direct observation, clinical cases	
Result	: Both patients got complaint-free.	
Test condition	: Two patients suffering from palindromic rheumatism were put on a menthol-free (among other substances) diet.	
Test substance	: unspecified isomer	
Reliability	: (4) not assignable	
	Documentation insufficient	
24.02.2003		(103)
Type of experience	: Direct observation, clinical cases	
Result	: Case report: methaemoglobinaemia (31% free hemoglobine) was diagnosed in a 15 w old baby who had been treated topically with a menthol containing cough balm (other ingredients of the preparation: camphor, ol. eucal., ol. pinix., other essential oils). It was assumed, that menthol was dermally absorbed.	
Test substance	: Menthol as an oxidizing agent is associated with methemoglobinemia.	
Reliability	: not specified isomer	
	: (4) not assignable	
	Documentation insufficient for assessment	
24.02.2003		(86) (104) (105)
Type of experience	: Direct observation, clinical cases	
Result	: Experience with human exposure: overdosage with menthol, particularly if it is continued over a long period can result in gastrointestinal distress, ataxia, stupor and convulsions; blood dyscrasias have been reported. (no details)	
Reliability	: (2) valid with restrictions	
	Limited documentation	
Flag	: Critical study for SIDS endpoint	
24.02.2003		(82)
Type of experience	: Direct observation, poisoning incidents	
Result	: Human: Oral intake of 8 or 9 g menthol were not lethal. The symptoms observed were a cold burning sensation in mouth, throat and esophagus, a cold sensation on the mucous membranes of the nose, on the skin of the hand and feet, and fatigue.	
Test substance	: unspecified isomer	
Reliability	: (4) not assignable	
	Documentation insufficient	
23.05.2002		(106) (107) (108)
Type of experience	: Direct observation, poisoning incidents	
Result	: One hour later she had the following symptoms: fatigue, pallor, cold limbs, cyanotic face, irregular and spasmodic respiration, irregular and rapid	

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		pulse, vomiting. The pulse interrupted completely for some seconds, while the diaphragm contracted convulsively.	
Test condition	:	Case report: A 4 1/2-year old girl ingested 3 bonbons, each containing 2 mg menthol (unspec. isomer).	
Test substance	:	unspecified isomer	
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment.	
Flag	:	Critical study for SIDS endpoint	
24.02.2003			(109)
Type of experience	:	Direct observation, poisoning incidents	
Result	:	Case report: Sucking of eucalyptol and menthol bonbons caused abundant formation of aphthae on the mouth mucosa. Clearance of the symptoms was observed on avoidance of the bonbons for some time.	
Test substance	:	unspecified isomer	
Reliability	:	(4) not assignable Documentation insufficient	
24.02.2003			(110)
Type of experience	:	Direct observation, poisoning incidents	
Result	:	An acute intoxication was observed in an infant after cutaneous application of menthol-containing medications. No further information from abstract available.	
Test substance	:	unspecified isomer	
Reliability	:	(4) not assignable Documentation insufficient for assessment	
24.02.2003			(111)
Type of experience	:	Direct observation, poisoning incidents	
Result	:	Baby was somnolent and hypotonic and had cornea erosions. Menthol may give rise to hypersensitivity reactions including contact dermatitis, apnea, and instant collapse, but no ocular side effects were reported before this clinical case. Conclusion: It is not clear, if the symptoms resulted from inhalation of fumes.	
Test condition	:	Rhino-Caps are used in an inhalation therapy for relief of nasal congestion. One Rhino-Caps capsule contains 25 mg Camphor, 125 mg eucalyptol, 55 mg menthol 120 mg terpineol and 5 mg chlorothymol.	
Reliability	:	Case-report: a 4 month old baby was exposed by inhalation to Rhino-Cap among other medications (after emptying the content of the capsule on a pillow). (4) not assignable Insufficient documentation for assessment	
24.02.2003			(112)
Type of experience	:	Direct observation, poisoning incidents	
Result	:	Case report: 62-year old man experienced full-thickness skin and muscle necrosis and persistent interstitial nephritis, due to excessive percutaneous absorption of a topical ointment, containing 18.3% methyl salicylate and 16 % menthol.	

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		The barrier function of the skin has possibly been destroyed by menthol and methyl salicylate was systemically absorbed through the damaged skin.	
Reliability	:	(4) not assignable Insufficient documentation for assessment.	
24.02.2003			(113)
Type of experience	:	Human – Epidemiology	
Result	:	For specific histological types of lung cancer (squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and adenocarcinoma) there was no indication of an association with menthol usage.	
Test condition	:	The effect of smoking mentholated cigarettes on lung cancer risk is studied. Investigated were current cigarette smokers: 588 male lung cancer cases, 914 male controls 456 female lung cancer cases and 410 female controls The prevalence of menthol usage did not differ between cases and controls of either sex.	
Reliability	:	(2) valid with restrictions co-exposure to cigarette smoke	
24.02.2003			(114)
Type of experience	:	Human – Epidemiology	
Result	:	Menthol was not a risk factor for cancer. The use of mentholated cigarettes is unlikely to be an important independent factor in oropharyngeal cancer.	
Test condition	:	The following hypothesis was tested: Smoking mentholated cigarettes increases the risk of cancer of the oral cavity and pharynx, a cancer with a 50% higher incidence in black Americans compared with whites.	
		194 male and 82 female persons as test subjects and 845 male and 411 female controls were part of the study.	
Reliability	:	(2) valid with restrictions co-exposure to cigarette smoke	
24.02.2003			(115)
Type of experience	:	Human – Epidemiology	
Result	:	No change in risk for males ever-smoking menthol versus those never smoking menthol cigarettes could be observed. For women, however, there was an increased risk.	
		Because of the limitations of the study the issue of menthol cigarette smoking and oesophageal cancer is not resolved.	
Test condition	:	The present study test whether menthol cigarette smoking is related to oesophageal cancer. Data from a large hospital-based case-control study are used.	
Reliability	:	(2) valid with restrictions co-exposure to cigarette smoke	
24.02.2003			(116)
Type of experience	:	Human – Epidemiology	
Result	:	The relative risk of lung cancer associated with mentholation compared with nonmentholated cigarettes was 1.45 in men and 0.75 in women. Conclusion: There is an increased risk of lung cancer associated with mentholated cigarette use in male smokers but not in female smokers.	
Test condition	:	The association of mentholated cigarette use with lung cancer in men and	

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Reliability	:	women was examined in a cohort study. The study population consisted of 11761 members (5771 men, 3990 women). (2) valid with restrictions co-exposure to cigarette smoke	(117)
24.02.2003			
Type of experience	:	Human – Epidemiology	
Result	:	Menthol (n=49) smokers had larger puff volumes, higher cotinine levels and shorter time to first cigarette compared to non-menthol smokers*.	
Test condition	:	* statistically significant 95 women (48 Black, 47 White) menthol-cigarette smokers (n=27 in Blacks, n=22 in Whites) Investigated was:	
		smokin topography, plasma continine, plasma nicotine, expired carbon monoxide, time to first cigarette, smoking history	
Test substance	:	unspecified isomer	
Reliability	:	(2) valid with restrictions co-exposure to cigarette smoke	
24.02.2003			(118)
Type of experience	:	Human - Epidemiology	
Result	:	Lung-cancer risk from smoking mentholated cigarettes resembles the risk from smoking non-mentholated cigarettes.	
Test condition	:	Association between menthol cigarette smoking and lung-cancer risk among smokers was studied. Population: 337 incident lung cancer Controls: 478	
Test substance	:	unspecified isomer	
Reliability	:	(2) valid with restrictions co-exposure to cigarette smoke	
24.02.2003			(119)
Type of experience	:	Human – Epidemiology	
Result	:	Menthol smokers may be chronically less aroused and more sensitive to the effects of nicotine than non-menthol smokers.	
Test condition	:	The psychophysiological and subjective effect of smoking menthol versus non-menthol cigarettes was investigated using mentholated and not-mentholated denicotinized cigarettes. Parameters: EEG Heart rate (HR) mental alertness muscular relaxation anxiety/nervousness desire to smoke usual brand	
Reliability	:	22 participants (27,4 years, SD 4.1, range 21-35), 12 menthol smokers and 10 non-menthol smokers. (2) valid with restrictions co-exposure to cigarette smoke	(120)
24.02.2003			
Type of experience	:	other: hyposmia	
Result	:	The examination showed evidently a diminution of the smell acuity.	

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- Test condition** : 25 employees exposed to menthol are olfactometrically examined.
Control group: 25 employees working in the same plant, but are not exposed to menthol
- Test substance** : unspecified isomer
- Reliability** : (4) not assignable
Documentation insufficient
- 02.05.2002 (121)
- Type of experience** : other: influence on taste receptors
- Result** : 0.4-4 ug per ml: decreased the taste threshold up to 60%;
larger amounts increased the threshold up to 60 %;
With 40-400 ug a decrease in threshold was found in 1/4 individuals only
and it is always preceded by an increase.
- Test condition** : Test subjects: two female and two male students (24-28 years).
Investigation of the influence of menthol on the sensitivity of taste receptors
in man by dropping solutions of menthol on the tip of the tongue
- Reliability** : (3) invalid
Invalid test system.
- 17.01.2002 (122)
- Type of experience** : other: specific investigations
- Result** : Menthol given by steam inhalation to urethanized rabbits augmented the
soluble mucus content and lowered the specific gravity of respiratory tract
fluid (less than 20 micrograms/kg bw).
- Test substance** : unspecified isomer
- Reliability** : (4) not assignable
Documentation insufficient for assessment
- 24.02.2003 (123)
- Type of experience** : other: Animals - Taste and Thermoreceptors
- Result** : Menthol elicited a slowly increasing activity in all gustatory fibres of the
chorda tympani nerve; adding menthol changed the gustatory response of
the sapid solutions.
Thermoreceptors are influenced (the threshold of the menthol effect lies
between a concentration 1:1,000,000 and 1:500,000).
- Test condition** : Study on the effect of menthol on the excitability of the gustatory receptors
and on thermoreceptors in cats.
- Test substance** : unspecified isomer
- Reliability** : (2) valid with restrictions
Limited documentation
- 24.02.2003 (124) (125)
- Type of experience** : Other
- Result** : Menthol as a flavoring content in toothpaste, cigarettes and hard candy
may cause oral sensitivities or mucosal contact sensitization reactions.
Details under 5.3 Sensitization.
- Reliability** : (2) valid with restrictions
limited documentation
- 24.02.2003 (126)
- Type of experience** : other: Food intolerance
- Result** : 73 subjects reported food allergy or intolerance reactions and 16 % (12 of
73) of the self-reported reactions could be objectively confirmed.
Only one person, a 58 year old woman reported food intolerance reactions

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	caused by menthol. This could be confirmed by a menthol challenge. The subject reported aggravation of aphthae 1 h after administration.
Test condition	: The prevalence of food allergy and intolerance was studied in a random sample of the Dutch adult population (n=1484). First the self-reported reactions were investigated by questionnaire. In a clinical follow-up study, it was determined in how many cases this self-reported food allergy or intolerance reactions could be objectively confirmed by double-blind placebo-controlled food challenge.
Reliability 24.02.2003	: (1) valid without restriction (127)
Type of experience	: other: ADI
Result	: Menthol's ADI increased from 0,2 mg/kg bw up to 4 mg/kg bw.
Reliability	: (1) valid without restriction Expert Committee report
Flag 24.02.2003	: Critical study for SIDS endpoint (128) (129)
Type of experience	: other: fatal dose
Result	: the fatal dose of menthol in man has been estimated to be about 2 g.
Reliability	: (4) not assignable Documentation insufficient.
13.11.2002	(88)
Type of experience	: other: fatal dose
Result	: The probable letal dose for man is 50-500 mg/kg bw.
Reliability	: (4) not assignable Documentation insufficient.
13.11.2002	(95)
Type of experience	: other: fatal dose
Result	: The fatal dose of menthol is approximately 1 g/kg bw.
Reliability	: (4) not assignable Documentation insufficient.
13.11.2002	(130)
Type of experience	: other: glucose-6-phosphate dehydrogenase deficiency
Result	: Treated babies developed significantly more often severe jaundice.
	Conclusions:
	- Neonates were unable to conjugate menthol.
	- Use of menthol-containing products on neonates should be discontinued (especially in communities where the incidence of glucose-6-phosphate dehydrogenase deficiency is high).
Test condition	: 60 glucose-6-phosphate dehydrogenase-deficient babies were studied: in 30 babies the umbilical cord was dressed daily for 5 days after birth with "mentholated" powder 30 babies were not treated with the powder and served as controls.
Reliability	: (2) valid with restrictions Limited documentation
Flag 24.02.2003	: Critical study for SIDS endpoint (131)
Type of experience	: other: risk group

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- Result** : All incidents reported in connection with the use of menthol ointments are examined and analysed in detail. Serious clinical cases are often combined with a very high amount of applied menthol and the application to the nostrils.
- Symptoms are:
Laryngospasms, dyspnoe, hyperactivity, tremor, spasm of the glottis, hypersensitivity, drowsiness, cyanosis.
- The author assumes, that laryngospasms, spasm of the glottis, dyspnoe, cyanosis are not a poisoning effect of menthol but a reflexory reaction of the nervus trigeminus (Kratschmer reflex).
- A special risk group are newborn babies:
- high resorption capacity
- detoxification mechanism (glucuronidation) is not fully developed.
- Reliability** : (2) valid with restrictions
Limited documentation
- Flag** : Critical study for SIDS endpoint (94)
24.02.2003
- Type of experience** : other: ADI
- Result** : Estimate of acceptable daily intake (ADI) for man in 1976 by WHO: 0 - 0.2 mg/kg bw.
- Reliability** : (1) valid without restriction
Expert Committee report
- Flag** : Critical study for SIDS endpoint (132)
04.03.2003

5.11 ADDITIONAL REMARKS

- Type** : Behaviour
- Result** : After a single i.m. or oral administration of menthol to rats, a choleric effect was noted.
- Test substance** : unspecified isomer
- Reliability** : (4) not assignable
Documentation insufficient. (37)
25.02.2003
- Type** : Behaviour
- Result** : ED 50 rats: 35 mg/kg bw.
- Test condition** : ED 50 for shaking after i.p. injection in rats was determined.
Effect: more than 10 times shaking in the 10 min interval after injection.
Vehicle: 50/50 v/v ethanol water.
- Reliability** : (2) valid with restrictions
Limited documentation. (35)
25.02.2003
- Type** : Biochemical or cellular interactions
- Result** : In an in vitro study with human liver samples menthol (isomer unsp.) inhibited the glucuronidation of 7-hydroxy-4-methylcoumarin (45 % inhibition).

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Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions non-standard in vitro system	
25.02.2003		(133)
Type	: Biochemical or cellular interactions	
Result	: Menthol depressed the isolated heart of both the frog and the rabbit directly and dilated the coronary vessels. The frequency of beat was diminished, the power was increased. Ultimately the heart stopped in diastole.	
Test substance	: unspecified isomer	
Reliability	: (4) not assignable Documentation insufficient	
25.02.2003		(36) (134) (135)
Type	: Biochemical or cellular interactions	
Result	: Menthol, a specific substrate for GT2a isoform of UDP-glucuronosyltransferase, competitively inhibited glucuronidation of 2,2-di(isopropoxycarbonyl)ethylene-1,1-dithiol.	
Test condition	: Investigations were performed in rats and rabbits hepatic microsomes.	
Test substance	: Unspecified isomer	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(136)
Type	: Biochemical or cellular interactions	
Result	: Menthol possesses potent Ca ²⁺ channel-modulating properties. Menthol blocks dihydropyridine insensitive Ca ²⁺ channels in neuronal cells of chick, rat and human origin (this is supposed to be the reason for the cooling feeling).	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(137) (138)
Type	: Biochemical or cellular interactions	
Result	: Menthol down-regulated IL-6 receptors on AF 10 cells at concentrations that inhibited the growth of the cells.	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions non-standard test system	
25.02.2003		(139)
Type	: Biochemical or cellular interactions	
Result	: Thiobarbiturate acid reactive substances were increased.	
Test condition	: In vitro addition of menthol on hepatic lipid peroxidation was studied.	
Test substance	: unspecified isomer	
Reliability	: (4) not assignable Documentation insufficient.	
25.02.2003		(140)
Type	: Biochemical or cellular interactions	

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Result	: Menthol has a high choleretic effect.	
Test condition	: Wistar rats; 260mMol/kg bw in olive oil, gavage. Menthol was tested among other terpenes.	
Reliability	: (2) valid with restrictions Limited documentation	
25.02.2003		(141)
Type	: Biochemical or cellular interactions	
Result	: Findings suggest, that menthol acts on two types of Ca channels coexisting on the membrane of cultured sensory neurons: - blocks currents through low voltage- activated Ca channel - facilitates inactivation gating of the classical high voltage- activated Ca channel.	
Test condition	: Cultured dorsal root ganglion cells from chick and rat embryos are used.	
Reliability	: (2) valid with restrictions non-standard test system	
25.02.2003		(138)
Type	: Biochemical or cellular interactions	
Result	: In the micromolar range, menthol (unspecified isomer) exerts a depressive action on the low-threshold channel (LVA) and shows a modulatory effect on the high-threshold channel (HVA), in that it speeds up its inactivation.	
Test condition	: In vitro study of block and modulation of neuronal Ca channels performed on primary cultures of chick dorsal root ganglia.	
Reliability	: (2) valid with restrictions non-standard test system	
25.02.2003		(142)
Type	: Cytotoxicity	
Result	: The 50 % inhibitory concentration (IC ₅₀) for the cellular and subcellular systems ranged from 0.32 mM to 0.76 mM - trachea from chicken embryos: 5 mM menthol completely stopped the ciliary activity within 7 min, while it took 38 min to reach ciliostasis in a 1 mM solution of menthol. - isolated hamster brown adipocytes: At a concentration of 0.5 mM menthol the receptor mediated respiratory stimulation was markedly inhibited while the intracellular mitochondrial functions were still unaffected. - rat liver mitochondria: Increase in the "state 4" respiratory rate (at 1.0 mM) and osmotic swelling (at 0.5 mM).	
Test condition	: The toxicity of menthol (unspecified isomer) in concentrations varying from 0.1 mM to 5 mM was tested in 4 different in vitro test systems: -trachea from chicken embryos -Ascites sarcoma BP 8 cells -isolated hamster brown adipocytes -rat liver mitochondria	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions non validated test systems	
25.02.2003		(143) (144)
Type	: Metabolism	
Result	: Menthol is coupled with glucuronic acid (phase-II hepatic detoxication	

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Test condition	: mechanism).	
	: In vitro metabolic studies with liver microsomal fractions of several mammalian species (pig, rat, guinea pig).	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions	
	Limited documentation	
25.02.2003		(145) (146)
Type	: Metabolism	
Result	: 2 g: < 1 hr after administration in urine 6 h: 90 % recovery 3.5 g: 24 h: > 90 % recovery	
Test condition	: 2 and 3.5 g of menthol were given to rabbits by stomach tube. Menthol glucuronides are determined.	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions	
	Limited documentation	
25.02.2003		(147)
Type	: Metabolism	
Test condition	: In patients having drug-induced liver damages, a menthol loading-test (single oral administration of 2 g menthol) can be used for investigating the biotransforming ability of the liver: the excretion of menthol glucuronide with urine is determined as a control parameter and compared to the corresponding values of healthy normal and pathological control groups.	
Test substance	: unspecified isomer	
Reliability	: (2) valid with restrictions	
	Limited documentation	
25.02.2003		(148) (149)
Type	: Metabolism	
Result	: Control: 100 % PB-induced: 110 % b-NF-induced: 130 %	
Test condition	: The activity of hepatic UDP-Glucuronosyltransferase from control and (Phenobarbital PB and b-Naphthoflavone b-NF) induced pigs towards menthol was studied.	
Test substance	: unspecified isomer	
Reliability	: (4) not assignable	
	Documentation insufficient	
25.02.2003		(145)
Type	: Metabolism	
Result	: Menthol was a potent inhibitor.	
Test condition	: The inhibition of glucuronidation of 7-hydroxy-4-methylcoumarin by human liver microsomes was studied.	
Test substance	: not specified isomer	
Reliability	: (2) valid with restrictions	
	Limited documentation	
25.02.2003		(133)
Type	: Metabolism	

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- Result** : In inhibitory studies menthol - as a specific substrate for GT2a isoform - competitively inhibited glucuronidation of the dithiol.
- Test condition** : The kinetic activity of UDP-glucuronosyltransferases (UDPGT) toward a dithiol metabolite of malotilate, 2,2-di(isopropoxycarbonyl)ethylene-1,1-dithiol was investigated using rat and rabbit hepatic microsomes.
- Phenobarbital, an inducer of the GT2 isoform of UDPGT, increased rat microsomal UDPGT activity towards the dithiol.
- Reliability** : (2) valid with restrictions
Limited documentation
- 25.02.2003 (136)
- Type** : other: QSAR
- Result** : Menthol is classified as a reactive chemical.
- Test condition** : Structure-activity relationships of volatile organic chemicals as sensory irritants are studied using the database of Schaper, M., Development of a database for sensory irritants and its use in establishing occupational exposure limits. Am Ind Hyg Assoc J 54, 488-544, 1993 (database in based on the sensory irritating potency obtained in mice (RD50))
- Test substance** : unspecified isomer
- Reliability** : (2) valid with restrictions
non-validated SAR
- 25.02.2003 (150)
- Type** : other: QSAR
- Remark** : Basis was data set from Carpenter, C.P. and Smyth, H.F., Chemical burns of the rabbit cornea, American Journal of Ophthalmology 49, 1363-1372, 1946
- Result** : Quantitative structure-activity relationships (QSARs) for the eye irritation potential was done. Predicted value of eye score from neutral network analysis: 7 (irritating)
- Test substance** : unspecified isomer
- Reliability** : (2) valid with restrictions
non-validated SAR
- Flag** : Critical study for SIDS endpoint
- 25.02.2003 (151)
- Type** : other: cooling function of menthol
- Result** : Menthol is an efficient cooling ingredient with a rapid effect but it has 3 major inconveniences:
- irritant in higher concentrations; it is not recommended to use in products which come into contact with mucous membranes (eye)
- very strong characteristic odour which is not easy to mask
- effect is only of relatively short duration
- Conclusions: Menthol derivatives are more effective cooling ingredients
- Reliability** : (2) valid with restrictions
Limited documentation
- 25.02.2003 (152)
- Type** : other: pharmacology of menthol
- Result** : Among other contents this review is concerned with the pharmacology of menthol regarding the respiratory system and the skin. Important facts are:
- General conclusion of a symposium - debating the safety of menthol

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- medication - was that commonly used vaporub remedies were safe to use in infants but that they should not be applied directly to the nostrils.
- Reliability** : (4) not assignable
Secondary literature
25.02.2003 (153)
- Type** : other: pharmacology of menthol
- Result** : Traditional therapy of atopic dermatitis, ie, use of menthol (among others) is often very effective.
- Reliability** : (2) valid with restrictions
Limited documentation
25.02.2003 (154)
- Type** : other: pharmacology of menthol
- Result** : Menthol acts as a local anaestheticum and it reduces inflammation, when added to an existing medication.
- Test condition** : Pharmacological effect of 4 -chlorophenol-campher-menthol (medicamentation) is described.
- Reliability** : (2) valid with restrictions
Limited documentation
25.02.2003 (155)
- Type** : other: pharmacology of menthol
- Result** : It is believed that menthol negatively influences the mucociliary clearance, which means that the removal of the mucus is slowed.
- Reliability** : (4) not assignable
No further reference.
25.02.2003 (156)
- Type** : other: skin penetration
- Result** : In vitro: Menthol showed the most potent enhancing effect.
In vivo: formulation containing 0.05% nonivamide, 5% menthol, 20% ethanol showed higher penetration rate and an acceptable degree of skin irritation.
- Test condition** : Influences of penetration enhancers (azone, cinnamic acid, cinnamyl alcohol, menthol, nonivamide, menthol&nonivamide) regarding the percutaneous absorption and skin irritation of ketoprofen formulations through rat skin is studied in vitro and in vivo.
- Reliability** : (2) valid with restrictions
Limited documentation
25.02.2003 (157)
- Type** : other: smoking
- Result** : Menthol and other additives that produce a sensation of coolness but without a mint flavour have also been used in cigarettes. There is no evidence that these additives result in a higher risk.
- Reliability** : (2) valid with restrictions
co-exposure to cigarette smoke
25.02.2003 (158)
- Result** : The percentage of the administered dose excreted with the urine was between 10 and 90 %

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Test condition : depending on individuum and environmental influences.
: 0.2-40 mg menthol was orally administered to 3 volunteers once and the 12
h urine was collected. The menthol content in the urine was gas-
chromatographically analyzed.

Reliability : (2) valid with restrictions
Limited documentation

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6. Analyt. Meth. for Detection and Identification

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6.1 ANALYTICAL METHODS**6.2 DETECTION AND IDENTIFICATION**

7. Eff. Against Target Org. and Intended Uses

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7.1 FUNCTION**7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED****7.3 ORGANISMS TO BE PROTECTED****7.4 USER****7.5 RESISTANCE**

8. Meas. Nec. to Prot. Man, Animals, Environment

Id

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8.1 METHODS HANDLING AND STORING**8.2 FIRE GUIDANCE****8.3 EMERGENCY MEASURES****8.4 POSSIB. OF RENDERING SUBST. HARMLESS****8.5 WASTE MANAGEMENT****8.6 SIDE-EFFECTS DETECTION****8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER****8.8 REACTIVITY TOWARDS CONTAINER MATERIAL**

9. References

Id 1490-04-6
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- (1) GESTIS Stoffdatenbank 5-Methyl-2-(1-methylethyl)cyclohexanol, <http://www.hvbg.de/d/bia/fac/zesp/zesp.htm> (Print 25.03.2002)
- (2) Haarmann & Reimer GmbH: Chemical Safety Data Sheet "Menthol Liqu.", revision 17.4.2002
- (3) Verschueren K (1996) Handbook of Environmental Data on Organic Chemicals, 3rd Ed., John Wiley & Sons Inc. New York
- (4) Hopp, R., Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties, Recent Advances Tobacco Sci 19, 3 - 46 (1993)
- (5) The Merck Index, 12th Edition. Ed. by Budavari et al., Whitehouse Station, NJ (1996)
- (6) Griffin S, Wyllie SG, Markham J (1999) Determination of octanol-water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography. J Chromatography A 864: 221 - 228
- (7) Bayer AG 2002, Calculation of log Pow with SRC-KOWWIN v. 1.66 (2000)
- (8) Hazardous Substances Data Bank, print from 01/14/2002
- (9) Suzuki, T., Journal of Computer-Aided Molecular Design, 5 (1991) 149-166
- (10) Seidell, A. (1941), Solubilities of Organic Compounds, 2nd ed., Vol. II, D. van Nostrand Company, Inc. New York
- (11) Wakita K, Yoshimoto M, Miyamoto S, Watanabe H (1986) A Method for Calculation of the Aqueous Solubility of Organic Compounds by Using New Fragment Solubility Constants. Chem Pharm Bull 34: 4663 - 4681
- (12) Calculation of the OH Rate Constant with SRC-AOP v1.90
- (13) Hazardous Substances Data Bank, print from 09/05/2001
- (14) Jüttner, F.: Water Sci. Technol. 40(6), 123-128 (1999)
- (15) Jüttner, F., Wat. Sci. Tech. Vol 25, No. 2, pp 155-164, 1992
- (16) Bayer AG (2003): Calculation of Mackay Distribution Level I
- (17) EC, Technical guidance document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 1488/94 on risk assessment for existing substances. European Chemicals Bureau, Ispra, Italy (1996)
- (18) Pitter P (1976) Determination of Biological Degradability of Organic Substances. Water Res 10: 231 - 235
- (19) Richardson ML, Bowron JM (1985) The Fate of Pharmaceutical Chemicals in the Aquatic Environment. J Pharm Pharmacol 37: 1 - 12
- (20) Gunnatilleka, A.D. & Poole, C.F.: Anal. Commun. 36(6), 235-242 (1999)
- (21) Chicu, S. A. et al. (2000), An approach to calculate the toxicity of simple organic molecules on the basis of QSAR analysis in *Hydractinia echinata* (Hydrozoa, Cnidaria), Quant. Struct. - Act. Relat. 19, 227-236

9. References

Id 1490-04-6
Date 18.03.2003

- (22) Nidiry, E. S. J. (1998), Structure-Fungitoxicity Relationships of the Monoterpenoids of the Essential Oils of Peppermint (*Mentha piperita*) and Scented Geranium (*Pelargonium graveolens*), J. Essent. Oil. Res. 10, 628-631
- (23) Westcott, L.C. & Winston, M.L.: Canad. Entomol. 131(3), 363-371 (1999)
- (24) Kevan, S.D. et al. (1999), Feeding menthol to honeybees (Hymenoptera: Apidae): Entry and persistence in Haemolymph without causing mortality, The canadian Entomologist 131, 279-281
- (25) Clegg, R.J. (1982) The Mechanism of Cyclic Monoterpene Inhibition of Hepatic 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase in Vivo in the Rat, J. Biol. Chem. 257, 2294-2299
- (26) Atzl, G. et al (1972), Determination of Etheral Oils from the Urine by Gas-Liquid Chromatography, Chromatographia 5, 250-255
- (27) Quick, A.J. (1928) Quantitive Studies of β -Oxidation, J. Biol. Chem. 80, 535-541
- (28) Somerville, K.W. et al (1984) Delayed release peppermint oil capsules (Colpermin) for the spastic colon syndrome: a pharmacokinetic study, Br. J. Clin. Pharmacol. 18, 638-640
- (29) Kaffenberger, R.M. and Doyle, M.J. (1990), Determination of menthol and menthol glucuronide in human urine by gas chromatography using an enzyme-sensitive internal standard and flame ionization detection, J. Chromatogr. 527, 59-66
- (30) Bolund, S. et al. (1967), A Menthol Loading Test for Glucuronide Synthesis Normal Values. Scand. J. Clin. Lab. Invest. 19, 288-290
- (31) Haarmann & Reimer GmbH (1980), Menthol flüssig - Akute Toxizität an Mäusen. Prepared for: 3450 Holzminden, 1-
- (32) Jenner, P.M. et al. (1964), Food Flavours and Compounds of Related Structure I. Acute Oral Toxicity, Fd Cosmet. Toxicol. 2, 327-343
- (33) Pellacani (1883), Pharmacology of the camphor group (Original Title: Zur Pharmakologie der Kamphergruppe). Arch. Exp. Path. Pharm. 17, 377, cited in: Schwenkenbecher, A. (1908), About menthol toxication in human (Original title: Ueber Mentholvergiftung des Menschen), Muenchner Medizinische Wochenschrift 55, 1495-1496
- (34) Macht, D. (1939), Comparative Pharmacology of Menthol and its Isomers, Arch. Int. Pharmacodyn. 63, 43-58
- (35) Wei, E. (1983), AG-3-5: A Chemical which Produces Sensations of Cold, Environment, drugs and thermoregulation: 5th International Symposium on the Pharmacology of Thermoregulation, Saint-Paul-de-Vence, November 1-5, 183-186, Karger, Basel
- (36) Heathcote, R.St.A. (1922), The action of camphor, menthol and thymol on the circulation, J. Pharm. and Exp. Therapeutics 21, 177-190
- (37) Foerster, W. and Oettel, H. (1954), Über die choleretische Wirkung hydroaromatischer Carbonsäuren, Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol. 222, 244-247
- (38) Haarmann & Reimer GmbH (1989), Assessment of the skin irritant effect of HR 89/620006 in rabbits, Scantox - biological laboratory ltd lab no. 11876, 16.08.1989
- (39) Haarmann & Reimer GmbH (1980), menthol fluid (RF Menthol), medical report, Prof. Dr. Stüttgen; 18.04.1980

9. References

Id 1490-04-6
Date 18.03.2003

- (40) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/620006 in rabbits, Scantox – biological laboratory ltd lab no. 11872, 16.08.1989
- (41) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/620006 in rabbits, Scantox - biological laboratory ltd lab no. 11756, 02.05.1989
- (42) Carpenter, C.P. and Smyth, H.F. (1946) Chemical burns of the rabbit cornea, Am J. Ophthalmol. 29, 1363-1372
- (43) Gordon, V.C. (1992), Utilization of Biomacromolecular In Vitro Assay Systems in the Prediction of In Vivo Toxic Responses, Lens Eye Toxic. Res. 9(3+4), 211-227
- (44) Baer, R.L. et al. (1955), STUDIES ON ALLERGIC SENSITIZATION TO CERTAIN TOPICAL THERAPEUTIC AGENTS, Arch. Dermatol. 71, 19-23
- (45) Blondeel, A. et al. (1978), Contact allergy in 330 dermatological patients, Contact Dermatitis 4, 270-276
- (46) Jarisch, R. and Sandor, I. (1978), Epicutanstandardtestung: Ergebnisse aus fünf Jahren und ihre Auswirkungen auf zukünftige Untersuchungen, Z. Hautkr. 53, 462-470
- (47) Rudzki, E. and Kleniewska, D. (1971), Kontaktallergie auf einige Lokaltheraeutika und Konservierungsmittel, Dermatologica 143, 36-42
- (48) Santucci, B. et al. (1987), Contact dermatitis to fragrances, Contact Dermatitis 16, 93-95
- (49) Rudzki, E. and Kleniewska, D. (1970), The Epidemiology of Contact Dermatitis in Poland, Br. J. Derm. 83, 543-545
- (50) Legiec, C. et al. (1996) Alergia kontaktowa lekowa u chorych z owrzodzeniami i wypryskiem podudzi, Przegląd Dermatologiczny 83, 371-375
- (51) Morton, C.A. et al. (1995), Contact sensitivity to menthol and peppermint in patients with intra-oral symptoms, Contact Dermatitis 32, 281-284
- (52) Schnuch, A. and Geier, J. (1995), Epikutantestung mit dem DKG-Analblock, Dermatosen 43, 81-82
- (53) Kanerva, L. et al. (2001), A Multicenter Study of Patch Test Reactions With Dental Screening Series, Am. J. Contact Dermat. 12(2), 83-87
- (54) Camarasa, G. and Alomar, A. (1978), Menthol dermatitis from cigarettes, Contact Dermatitis 4, 169-170
- (55) Paschoud, J.-M. (1963), Quelques cas d'eczéma de contact avec sensibilisation de groupe, Dermatologica 127, 349-364
- (56) Dooms-Goossens, A. et al. (1977), Turpentine-induced hypersensitivity to peppermint oil, Contact Dermatitis 3, 304-308
- (57) Gronemeyer, W. (1939), Allergische Reaktion gegen Menthol, Deutsche Medizinische Wochenschrift 65, 756-757
- (58) Oshima, H. et al. (1991), Epidemiologic study on occupational allergy in the dental clinic, Contact Dermatitis 24, 138-139
- (59) Campiglio, R. et al. (1984), ASPETTI DELLA PATOLOGIA ALLERGICA NELL'INDUSTRIA COSMETICA, Med. Lav. 75, 407-411

9. References

Id 1490-04-6
Date 18.03.2003

- (60) Corazza, M. et al. (1996), Allergic contact dermatitis from benzyl alcohol, *Contact Dermatitis* 34, 74-75
- (61) Lewis, F.M. et al. (1995), Contact sensitivity to food additives can cause oral and perioral symptoms, *Contact Dermatitis* 33, 429-430
- (62) Mose, V.T. (1974), Allergiske reaktioner mod tandpasta, *Tandlaegebladet* 78, 325-328
- (63) v. Preyss, J.A. (1962), Allergie gegen Lösungsmittel, insbesondere Terpentinöl, *Berufsdermatosen* 10, 214-217
- (64) Downs, A.M.R. et al. (1998), Contact sensitivity in patients with oral symptoms, *Contact Dermatitis*, 39, 258-259
- (65) Fleming, C.J. and Forsyth, A. (1998), D5 patch test reactions to menthol and peppermint, *Contact Dermatitis* 38, 337-365
- (66) Francalani, S. et al. (2000), Multicentre study of allergic contact cheilitis from toothpastes, *Contact Dermatitis* 43, 216-222
- (67) Mochida, K. and Sugai, T. (1991) A Probable Case of Systemic Contact Dermatitis from Parabens, *Skin Res.* 33, 198-203, suppl. 11
- (68) Shah M, Lewis M and Gawkodger DJ (1996) Contact allergy in patients with oral symptoms: a study of 47 patients. *American Journal of Contact Dermatitis* 7: 146-151
- (69) Papa, C.M. and Shelley, W.B. (1964), Menthol Hypersensitivity, *J. Am. Med. Ass.* 189, 546-548 (100-102)
- (70) Chrisman, B.B. (1978), Menthol and Dermatitis, *Arch. Dermatol.* 114, 286
- (71) Kawane, H. (1996), Menthol and aspirin-induced asthma, *Respir. Med.* 90, 247
- (72) Subiza, J. et al. (1992), Toothpaste flavor-induced asthma, *J. Allergy Clin Immunol* 90, 1004-1006
- (73) dos Santos, M.A. (2001) Menthol-Induced Asthma: A Case Report, *J. Investig. Allergol. Clin. Immunol.* 11(1), 56-58
- (74) McGowan, E.M. (1966), Menthol Urticaria, *Arch. Derm.* 94, 62-63
- (75) Imaizumi, K. et al. (1985), Effect of Essential Oils on the Concentration of Serum Lipids and Apolipoproteins in Rats, *Agric. biol. Chem.* 49, 2795-2796
- (76) Sperling, F. and Ewenike, H.K.U. (1972), Changes in Ld50 of Parathion and Heptachlor Following Turpentine Pretreatment, *Environmental Research* 5, 164-171
- (77) Fishman, W.H. (1940), Studies on β -Glucuronidase, *J. Biol. Chem.* 136, 229-236
- (78) Fox, N. (1939), The Effect of Camphor, Eucalyptol and Menthol on the Nasal Mucosa, *Arch. Otolaryng.* 11, 48-54
- (79) Alarie, Y. (1990), Unpublished data (cited in Federal Register, 1976) cited in Bibra, Toxicity Profiles, The British Industrial Biological Research Association, Carshalton Surrey, 1-9
- (80) Stoner, G.D. et al. (1973), Test for Carcinogenicity of Food Additives and Chemotherapeutic Agents by the Pulmonary Tumor Response in Strain A Mice, *Cancer Research* 33, 3069-3085

9. References

Id 1490-04-6
Date 18.03.2003

- (81) Berger, H. et al. (1967), Über Methämoglobinämie unter Mentholeinwirkung sowie erste Versuche mit dem Impedance- Pneumographen über die Beeinflussung der Atmung, 120-134, In: Menthol and Menthol-containing external remedies, ed. Dost, F.H. and Leiber, B., Georg Thieme Verlag Stuttgart
- (82) Dukes, M.N.G. (1980), Camphor and menthol; volatile oils, ed. Meyler's side effects of drugs: an encyclopaedia of adverse reactions and interactions. 9th ed. Amsterdam: Excerpta Medica, p. 279
- (83) Kleinschmidt, H. (1935), Mentholschädigungen bei Säuglingen?, Die Medizinische Welt 23, 843-844
- (84) Klink, K. (1967), Klinische Beobachtungen nach Verabreichung von WickVapoRub bei Kleinkindern, 82-86, In: Menthol and Menthol-containing external remedies, ed. Dost, F.H. and Leiber, B., Georg Thieme Verlag Stuttgart
- (85) Kuschinsky, G. (1970), Menthol, 656-657, In: Taschenbuch der modernen Arzneibehandlung - 5th ed., Georg Thieme Verlag Stuttgart
- (86) Lesoine, W. (1965), Gefahren und Komplikationen bei der Anwendung mentholhaltiger Präparate in der HNO-Heilkunde, HNO fuer die aerztliche Praxis 13, 238-239
- (87) Lublinski, W. (1912), Die lokale Mentholanwendung in der Nase und ihre Gefahr im frühen Kindesalter, Berliner Klinische Wochenschrift 49, 261-262
- (88) Martindale, W. (1982), Menthol, The Extra Pharmacopoeia - 28th edition, ed. Reynolds, J.E.F. and Prasad, A.B., The Pharmaceutical Press, London, p. 352
- (89) Melis, K. et al. (1989), Metabolic investigation of a patient with Rett syndrome, Eur. J. Pediatr. 148, 786-787
- (90) Melis, K. et al. (1990), Accidental Nasal Eucalyptol and Menthol Instillation, Acta Clin. Belg. Suppl. 13, 101-102
- (91) Meyler, L. (1963), Side Effects of Drugs. Adverse Reactions as reported in the Medical Literature of the World, 1963-1965, Volume V, p. 529. Excerpta Medica Foundation Amsterdam
- (92) Lobitz, W.C. (1962), Cold and Heat Hypersensitivity, Dermatoses due to Environmental and Physical Factors, 104-118
- (93) Larkin, V.P. and Castellano, J.C. (1967), Laryngoscopic findings in acute respiratory infections treated with and without a mentholated rub, In: Menthol & Menthol-containing. Ext. Remedies, 108-119
- (94) Leiber, B. (1967), Menthol - eine kritische Bestandsaufnahme; in Menthol and menthol-containing external remedies. Use, mode of effect and tolerance in children. International Symposium, Paris, April 1966. Proceedings. Pages 7-32. Dost, F.H. and Leiber, B. (editors). Georg Thieme Verlag, Stuttgart
- (95) Gleason, M.N. et al. (1969), Clinical toxicology of commercial products, 3rd ed., The Williams and Wilkins Co., Philadelphia: cited in WHO Food Additives Series No. 10, p.67 (1976)
- (96) O'Mullane, N.M. et al. (1982) Adverse CNS Effects of Menthol-containing Olbas Oil, Lancet 1/8281, 1121
- (97) Fisher A. (1993), Allergic Contact Dermatitis, Phys. Sportsmed 21(39), 65-72

9. References

Id 1490-04-6
Date 18.03.2003

- (98) Fisher, A.A. (1986), Menthol Contact Dermatitis, 3rd ed. Lea and Febiger
- (99) Fisher, A.A. (1986), Reactions to Menthol, *Cutis* 38, 17-18
- (100) King, T.H. and Perez-Figaredo, R.A. (1988), Plant-Derived Dermatologic Drugs, *J. Assoc. Military Dermatologists* 14, 26-31
- (101) Highstein, B. and Zeligmann, I. (1951), Nonthrombocytopenic Purpura Caused by Mentholated Cigarettes, *J. Amer. med. Ass.* 146, 816
- (102) Luke, E. (1962), Addiction to Mentholated Cigarettes, *Lancet* 1, 110-111
- (103) Williams, B. (1974), Palindromic rheumatism, *Med. J. Aust.* 1, 455-456
- (104) Pape, M. (1962), Über einen Fall von Methämoglobinämie beim jungen Säugling, *Kinderaerztliche Praxis* 30, 245-247
- (105) Vessely, M.B. and Zitsch, R.P. (1993), Topical anesthetic-induced methemoglobinemia: A case report and review of the literature, *Otolaryngol. Head Neck Surg.* 108(6), 763-767
- (106) Moeschlin S., Menthol-Pfefferminze, Klinik und Therapie der Vergiftungen, Georg Thieme Verlag, Stuttgart, 7. Auflage, 583-584,
- (107) Schwenkenbecher, A. (1908) Ueber Mentholvergiftung des Menschen, *Muenchener Medizinische Wochenschrift* 55, 1495-1496
- (108) Wirth, W., Gloxhuber (1994), Menthol, *Toxikologie-Giftpflanzen und Pflanzengifte*, 405-406
- (109) Champeau, M. (1935), Accidents graves attribués à l'ingestion de 6 milligrammes de menthol chez une enfant de quatre ans et demi, *Strasbourg Med.* 95, 553-554
- (110) Ochsenius. M. *Inch. med. Wschr.* 48, 2201 (1931) cited in: Urbach, E. und Wiethe, C.: Salbei-, Zitronen- und Menthol-Ueberempfindlichkeit. in: Fuehner, H. (ed.): *Sammlung von Vergiftungsfaellen*, 3rd. vol., 254, Verlag von F.C.W. Vogel, Berlin (1932).
- (111) Dupeyron, J. et al. (1976), Intoxication aigue du nourrisson par application cutanée d'une pommade révulsive locale et antiseptique pulmonaire, *Eur.J.Toxicol.Environ.Hyg.* 9(5), 313-320
- (112) Soen, G. et al (1992) Corneal Erosions and Encephalopathy Following Exposure to "Rhino-caps", *J Pediatr Ophthalmol Strabismus*, 29, 191
- (113) Heng, M.C. (1987), Local Necrosis and Interstitial Nephritis Due to Topical Methyl Salicylate and Menthol, *Cutis*, 442-444
- (114) Kabat, G.C. and Hebert, J.R. (1991), Use of Mentholated Cigarettes and Lung Cancer Risk, *Cancer Res.* 51, 6510-6513
- (115) Kabat, G.C. and Hebert, J.R. (1994), Use of Mentholated Cigarettes and Oropharyngeal Cancer, *Epidemiology* 5(2), 183-188
- (116) Hebert, J.R. and Kabat, G.C. (1989), Menthol Cigarette Smoking and Oesophageal Cancer, *Int. J. Epidemiol.* 18(1), 37-44
- (117) Sidney, S., et al. (1995), Mentholated Cigarette Use and Lung Cancer, *Arch. Intern. Med.* 155(7), 727-732

9. References

Id 1490-04-6
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- (118) Ahijevych, K. and Parsley, L.A. (1999), SMOKE CONSTITUENT EXPOSURE AND STAGE OF CHANGE IN BLACK AND WHITE WOMEN CIGARETTE SMOKERS, *Addictive Behaviours* 24(1), 115-120
- (119) Carpenter, C.L. et al. (1999), Mentholated Cigarette Smoking and Lung-Cancer Risk, *Ann. Epid.*, 114-120, 1999
- (120) Pritchard, W.S. et al. (1999), Little evidence that "denicotinized" menthol cigarettes have pharmacological effects: an EEG/heart-rate/subjective -response study, *Psychopharmacology* 143(3), 273-279
- (121) Naus, A. (1968), Alterations of the Smell Acuity Caused by Menthol, *J. Laryngol. Otol.* 82, 1009-1011
- (122) Skouby, A.P. and Zilstorff-Pedersen, K. (1955), The Influence of Acetylcholine, Menthol and Strychnine on Taste Receptors in Man, *Acta Physiol. Scand.* 34, 250-256
- (123) Boyd, E.M. and Sheppard, E.P. (1969), A BRONCHOMUCOTROPIC ACTION IN RABBITS FROM INHALED MENTHOL AND THYMOL, *Arch. int. Pharmacodyn.* 182, 206-214
- (124) Hellekant, G. (1969), The Effect of Menthol on Taste Receptors, *Acta physiol. scand.* 76, 361-368
- (125) Hensel, H. and Zotterman, Y. (1951), The Effect of Menthol on the Thermoreceptors, *Acta Physiol. Scand.* 24, 27-34
- (126) Taylor, S. and Dormedy E. (1998) in Taylor S. (ed.) *Advances in food and nutrition research, The role of flavoring substances in food allergy and intolerance*, Academic Press, Inc., 1-44
- (127) Niestijl Jansen JJ et al. (1994), Prevalence of food allergy and intolerance in the adult Dutch population, *J All Clin Imm* 93, 446-456
- (128) BIBRA Bull. 39 (2000), International 51st JECFA report on food additives, WHO, 174-175
- (129) Joint FAO/WHO Expert Committee on Food Additives, Fifty-first meeting; Geneva, 9-18 June 1998, <http://www.who.int/pes/jecfa/summary51.htm>, 9.8.99
- (130) Morton, J.F. (1977), *Major Medicinal Plants*. Springfield, Ill: Thomas, C.C. , cited in: King, T.H. and Perez-Figaredo, R.A. (1988), *J. Assoc. Military Dermatologists* 14, 26-31
- (131) Olowe, S.A. and Ransome-Kuti, O. (1980), The Risk of Jaundice in Glucose-6-phosphate Dehydrogenase Deficient Babies Exposed to Menthol, *Acta Paediatr. Scand.* 69, 341-345
- (132) FAO/WHO (1976), Menthol - Allocation of acceptable daily intakes (ADI), WHO food additives series No. 10: Toxicological evaluation of certain food additives, Food and Agriculture Organisation of the United Nations World Health Organisation, 2-5
- (133) Irshaid, Y.M. et al (1990), Glucuronidation of 7-hydroxy- 4-methylcoumarin by human liver microsomes. Inhibition by certain drugs, *Eur. J. Drug. Metab. Pharmacokinet.* 15(4), 295-301
- (134) Joachimoglu. *Arch. Exp. Path. Pharm.* lxxx, 259 (1916/7) cited in: Heathcote, R.St.A.: The action of camphor, menthol and thymol on the circulation. *J. Pharm. and Exp. Therapeutics* 21, 177-190 (1922)
- (135) Pellacani (1883), *Arch. Exp. Path. Pharm.* xvii, 369 cited in: Heathcote, R.St.A. (1922), The action of camphor, menthol and thymol on the circulation. *J. Pharm. and Exp. Therapeutics* 21, 177-190

9. References

Id 1490-04-6
Date 18.03.2003

- (136) Nakaoka, M. (1990), Kinetic characteristics of UDP-glucuronosyltransferases towards a dithiol metabolite of malotilate in hepatic microsomes of rats and rabbits, *Xenobiotica*, 20(6), 619-627
- (137) Sidell, N. et al. (1990), Menthol Blocks Dihydropyridine-Insensitive Ca²⁺ Channels and Induces Neurite Outgrowth in Human Neuroblastoma Cells, *J. Cell. Physiol.* 142, 410
- (138) Swandulla, D. et al. (1987), Effect of menthol on two types of Ca currents in cultured sensory neurons of vertebrates, *Pfluegers Arch.*, 409, 52-59
- (139) Sidell, N. et al. (1991), Retinoic Acid-induced Growth Inhibition of a Human Myeloma Cell Line via Down-regulation of IL-6 Receptors, *J Immunol.* 146, 3809-3814
- (140) Fadhel, Z. and Abdul-Rahman, S. (1999), Effect of Mentha Piperita and Menthol on CCL4-Induced Hepatic Lipid Peroxidation in Female Rats, *Toxicologist* 48, 279
- (141) Moersdorf, K. (1966), Cyclische Terpene und ihre choleretische Wirkung, *Chim. Ther.* 7, 442-443
- (142) Carbone, E. et al. (1988), BLOCK AND MODULATION OF NEURONAL Ca CHANNELS BY CATECHOLAMINES, CYCLIC ALCOHOLS AND NICKEL, *Symposia in Neuroscience* 6, 97-106
- (143) Bernson, V.S.M. and Pettersson, B. (1983), THE TOXICITY OF MENTHOL IN SHORT-TERM BIOASSAYS, *Chem.-Biol. Interactions* 46, 233-246
- (144) Curvall, M. et al. (1984), AN EVALUATION OF THE UTILITY OF FOUR IN VITRO SHORT TERM TESTS FOR PREDICTING THE CYTOTOXICITY OF INDIVIDUAL COMPOUNDS DERIVED FROM TOBACCO SMOKE, *Cell Biol. Toxicol.* 1, 173-193
- (145) Boutin, J.A. et al. (1981), THE ACTIVITY OF HEPATIC UDP-GLUCURONOSYLTRANSFERASE FROM CONTROL AND INDUCED PIGS TOWARDS 17 HYDROXYLATED AGLYCONES, *IRCS J. Med. Sci.* 9(7), 633-634
- (146) Boutin, J.A. et al. (1985), HETEROGENEITY OF HEPATIC MICROSOMAL UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES, *Biochemical Pharmacology* 34, 2235-2249
- (147) Quick, A.J. (1924), The Synthesis of Menthol Glycuronic Acid in the Rabbit, *J. Biol. Chem.* 61, 679-683
- (148) Horvath, T. et al. (1989), Drug Metabolism in Drug-Induced Liver Diseases: Pathogenetic Role of Active Metabolites, *Acta Physiologica Hungarica* 73, 293-304
- (149) Horvath, T. et al. (1990), Ein neuer Aspekt der Leberfunktion: Quantitative Charakterisierung der Biotransformationskapazität bei chronischen Lebererkrankungen mit Testsubstanzen, *Z. Klin. Med.* 45, 1705-1708
- (150) Alarie, Y. (1998), Structure-activity relationships of volatile organic chemicals as sensory irritants, *Arch. Toxicol.* 72(3), 125-140
- (151) Barratt, M. (1997), QSARS for the Eye Irritation Potential of Neutral Organic Chemicals, *Toxicol. in Vitro* 11, 1-8
- (152) Jacobs, P. and Johncock W. (1999), Some like it cool, *Parfuem. Kosmet.* 80(4), 26, 28-31
- (153) Eccles, R. (1994) Review: menthol and related cooling compounds, *J. Pharm. Pharmacol.* 46, 618-630

9. References

Id	1490-04-6
Date	18.03.2003

- (154) Millikan, L. (1984), Atopic dermatitis, *Postgrad. Med* 76, 139-146
- (155) Thesen R. et al (1993), Ganz oder teilweise "negativ" bewertete Arzneistoffe, *Pharmaz. Z.* 138(45), 44-48
- (156) Hendriks, H. (1998), Pharmaceutical Aspects of Some Mentha Herbs and Their Essential Oils, *Perfum. Flavor.* 23 (nov), 15-23
- (157) Wu, P.C. et al (2001), Evaluation of percutaneous absorption and skin irritation of ketoprofen through rat skin: in vitro and in vivo study, *Int. J. Pharm.* 222(2), 225-235
- (158) WHO, IPCS (1999) EHC No. 211, Health Effects of Interactions between Tobacco use and exposure to other agents, p. 15
- (159) Gleispach, H. and Schandara, E. (1970) *Z. Anal. Chem.* 252, 140-143

10. Summary and Evaluation

Id

1490-04-6

Date

18.03.2003

10.1 END POINT SUMMARY**10.2 HAZARD SUMMARY****10.3 RISK ASSESSMENT**

I U C L I D Data Set

Existing Chemical : ID: 89-78-1
CAS No. : 89-78-1
EINECS Name : DL-menthol
EC No. : 201-939-0
Molecular Formula : C₁₀H₂₀O

Producer related part
Company : Bayer AG
Creation date : 06.08.1992

Substance related part
Company : Bayer AG
Creation date : 06.08.1992

Status :
Memo : X AKTUELL EG/ICCA

Printing date : 18.03.2003
Revision date : 02.06.1994
Date of last update : 18.03.2003

Number of pages : 1

Chapter (profile) : Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile) : Reliability: without reliability, 1, 2, 3, 4
Flags (profile) : Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE),
Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information

Id	89-78-1
Date	18.03.2003

1.0.1 APPLICANT AND COMPANY INFORMATION

23.10.2001

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR**1.0.3 IDENTITY OF RECIPIENTS****1.0.4 DETAILS ON CATEGORY/TEMPLATE****1.1.0 SUBSTANCE IDENTIFICATION****1.1.1 GENERAL SUBSTANCE INFORMATION**

Purity type	:	
Substance type	:	organic
Physical status	:	solid
Purity	:	
Colour	:	white
Odour	:	minty
Flag	:	Critical study for SIDS endpoint

03.06.2002

1.1.2 SPECTRA**1.2 SYNONYMS AND TRADENAMES****5-METHYL-2-(1-METHYLETHYL)-CYCLOHEXANOL, RACEMATE**

Flag	:	Critical study for SIDS endpoint

03.06.2002

CYCLOHEXANOL; 5-METHYL-2-(1-METHYLETHYL)-

Flag	:	Critical study for SIDS endpoint

10.10.2001

DL-MENTHOL

Flag	:	Critical study for SIDS endpoint

MENTHOL

Flag	:	Critical study for SIDS endpoint

1. General Information

Id	89-78-1
Date	18.03.2003

03.06.2002

1.3 IMPURITIES**1.4 ADDITIVES****1.5 TOTAL QUANTITY****1.6.1 LABELLING**

Labelling	: provisionally by manufacturer/importer
Specific limits	:
Symbols	: Xi, , ,
Nota	: , ,
R-Phrases	: (38) Irritating to skin
S-Phrases	: (25) Avoid contact with eyes
Flag	: Critical study for SIDS endpoint

1.6.2 CLASSIFICATION

Classified	: provisionally by manufacturer/importer
Class of danger	: irritating
R-Phrases	: (38) Irritating to skin
Specific limits	:
Flag	: Critical study for SIDS endpoint

1.6.3 PACKAGING**1.7 USE PATTERN**

Type of use	: type
Category	: Wide dispersive use
Remark	: L-Menthol, D/L-menthol and menthol liquid are widely used as flavoring, disinfectant and cooling compounds in confectionery products, liqueurs, chewing gums, toothpastes, cosmetics and common cold ointments and medications and cleaning/washing agents and in veterinary activities.
Flag	: Critical study for SIDS endpoint
24.07.2002	
Type of use	: industrial
Category	: Chemical industry: used in synthesis
Remark	: To produce L-menthol, D/L-menthol is transesterified with

1. General Information

Id 89-78-1
Date 18.03.2003

Flag : methylbenzoate and further manufactured. Resulting products are L- and D-menthol.
03.06.2002 : Critical study for SIDS endpoint

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type : Production

Remark : D/L-menthol is produced via reaction of m-cresol with propen to thymol, and hydrogenation of thymol, resulting in 4 isomers: D/L-neomenthol, D/L-neoisomenthol, D/L-menthol and D/L-isomenthol. D/L-menthol is isolated by fractional distillation.

03.06.2002

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

Classified by : other: Bayer AG
Labelled by : other: Bayer AG
Class of danger : 1 (weakly water polluting)

1.8.4 MAJOR ACCIDENT HAZARDS

Legislation : Störfallverordnung (DE)
Substance listed : no
No. in Seveso directive :

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1. General Information

Id	89-78-1
Date	18.03.2003

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS**1.9.2 COMPONENTS****1.10 SOURCE OF EXPOSURE****1.11 ADDITIONAL REMARKS****1.12 LAST LITERATURE SEARCH**

Type of search : Internal and External
Chapters covered : 5
Date of search : 01.09.2001

Remark : Human Health: last literature search September 2001: CAS number search in external and internal databases, e.g. Biosis, Embase, Toxline, Scisearch
Flag : Critical study for SIDS endpoint
 03.07.2002

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 14.01.2002

Remark : Physico-chemical properties / Environment / Ecotoxicology : last literature search January 2002: CAS number search in external and internal databases, e.g. HSDB, Aquire.
Flag : Critical study for SIDS endpoint
 29.07.2002

1.13 REVIEWS

Memo : Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties
 03.06.2002

(1)

2. Physico-Chemical Data

Id 89-78-1
Date 18.03.2003

2.1 MELTING POINT

Value : ca. 30 - 32 °C
Sublimation :
Method :
Year : 2002
GLP : no data
Test substance : other TS: typical for technical intermediate

Flag : Critical study for SIDS endpoint
18.03.2003

(2) (3)

Value : 38 °C
Sublimation :
Method :
Year : 1996
GLP : no data
Test substance : no data

18.03.2003

(4)

Sublimation :
Method : other: DIN 51556
Year :
GLP :
Test substance :

Remark : Freezing temperature ca. 27 °C
03.06.2002

(2)

Sublimation :
Method :
Year : 1972
GLP : no data
Test substance : no data

Remark : Freezing point of (+/-) menthol: 27 - 28 °C, rising on prolonged stirring to 30 - 32 °C
18.03.2003

(5)

2.2 BOILING POINT

Value : 216 °C at

Flag : Critical study for SIDS endpoint
24.07.2002

(2) (4)

2.3 DENSITY

Type : density
Value : .903 g/cm³ at 15 °C

18.07.2002

(4)

2. Physico-Chemical Data

Id 89-78-1
Date 18.03.2003

Type : density
Value : .895 g/cm³ at 20 °C
Method : other: DIN 51757
Year :
GLP :
Test substance :

Flag : Critical study for SIDS endpoint
05.02.2002

(2)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : 1.3 hPa at 55 °C
Decomposition :
Method :
Year : 1977
GLP : no
Test substance : no data

18.03.2003

(6) (2)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : 3.4 at °C
pH value :
Method : other (measured)
Year : 1999
GLP : no data
Test substance : other TS: purity not reported but HPLC applied

Method : Reversed-phase high-performance liquid chromatography
Flag : Critical study for SIDS endpoint
14.03.2003

(7)

Partition coefficient : octanol-water
Log pow : 3.38 at °C
pH value :
Method : other (calculated): SRC-KOWWIN v. 1.66
Year : 2002
GLP :
Test substance :

18.03.2003

(8)

Partition coefficient : octanol-water
Log pow : 3.2 at °C
pH value :
Method : other (calculated): Leo, A. CLOGP-3.54 MedChem Software 1989.
Daylight, Chemical Information Systems, Claremont, CA 91711, USA
Year : 1991
GLP :

2. Physico-Chemical Data

Id 89-78-1
Date 18.03.2003

Test substance	:		
06.03.2003			(9)
Method	:		
Year	:	1979	
GLP	:	no data	
Test substance	:	no data	
Result	:	log partition oils/water 2.27 resp. 2.40 according to 2 cited references	
18.03.2003			(10)
Partition coefficient	:	octanol-water	
Log pow	:	3.3 at °C	
pH value	:		
Remark	:	The reference notes both Cas-No. 2216-51-5 and 15356-70-4 (former CAS -No. for 89-78-1)	
06.03.2003			(11)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water	
Value	:	508 mg/l at 20 °C	
pH value	:		
concentration	:	at °C	
Temperature effects	:		
Examine different pol.	:		
pKa	:	at 25 °C	
Description	:		
Stable	:		
Deg. product	:		
Method	:	other: flask method	
Year	:	1990	
GLP	:		
Test substance	:	other TS: HR product 131136, d,l-Menthol, purity 99.4 %	
Flag	:	Critical study for SIDS endpoint	
07.03.2003			(12)
Solubility in	:	Water	
Value	:	456 mg/l at 25 °C	
pH value	:		
concentration	:	at °C	
Temperature effects	:		
Examine different pol.	:		
pKa	:	at 25 °C	
Description	:		
Stable	:		
06.03.2003			(13) (14)
Solubility in	:	Water	
Value	:	431 mg/l at 20 °C	
pH value	:		
concentration	:	at °C	
Temperature effects	:		

2. Physico-Chemical Data

Id 89-78-1
Date 18.03.2003

Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 2002
GLP : no data
Test substance : no data

18.03.2003 (2)

2.6.2 SURFACE TENSION**2.7 FLASH POINT**

Value : 92 °C
Type : closed cup

05.02.2002 (2)

2.8 AUTO FLAMMABILITY**2.9 FLAMMABILITY****2.10 EXPLOSIVE PROPERTIES**

Result : other: lower limit 0.80 Vol%, upper limit 7.00 Vol%

06.05.2002 (2)

2.11 OXIDIZING PROPERTIES**2.12 DISSOCIATION CONSTANT****2.13 VISCOSITY**

Value : 6 - mm²/s (static) at 50 °C
Result :

05.02.2002 (2)

2.14 ADDITIONAL REMARKS

Memo : Ignition temperature ca. 405 °C

2. Physico-Chemical Data

Id	89-78-1
Date	18.03.2003

Method	:	DIN 51794	
03.06.2002			(2)

Memo	:	Refractive index nD20 = 1.4615	

Flag	:	Critical study for SIDS endpoint	
03.06.2002			(4)

3. Environmental Fate and Pathways

Id 89-78-1
Date 18.03.2003

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight

Method : structure estimation method
Result : Rate constant: $k = 2.4 \times 10^{-11}$ cm³/molecule/sec at 25 degrees C;
considering an atmospheric OH-radical concentration of 5×10^5 OH-radicals/cm³, the half-life is about 16 h

Reliability : (2) valid with restrictions
accepted calculation procedure

Flag : Critical study for SIDS endpoint
29.07.2002

(15)

3.1.2 STABILITY IN WATER

Deg. product :
Method : other (calculated)
Year :
GLP :
Test substance :

Result : volatilization half-lives for a model river (1 m deep, flow-rate 1 m/sec, wind velocity 3 m/sec) and a model lake (1 m deep, flow-rate 0.05 m/sec, wind velocity 0.5 m/sec) are estimated to be 2 and 18 days

Reliability : (2) valid with restrictions
accepted calculation procedure derived from L-menthol cause of structural similarities

Flag : Critical study for SIDS endpoint
30.07.2002

(16)

3.1.3 STABILITY IN SOIL**3.2.1 MONITORING DATA****3.2.2 FIELD STUDIES****3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS**

Type : volatility
Media : water - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year : 2003

3. Environmental Fate and Pathways

Id 89-78-1
Date 18.03.2003

Result : Based on a water solubility of 508 mg/l and a vapour pressure of 8.5 Pa (see chapter 2), the Henry's law constant is calculated to be 2.62 Pa x m³/mol at 25°C

Reliability : (2) valid with restrictions
Generally accepted calculation method

Flag : Critical study for SIDS endpoint

14.03.2003

(17)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water

Method : Calculation according Mackay, Level I

Year : 2003

Result : air: 39.5 %
water: 43.8 %
soil: 8.7 %
sediment: 7.9 %
biota: 0.0055 %

Test condition : Base data for calculation:
temperature: 20 °C
molar mass: 156.27 g/mol
vapour pressure: 8.5 Pa
water solubility: 508 g/m³
log Kow: 3.4
environmental compartments:
- air: 6*10⁹ m³, 1.2 kg/m³
- water: 7*10⁶ m³, 1000 kg/m³
- soil: 4.5 *10⁴ m³, 1500 kg/m³, 2 % org. C
- sediment: 2.1*10⁴ m³, 1300 kg/m², 5 % org. C
- susp. sediment: 35 m³, 1500 kg/m³, 16.7 % org. C
- aerosol: 0.12 m³, 1500 kg/m³
- aquatic biota: 7 m³, 1000 kg/m³, 5 % fat

Reliability : (2) valid with restrictions
Generally accepted calculation method

Flag : Critical study for SIDS endpoint

14.03.2003

(17)

Media : water - soil

Method : other (calculation)

Year :

Result : Using the equation log Koc = 0.52 log Kow + 1.02 and based on a log Kow of 3.40 (see chapter 2) a Koc value of 614 can be calculated for the distribution between the organic phase of soil and pore water

Reliability : (2) valid with restrictions
Generally accepted calculation method

Flag : Critical study for SIDS endpoint

07.03.2003

(18)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic

3. Environmental Fate and Pathways

Id 89-78-1
Date 18.03.2003

Inoculum : activated sludge
Concentration : 100 mg/l related to Test substance related to
Contact time : 28 day(s)
Degradation : 0 (±) % after 28 day(s)
Result :
Deg. product :
Method : other: corresponding to OECD 301C
Year : 1992
GLP :
Test substance : other TS: not clear

Remark : TS not clear. The reference notes two CAS -No.: 2216-51-5 and 15356-70-4 (= 89-78-1)
Test condition : sludge concentration 30 mg/l
Reliability : (3) invalid
 Biodegradation possibly affected by toxicity of the substance at the concentration tested
Flag : Critical study for SIDS endpoint
 05.03.2003

(11)

3.6 BOD5, COD OR BOD5/COD RATIO

COD
Method :
Year :
COD : 2767 mg/g substance
GLP :
Remark : ThOD: 2970 mg/g
Reliability : (4) not assignable
 Original reference in Czech
 30.07.2002

(19)

3.7 BIOACCUMULATION**3.8 ADDITIONAL REMARKS**

4. Ecotoxicity

Id 89-78-1
Date 18.03.2003

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	: static
Species	: Brachydanio rerio (Fish, fresh water)
Exposure period	: 96 hour(s)
Unit	: mg/l
LC0	: 11.3
LC50	: 17.6
LC100	: 26.2
Limit test	: no
Analytical monitoring	: yes
Method	: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	: 1990
GLP	: yes
Test substance	: other TS: HR product 131136, d,l-Menthol, purity 99.4 %
Remark	: analyt. monitoring: GC D,L-menthol (rac., solid) LC50: 17.2 mg/l (geom. mean between LC0 and LC100) LC50: 17.6 mg/l (calculated with probit analysis). This value is not reported in the original study. All effect values reported in the study based on nominal concentrations. However it can be derived the effective values. The results here given based on real concentrations (LC0=11.3 mg/l, LC100=26.2 mg/l).
Result	: RESULTS: EXPOSED - Nominal/measured concentrations: m.(mg/l) 7.8 11 16 22 31 c. (mg/l) (0 h) 7.2 9.9 11.5 17.1 26.5 c. (mg/l) (24 h) 6.7 9.7 13.2 19.5 25.8 c. (mg/l) (48 h) 6.3 9.2 12.3 18.9 c. (mg/l) (72 h) 6.1 9.1 10.8 19.1 c. (mg/l) (96 h) 5.9 8.7 8.9 18.8 - Effect data (Mortality): Mortality, visible abnormalities of fishes - Concentration / response curve: There were no dead fishes in tanks with concentration: 7.8, 11 and 16 mg/l. 22 mg/l hours (h) 0 24 48 72 96 Mortality (%) 0 80 90 90 90 31 mg/l Mortality (%) 0 100 - Effect concentration vs. test substance solubility: Despite bad solubility and high volatility of substance the required concentration was not reached during testing procedure. Undissolved substance particles remained on the water surface at the start of the test: After 2 hours there were no particles visible at concentration 7.8 mg/l, after 6 hours the particles in tanks with concentration 11 mg/l and 16 mg/l disappeared and after 24 hours there were no undissolved particles in any fish tank visible. - Other effects: RESULTS: CONTROL: No dead fish - Number/percentage of animals showing adverse effects: 7.8 mg/l hours (h) 2 24 48 72 96 7.8 mg/l - - - - - 11 mg/l - - 100%A 90%A 10%B 10%B 16 mg/l - 80%A 80%A 90%A 90%A

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20%B 20%B 10%B 10%B
 22 mg/l 90%B 20%B 10%B 10%B 10%B
 10%A

31 mg/l 100%B

- Nature of adverse effects:

A: slow and inactive swimming behaviour

B: loss of equilibrium (uncontrolled movements)

Reliability

: (2) valid with restrictions

Guideline study; effective concentrations decreased below 80% of the nominal during the test period

Flag

07.03.2003

: Critical study for SIDS endpoint

(20)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : 15.7
EC50 : 71
EC100 : 125
Limit Test : no
Analytical monitoring : no
Method : other: Daphnien-Schwimmunfaehigkeits-Test, UBA-Verfahrensvorschlag
 Mai 1984, Bestimmung der Schwimmunfaehigkeit beim Wasserfloh
 Daphnia magna, EC0, EC50, EC100, 24h, static
Year : 1990
GLP : yes
Test substance : other TS: HR product 131136, d,l-Menthol, purity 99.4 %

Remark : D/L-menthol (rac., solid)
 EC50 = 44.3 mg/l (geom. mean between LC0 and LC100)
 EC50 = 71 mg/l (calculated with probit analysis). This value was not reported in the original study.
 To produce the stock solution, 300 mg/l was weighed into water and after treatment with an Ultra-Turrax for 60 sec., stirred on a magnetic stirrer for two hours. In order to avoid uneven distribution of undissolved particles, the solution was kept in movement when preparing the test concentrations.

Result : RESULTS: EXPOSED
 - Nominal concentrations:
 250 mg/l, 125 mg/l, 63 mg/l, 31 mg/l, 15.7 mg/l, 7.8 mg/l,
 4.0 mg/l, 2.0 mg/l, no analytical monitoring
 - Effect data (Immobilisation):
 Immobilisation of Daphnia magna
 - Concentration / response curve:
 - Cumulative immobilisation:
 after 24 hours, 2 replicates:
 conc. (mg/l) 250 125 63 31 15.7 7.8 4.0 2.0
 immobile
 Daphnia (%) 100 100 35 25 0 0 0 0
 - Effect concentration vs. test substance solubility:
 The water solubility of test substance is low. To produce the stock solution, 300 mg/l was weighed into water and after treatment with an Ultra-Turrax for 60 sec., stirred on a magnetic stirrer for two hours. In order to avoid uneven distribution of undissolved particles, the solution was kept in movement when preparing the test concentrations.
 - Other effects:

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	RESULTS CONTROL: no immobile Daphnia
	RESULTS: TEST WITH REFERENCE SUBSTANCE
	Reference substance: Potassium dichromate
	- Results:
	conc. (mg/l) 0.5 1.0 1.5 2.0 3.0 4.0 5.0
	immobile
	Daphnia (%) 0 0 15 30 30 45 65
Test condition	: TEST ORGANISMS: Daphnia magna Straus, parthenogenetic female
	- Strain: clone, Bundesgesundheitsamt Berlin
	- Source/supplier: Lab breeding, Bayer AG Leverkusen
	- Age: 6-24 h
	STOCK AND TEST SOLUTION AND THEIR PREPARATION
	- Vehicle, solvent: Filtered surface water, Monheimer Kiesgrube
	DILUTION WATER: Filtered surface water
	- Source: Monheimer Kiesgrube
	TEST SYSTEM
	- Test type: static
	- Concentrations: see results
	- Renewal of test solution: no
	- Exposure vessel type: cylindric vessels, diameter: 4.0 cm, height: 6.5 cm
	- Number of replicates, individuals per replicate: 2 replicates with test substance, and 2 replicates with reference substance, 10 individuals in each test
	- Test temperature: between 19.9 and 20.0 °C
	- Dissolved oxygen: between 8.1 and 8.2 mg/l
	- pH: between 8.3 and 8.4
	- Adjustment of pH: no
	DURATION OF THE TEST: 24 hours
	TEST PARAMETER: Oxygen (mg/l), pH, Temperature (°C), Number of immobile Daphnia
	MONITORING OF TEST SUBSTANCE CONCENTRATION: no
Reliability	: (2) valid with restrictions
	Test procedure comparable to standard method and in accordance with general accepted scientific standards; detailed documentation of test procedure and test conditions but not analytical monitoring was conducted
Flag	: Critical study for SIDS endpoint
05.03.2003	

(21)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	: Scenedesmus subspicatus (Algae)
Endpoint	: growth rate
Exposure period	: 72 hour(s)
Unit	: mg/l
NOEC	: 5
LOEC	: 10
ErC50	: 16.2
Limit test	: no
Analytical monitoring	: yes
Method	: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	: 2000
GLP	: yes
Test substance	: other TS: HR product 131136, d,l-Menthol, purity 99 %
Remark	: The NOEC, LOEC and ErC50 values are given as nominal concentrations as the analytical control (TOC measurements) revealed that the test concentrations have not decreased below 80 % of the nominal. The test concentrations 1.25 and 2.5 mg/l were not analytically determined

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Result

- as the measured concentrations to be expected were below the detection limit of the TOC determination method (2 mg/l) d,l-menthol (rac., solid)
- : **RESULTS: EXPOSED**
- Nominal concentrations:
nominal concentration analytically determined conc.
(mg/l)
- | test substance (mg/l) | 0 hours | 72 hours |
|-----------------------|---------|----------|
| control | < 2 | 3 |
| w. algae* 5 | 5 | 3 |
| 1.25 | - | - |
| 2.5 | - | - |
| 5 | 4 | 3 |
| 10 | 8 | 7 |
| 20 | 15 | 13 |
| 40 | 29 | 26 |
| w. algae* 40 | 27 | 27 |
- * w. algae: without algal inoculum
- All values refer to TOC determination. According to the relevant product information 1 mg/l TOC equals to 1.3 mg/l of the test substance (empirical formula: C₁₀ H₂₀ O; molecular weight: 156.3 mg/l).
- Effect data/Element values:
- Cell density data:
- | Nominal concentr.
test substance
(mg/l) | Number of cells/ml
mean values (3 replicates) | | |
|---|--|----------|----------|
| | 24 hours | 48 hours | 72 hours |
| control* | 45600 | 188000 | 527000 |
| 1.25 | 41100 | 174000 | 541000 |
| 2.5 | 47800 | 180000 | 548000 |
| 5 | 44400 | 161000 | 452000 |
| 10 | 40000 | 118000 | 287000 |
| 20 | 32200 | 60000 | 50000 |
| 40 | 18900 | 15500 | 10000 |
- *control - 6 replicates
- Growth curves:
- | Nominal concentr.
test substance
(mg/l) | Growth (b) | Growth rate (r) |
|---|---------------|-----------------|
| control | 472000 (0.0) | 1.3 (0.0) |
| 1.25 | 461000 (2.3) | 1.3 (0.0) |
| 2.5 | 477000 (-1.1) | 1.3 (0.0) |
| 5 | 406000 (14.0) | 1.3 (0.0) |
| 10 | 277000 (41.3) | 1.1 (15.4) |
| 20 | 92200 (80.5) | 0.5 (61.5) |
| 40 | 14400 (96.9) | 0.0 (100.0) |
- Values in brackets indicate % inhibition [+] or % increase [-]
- RESULTS CONTROL: see above
- : (1) valid without restriction
Guideline study
- : Critical study for SIDS endpoint

Reliability**Flag**

07.08.2002

(22)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA**Type**

: aquatic

Species

: activated sludge

Exposure period

: 3 hour(s)

Unit

: mg/l

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EC10 : 117
EC50 : 306
EC5 : 89
EC90 : 800
Analytical monitoring : no
Method : OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year : 1989
GLP : yes
Test substance : other TS: HR product 131136, d,l-Menthol, purity 99.4 %

Remark : Direct weight
Reliability : (1) valid without restriction
 Guideline Study; detailed documentation of test procedure and test conditions

Flag : Critical study for SIDS endpoint

29.07.2002

(23)

4.5.1 CHRONIC TOXICITY TO FISH**4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES****4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS****4.6.2 TOXICITY TO TERRESTRIAL PLANTS****4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS****4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES****4.7 BIOLOGICAL EFFECTS MONITORING****4.8 BIOTRANSFORMATION AND KINETICS****4.9 ADDITIONAL REMARKS**

5. Toxicity

Id

89-78-1

Date

18.03.2003

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo	:	
Type	:	Metabolism
Species	:	rabbit
Number of animals		
Males	:	
Females	:	4
Doses		
Males	:	1 g/kg
Females	:	
Vehicle	:	
Method	:	
Year	:	1938
GLP	:	no
Test substance	:	other TS: D-, L- and D/L-menthol
Remark	:	The main objects of this study were to find out whether the optical and geometrical isomerism of the menthols influenced their conjugation with glucuronic acid in the body and whether the feeding of a D/L -menthol resulted in the excretion of a conjugated glucuronide containing more of one antipode than the other.
Result	:	After a single oral administration of 1 g/kg bw of menthol racemic to rabbits, 59 % of the applied test substance was excreted as glucuronide with the urine within 2 d.
Test condition	:	Urine was collected for 2 days and analysed for conjugated glucuronic acid by ether extraction.
Reliability	:	(2) valid with restrictions limited documentation
Flag	:	Critical study for SIDS endpoint
19.02.2003		

(24)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 2602 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	female
Number of animals	:	10
Vehicle	:	peanut oil
Doses	:	2000, 2500, 3000, 3500 mg/kg bw
Method	:	other
Year	:	1974
GLP	:	no
Test substance	:	other TS: menthol racemic 100
Result	:	MORTALITY: - Time of death: 1-3 days after application - Number of deaths at each dose: dose (mg/kg bw): number of deaths 2000 1/10 2500 4/10 3000 7/10 3500 10/10 CLINICAL SIGNS: narcotic status (no data available on exposure level at

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		which the clinical signs were observed)	
		NECROPSY FINDINGS: no data	
Test condition	:	ADMINISTRATION:	
		- Volume administered or concentration: 10-20 ml/kg	
		- Post dose observation period: 14 days	
		EXAMINATIONS:	
		deaths, clinical signs	
		No further information given on statistical methods and confidence limits.	
Reliability	:	(2) valid with restrictions	
		Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: no information on statistical methods and confidence limits.	
Flag	:	Critical study for SIDS endpoint	
19.02.2003			(25)
Type	:	LD50	
Value	:	= 2900 mg/kg bw	
Species	:	rat	
Strain	:	no data	
Sex	:	no data	
Number of animals	:		
Vehicle	:	no data	
Doses	:		
Method	:	other	
Year	:	1961	
GLP	:	no	
Test substance	:	other TS: not further specified	
Reliability	:	(4) not assignable	
		Secondary literature	
19.02.2003			(26) (27)
Type	:	LD50	
Value	:	= 3100 mg/kg bw	
Species	:	mouse	
Strain	:	no data	
Sex	:	no data	
Number of animals	:	10	
Vehicle	:	other: olive oil	
Doses	:	2000, 4000 mg/kg bw	
Method	:	other	
Year	:	1932	
GLP	:	no	
Test substance	:	other TS: not further specified	
Reliability	:	(4) not assignable	
		Documentation insufficient.	
19.02.2003			(28)
Type	:	other: lethal dose	
Value	:	1500 - 1600 mg/kg bw	
Species	:	cat	
Strain	:	no data	
Sex	:	no data	
Number of animals	:		
Vehicle	:	no data	
Doses	:	no data	
Method	:	other	
Year	:	1926	

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18.03.2003

GLP : no
Test substance : other TS: synthetic menthol (inactive, Fp.: 35-38 °C)

Reliability : (4) not assignable
 Documentation insufficient.

19.02.2003

(29)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 5000 mg/kg bw
Species : rabbit
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year : 1973
GLP : no
Test substance : other TS: not further specified

Reliability : (2) valid with restrictions
 Secondary literature from peer-reviewed journal

Flag : Critical study for SIDS endpoint

19.02.2003

(30)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : = 750 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1961
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
 Secondary literature

19.02.2003

(27)

Type : LD50
Value : = 670 mg/kg bw
Species : rat
Strain : other: white rats

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Sex : no data
Number of animals : 10
Vehicle : other: olive oil
Doses : 500, 600, 700, 800, 900, 1000, 1100 mg/kg bw
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1952
GLP : no
Test substance : other TS: not further specified

Result : MORTALITY:
- Time of death: 12 hours after application
- Number of deaths at each dose:
dose (mg/kg bw)/deaths
500/2/10
600/4/10
700/7/10
800/9/10
900/8/10
1000/9/10
1100/10/10

CLINICAL SIGNS: loss of equilibrium, partial to total relaxation, deep sleep with abolition of reflexes

Test condition : TEST ORGANISMS:
- Source: no data
- Age: no data
- Weight at study initiation: 90-120 g
- Controls: no data
ADMINISTRATION:
- Post dose observation period: the animals were observed until deaths or until return to normal behaviour
EXAMINATIONS:
deaths, clinical signs

Reliability : (2) valid with restrictions
limited documentation

04.03.2003

(31)

Type : LD50
Value : ca. 2000 mg/kg bw
Species : rabbit
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1961
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
Secondary literature

19.02.2003

(27)

Type : LD50
Value : = 865 mg/kg bw

5. Toxicity

Id 89-78-1
Date 18.03.2003

Species : guinea pig
Strain : no data
Sex : no data
Number of animals : 10
Vehicle : other: olive oil
Doses : 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 mg/kg bw
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1952
GLP : no
Test substance : other TS: not further specified

Result : MORTALITY:
- Time of death: 12 hours after application
- Number of deaths at each dose:
dose (mg/kg bw)/deaths
500/1/10
600/4/10
700/4/10
800/6/10
900/6/10
1000/7/10
1100/6/10
1200/7/10
1300/8/10
1400/10/10
CLINICAL SIGNS: loss of equilibrium, partial to total relaxation, deep sleep with abolition of reflexes

Test condition : TEST ORGANISMS:
- Source: no data
- Age: no data
- Weight at study initiation: 280-360 g
- Controls: no data
ADMINISTRATION:
- Post dose observation period: the animals were observed until deaths or until return to normal behaviour
EXAMINATIONS:
deaths, clinical signs
Reliability : (2) valid with restrictions
limited documentation

04.03.2003

(31)

Type : other: LD
Value : > 1500 mg/kg bw
Species : cat
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: not specified oil
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1926
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable

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19.02.2003	Documentation insufficient.	(29)
Type	: other: LD	
Value	: > 1000 mg/kg bw	
Species	: rat	
Strain	: no data	
Sex	: no data	
Number of animals	:	
Vehicle	: other: not specified oil	
Doses	:	
Route of admin.	: s.c.	
Exposure time	:	
Method	: other	
Year	: 1926	
GLP	: no	
Test substance	: other TS: not further specified	
Reliability	: (4) not assignable Documentation insufficient.	
19.02.2003		(29)
Type	: other: LD	
Value	: > 14000 mg/kg bw	
Species	: mouse	
Strain	: no data	
Sex	: no data	
Number of animals	:	
Vehicle	: other: not specified oil	
Doses	:	
Route of admin.	: s.c.	
Exposure time	:	
Method	: other	
Year	: 1926	
GLP	: no	
Test substance	: other TS: not further specified	
Reliability	: (4) not assignable Documentation insufficient.	
19.02.2003		(29)
Type	: LD50	
Value	: = 14200 mg/kg bw	
Species	: mouse	
Strain	: no data	
Sex	: no data	
Number of animals	:	
Vehicle	: no data	
Doses	:	
Route of admin.	: other: not specified	
Exposure time	:	
Method	: other	
Year	: 1962	
GLP	: no	
Test substance	: other TS: not further specified	
Reliability	: (4) not assignable Insufficient documentation for assessment.	
04.03.2003		(32)

5. Toxicity

Id 89-78-1
Date 18.03.2003

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : 100 %
Exposure : Semiocclusive
Exposure time : 4 hour(s)
Number of animals : 4
Vehicle : other: diethylphthalate (DEP)
PDII :
Result : moderately irritating
Classification :
Method : OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: HR 89/131136, purity: no data

Result : AVERAGE SCORE
100%/50%/25%/5%/1%/Vehicle
3.0/1.6/0.8/0.2/0.1/0.1 (erythema)
3.0/1.7/0.5/0.0/0.0/0.0 (oedema)
REVERSIBILITY: Yes
Day 7: 100%: in 3/4 - treated sites were covered with a layer of white-brown scales, 1/4 - thin layer of white scales
50%: 4/4 - white scales
25%: 1/4 - scattered white scales
Day 14: 100%: 4/4 - treated sites were covered with white to white-brown scales, underlying skin was intact
50%: 3/4 - treated sites showed scattered scale formation on intact skin.

Test condition : TEST ANIMALS:
- Strain: Chbb:HM (C.H.Boehringer/Biberach)
- Sex: female
- Source: Dr. Karl Thomae GmbH, Biberach an der Riss
- Age: no data
- Weight at study initiation: 2400-2700 g
- Number of animals: 4
- Controls: internal control (one part of skin)
ADMINISTRATION/EXPOSURE
- Preparation of test substance: dilutions of substance with DEP, concentrated test substance was moistened with DEP in the ratio 6:1
- Area of exposure: six different fields on back (two anterior, two centrally located and two posterior treatment sites)
- Concentration in vehicle: 100, 50, 25, 5 and 1 %, Vehicle
- Total volume applied: 0.5 ml
- Postexposure period: up to 14 days
- Removal of test substance: skin was washed with luke warm water and soap

Reliability : (2) valid with restrictions
purity of TS not stated

Flag : Critical study for SIDS endpoint
19.02.2003

(33)

Species : guinea pig
Concentration : no data
Exposure : Open
Exposure time : 14 day(s)
Number of animals : 20
Vehicle : no data
PDII :

5. Toxicity

Id 89-78-1
Date 18.03.2003

Result : not irritating
Classification : not irritating
Method : other
Year : 1974
GLP : no data
Test substance : other TS: menthol racemic 100

Test condition : Substance was rubbed into the skin for 30 s once daily.
Substance was applied 2 x 5 days, results were taken after 14 days.

Reliability : (3) invalid
Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.

17.12.2001 (34)

Species : human
Concentration : 8 %
Exposure : Occlusive
Exposure time : 48 hour(s)
Number of animals :
Vehicle : petrolatum
PDII :
Result : not irritating
Classification :
Method : other: closed patch-test
Year : 1973
GLP : no
Test substance : other TS: not further specified

Reliability : (4) not assignable
Secondary literature

05.12.2001 (35)

Species : rabbit
Concentration : no data
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals :
Vehicle : no data
PDII :
Result : slightly irritating
Classification :
Method : other
Year : 1973
GLP : no
Test substance : other TS: not further specified

Test condition : Substance applied full strength to intact or abraded skin
(no further data)

Reliability : (4) not assignable
Secondary literature

17.12.2001 (30)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : 40 %
Dose : .1 ml

5. Toxicity

Id 89-78-1
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Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 4
Vehicle : other: diethylphthalate (DEP)
Result : slightly irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol rac. HR 89/131136 DEP, purity: no data

Result : AVERAGE SCORE:
 - Cornea opacity: 0.8
 - Iris lesion: 0.0
 - Conjunctivae (Redness): 1.5
 - Conjunctivae (Chemosis): 0.4
 REVERSIBILITY: a slight reaction of conjunctiva was seen in one rabbit on day 7

Test condition : TEST ANIMALS:
 - Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya)
 - Sex: female
 - Source: Dr. Karl Thomae GmbH, Biberach an der Riss
 - Age: no data
 - Weight at study initiation: 2400-2800 g
 - Number of animals: 4
 - Controls: internal control (right eye)

Reliability : (2) valid with restrictions
 purity of TS not stated, short duration of experiment

Flag : Critical study for SIDS endpoint
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(36)

Species : rabbit
Concentration : 64 %
Dose : .1 ml
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 4
Vehicle : other: 40 % solution of d,l-menthol in DEP (HR 89/131136 DEP)
Result : slightly irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol rac, HR 89/131136

Result : AVERAGE SCORE
 HR 89/131136 64 %/Vehicle
 1.0/1.0 (cornea)
 0.0/0.0 (iris)
 2.1/1.9 (redness of conjunctivae)
 0.3/0.3 (chemosis, conjunctivae)
 The right eyes were treated with the vehicle (40% d/l-menthol in DEP) and the left eyes with the test article solution. Both articles had almost the same eye-irritating potential.
 REVERSIBILITY: yes, no reactions observed at day 7

Test condition : TEST ANIMALS:
 - Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya)
 - Sex: female
 - Source: Dr. Karl Thomae GmbH, Biberach an der Riss

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- Age: no data
- Weight at study initiation: 2600-2800 g
- Number of animals: 4
- Controls: internal control with vehicle (right eye)
- ADMINISTRATION/EXPOSURE
- Preparation of test substance: Test article was pulverized in a mortar and then diluted with vehicle (absolute concentration of substance in DEP is 64%).
- Vehicle: 40% d/l-menthol in DEP (HR 89/131136 DEP, previously tested by Scantox, lab.no.: 11753)

Reliability : (2) valid with restrictions
purity of TS not stated, no untreated controls

Flag : Critical study for SIDS endpoint

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(37)

Species : rabbit
Concentration : 60 %
Dose : .1 ml
Exposure time : 1 minute(s)
Comment : other
Number of animals : 8
Vehicle : other: olive oil
Result : not irritating
Classification : not irritating
Method : Draize Test
Year : 1974
GLP : no data
Test substance : other TS: menthol racemic 100

Test condition : Substance was initially applied in 10, 20 and 30 % solution.
The eyes of 4 animals were rinsed 1 minute after application with physiological saline, substance remained in the eyes of 4 animals. In a second step animals were treated with concentration of 40, 50 and 60 % .

Reliability : (2) valid with restrictions
limited documentation

04.03.2003

(34)

5.3 SENSITIZATION

Type : other: open repetitive dermal test
Species : guinea pig
Number of animals : 20
Vehicle : no data
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1974
GLP : no data
Test substance : other TS: menthol racemic 100

Test condition : Substance was rubbed into shaved skin for 30 sec once daily for 3x5 days.
After 5 days without application the test substance was rubbed into an untreated part of the skin.
Results were taken after 24 h, 2 and 3 days.

Reliability : (3) invalid
Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.

17.12.2001

(34)

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Type : other: Maximization test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1973
GLP : no
Test substance : other TS: dl-menthol, 8%

Result : The material produced no sensitization reactions.
Test condition : Number of volunteers: 25
Reliability : (2) valid with restrictions
 Data cited by a peer-reviewed standard reference journal
Flag : Critical study for SIDS endpoint
 19.02.2003

(35)

5.4 REPEATED DOSE TOXICITY

Type : Sub-chronic
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 13 w
Frequency of treatm. : continuously
Post exposure period : 1 w
Doses : 930, 1870, 3750, 7500, 15000 ppm
Control group : yes, concurrent vehicle
Method : other
Year : 1974
GLP : no
Test substance : other TS: USP grade was used

Result : ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX
 calculated relation of % in diet to mg/kg bw/d (data obtained from detailed food consumption table given in the study):
 930, 1870, 3750, 7500 or 15000 ppm =
 for male mice: 243, 488, 978, 1956, 3913 mg/kg bw/d
 for female mice: 290, 595, 1193, 2386, 4773 mg/kg bw/d

NOAEL for male mice: 1956 mg/kg bw/d, based on reduced body weight gain
 NOAEL for female mice: 2386 mg/kg bw/d, based on reduced body weight gain

- Time of death:
 male: control (1/week 12), 3750 ppm (1/week 13), 7500 ppm (1/week 7), 15000 ppm (1/week 1 and 1/week 2)
 female: control (1/week 5 and 1/week 6), 15000 ppm (1/week 11)
 - Number of deaths at each dose:
 control (1 male/2 female), 3750 ppm (1 male), 7500 ppm (1 male), 15000 ppm (2 male/1 female)
 TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
 - Mortality and time to death: no effect
 - Clinical signs: hunched appearance, localized alopecia, and urine stains

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(sporadically noted in a few animals in each group including controls)

- Body weight gain:
15000 ppm, m, f: decrease (5-10 % of controls)
- Food/water consumption: no effect
- Gross pathology: no effect
- Histopathology: no effect

RESPONSE/EFFECTS BY DOSE LEVEL which are not compound related:
Lung (peribronchial or perivascular lymphoid hyperplasia, lung congestion):
m: control 8/10, 7500 ppm 3/10, 15000 ppm 1/10
f: control 4/10, 7500 ppm 6/10, 15000 ppm 6/10
These findings revealed early spontaneous respiratory disease lesions.
Kidney (interstitial nephritis, nonsuppurative pyelitis):
m: control 3/10, 7500 ppm 0/10, 15000 ppm 2/10
f: control 1/10, 7500 ppm 5/10, 15000 ppm 2/10
These findings are noted as spontaneous lesions.

Test condition

: TEST ORGANISMS

- Age: 5 weeks
- Weight at study initiation: males: 16-23 g, females: 17-22g
- Number of animals/dose group: 10 male and 10 female

ADMINISTRATION / EXPOSURE

- Vehicle: corn oil

EXAMINATIONS:

- Clinical signs: appearance and behaviour was recorded weekly
- Mortality: yes (daily)
- Body weight: yes (weekly monitored)
- Food consumption: yes (weekly monitored)
- Water consumption: no
- Organ weights: no
- Ophthalmoscopic examination: no
- Haematology: no
- Biochemistry: no
- Urinalysis: no
- Histopathology: for the 7500 and 15000 ppm concentration groups

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
Organs examined and preserved mainly as described in OECD guideline 408 (Exceptions: No full histopathological examination was carried out on the aorta, peripheral nerve and spinal cord)
STATISTICAL METHODS: only standard deviations given

Reliability

: (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: no statistics and no data on organ weights

Flag

: Critical study for SIDS endpoint

07.08.2002

(38)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 13 w
Frequency of treatm. : continuously
Post exposure period : 1 w
Doses : 930, 1870, 3750, 7500, 15000 ppm
Control group : yes, concurrent vehicle
Method : other
Year : 1976
GLP : no

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Test substance	: other TS: USP grade was used
Result	: ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX calculated relation of % in diet to mg/kg bw/d (data obtained from detailed food consumption table given in the paper): 930, 1870, 3750, 7500 or 15000 ppm = for male rats: 59, 114, 231, 472, 937 mg/kg bw/d for female rats: 67, 142, 285, 521, 998 mg/kg bw/d NOAEL for male rats: 937 mg/kg bw/d NOAEL for female rats: 998 mg/kg bw/d - Time of death: no deaths - Number of deaths at each dose: none TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: no effect - Clinical signs: hunched appearance with wheezing, localized alopecia, urine stains, soft feces, and redness of the eye (sporadically noted in a few animals in each group including controls) - Body weight gain: no effect - Food/water consumption: no effect - Gross pathology: no effect - Histopathology: 15000 ppm males: Minimal increase in the severity of spontaneous interstitial nephritis (finding of questionable significance) No further dose-dependent observations.
Test condition	: TEST ORGANISMS - Age: 8 weeks - Weight at study initiation: males: 166-214 g, females: 121-149g - Number of animals/dose group: 10 male and 10 female ADMINISTRATION / EXPOSURE - Vehicle: corn oil EXAMINATIONS: - Clinical signs: appearance and behaviour was recorded weekly - Mortality: yes (daily) - Body weight: yes (weekly monitored) - Food consumption: yes (weekly monitored) - Water consumption: no - Organ weights: no - Ophthalmoscopic examination: no - Haematology: no - Biochemistry: no - Urinalysis: no - Histopathology: yes ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): Organs examined and preserved as described in OECD guideline 408 (Exceptions: No full histopathological examination was carried out on the aorta, peripheral nerve and the spinal cord) STATISTICAL METHODS: only standard deviations given
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: no statistics and no data on organ weights
Flag 07.08.2002	: Critical study for SIDS endpoint

(39)

5. Toxicity

Id 89-78-1
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Type :
Species : rat
Sex : male/female
Strain : no data
Route of admin. : other: diet
Exposure period : 5.5 w
Frequency of treatm. : daily
Post exposure period : no data
Doses : 0, 100 or 200 mg/kg bw/d
Control group : other: Yes, not specified
NOAEL : 200 mg/kg bw
Method : other: no data
Year : 1961
GLP : no
Test substance : other TS: L-menthol or D/L-menthol

Remark : other: Repeated dose study
Result : No adverse effects on weight gain, excretion of glucuronides, water, or electrolytes, or interference with central nervous system reactions to stimulants were observed

Test condition : NUMBER OF ANIMALS: 40 rats of each sex/dose
Reliability : (2) valid with restrictions
secondary citation from peer-reviewed expert document (FAO/WHO report 1999)

Flag : Critical study for SIDS endpoint
19.02.2003

(40)

Type : Chronic
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 103 w
Frequency of treatm. : continuously
Post exposure period : 2 w
Doses : 3750 or 7500 ppm (about 188 or 375 mg/kg bw/d)
Control group : yes, concurrent vehicle
NOAEL : = 188 mg/kg bw
NOAEL male rat : = 375 mg/kg bw
Method : other
Year : 1976
GLP : no
Test substance : other TS: purity of 100 % is assumed

Result : NOAEL (NOEL), LOAEL (LOEL): the overall NOAEL (188 mg/kg bw) is based on females showing decreased body weight gain with a difference greater than 10% between dosed and control rats.
TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Survival m: 31/50 (62 %), 33/50 (66 %); 34/50 (68 %)
- Survival f: 36/50 (72 %), 35/50 (70 %), 38/50 (76 %)
- Clinical signs: No dose-related clinical signs of toxicity.
- Body weight gain:
3750 ppm, m and f: slight body weight gain suppression: < 10% (estimated from graphic)
7500 ppm, m and f: slight body weight gain suppression, m: < 10%, f: < 14% (estimated from graphic)
- Food/water consumption: no effect
- Gross pathology: no effect
- Histopathology:

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Test condition	Chronic inflammation kidney: m: 29/49, 41/50, 41/50 (effect frequently found in aged male Fischer rats; considered as of questionable significance by the authors). : TEST ORGANISMS - Age: 9 weeks - Weight at study initiation: males: 165-180 g, females: 120-135 g (estimated from graphic) - Number of animals/dose group: 50 male, 50 female ACTUAL DOSE RECEIVED BY DOSE LEVEL mg/kg bw/d values calculated for rats with a bw of in average 400 g, food consumption of in average 20 g/d ADMINISTRATION / EXPOSURE - Vehicle: corn oil CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: yes (twice daily) - Mortality: yes (twice daily) - Body weight: yes (monitored every two weeks) - Organ weight: no - Food consumption: yes (monitored every two weeks) - Water consumption: no - Ophthalmoscopic examination: no - Haematology: no - Biochemistry: no - Urinalysis: no - Histopathology: yes ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): Organs examined and preserved as described in OECD guideline 451. STATISTICAL METHODS: Probabilities of survival: procedure of Kaplan and Meier. Possible dose-related effect on survival: method of Cox. Dose-related trends: Tarone's extensions of Cox' methods
Reliability	: (2) valid with restrictions No biochemistry/hematology performed, organ weights not determined
Flag 19.02.2003	: Critical study for SIDS endpoint
Type Species Sex Strain Route of admin. Exposure period Frequency of treatm. Post exposure period Doses Control group NOAEL Method Year GLP Test substance	: Chronic : mouse : male/female : B6C3F1 : oral feed : 103 w : continously : 1 w : 2000 or 4000 ppm (about 334 or 667 mg/kg bw/d) : yes, concurrent vehicle : = 667 mg/kg bw : other : 1976 : no : other TS: purity of 100 % is assumed
Result	: TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Survival m: 32/50 (62 %), 32/50 (64 %); 35/50 (70 %) - Survival f: 45/50 (90 %), 40/50 (80 %), 36/50 (72 %) - Clinical signs: no clinical signs of toxicity - Body weight gain: 2000 and 4000 ppm m and f: slight body weight gain

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	suppression: < 10% (estimated from graphic) - Food/water consumption: no effect - Gross pathology: no effect - Histopathology: see 5.7 Carcinogenicity
Test condition	: TEST ORGANISMS - Age: 6 weeks - Weight at study initiation: male: 23-25 g, female: 19-21 g (estimated from graphic) - Number of animals/dose group: 50 male, 50 female ACTUAL DOSE RECEIVED BY DOSE LEVEL mg/kg bw/d values calculated for mice with a bw of in average 30 g, food consumption of in average 5 g/d ADMINISTRATION / EXPOSURE - Vehicle: corn oil CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: yes (twice daily) - Mortality: yes (twice daily) - Body weight: yes (recorded every two weeks) - Organ weights: no - Food consumption: yes (recorded every two weeks) - Water consumption: yes - Ophthalmoscopic examination: no - Haematology: no - Biochemistry: no - Urinalysis: no - Histopathology: yes ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): Organs examined and preserved as described in OECD guideline 451 STATISTICAL METHODS: Probabilities of survival: procedure of Kaplan and Meier. Possible dose-related effect on survival: method of Cox. Dose-related trends: Tarone's extensions of Cox' methods
Reliability	: (2) valid with restrictions No biochemistry/hematology performed, organ weights not determined
Flag	: Critical study for SIDS endpoint
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5.5 GENETIC TOXICITY 'IN VITRO'

Type	: Ames test
System of testing	: S. typhimurium TA 98, TA 100, TA 2637
Test concentration	: 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 mg/plate
Cycotoxic concentr.	: 0.2 mg/plate
Metabolic activation	: with and without
Result	: negative
Method	: other: according to Ames et. al (1975)
Year	: 1985
GLP	: no data
Test substance	: other TS: purity not stated
Result	: No increases in mutant frequency were seen in any strain both in the absence or in the presence of metabolic activation.
Test condition	: Metabolic activation system: S9-mix from PCB induced BALB/c mice
	Vehicle: DMSO
	Negative control: DMSO
	Positive controls: AF-2 0.02 µg/plate (for TA 100 and TA 98 without S9 mix), 9-aminoacridine 0.2 mg/plate (for TA 2637 without S9 mix), 2-

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Reliability	:	aminoanthracene 0.05 mg/plate (for all used strains with S9 mix)	
	:	(2) valid with restrictions	
	:	purity of TS not stated	
Flag 19.02.2003	:	Critical study for SIDS endpoint	(42)
Type	:	Ames test	
System of testing	:	S. typhimurium TA 97, TA 98, TA 100, TA 1535	
Test concentration	:	3, 10, 33, 100, 166, 333, 666 µg/plate	
Cycotoxic concentr.	:	333 µg/plate	
Metabolic activation	:	with and without	
Result	:	negative	
Method	:	other: see reference Zeiger et.al.	
Year	:	1984	
GLP	:	no data	
Test substance	:	other TS: D/L-menthol, purity not stated	
Test condition	:	SYSTEM OF TESTING - Metabolic activation system: S9, Sprague-Dawley rat and male Syrian hamster livers respectively, induced with Aroclor 1254, mix contained 10 or 30% S9 ADMINISTRATION: - Number of replicates: 3 Vehicle and negative control: DMSO Positive controls in the absence of metabolic activation: sodium azide (for TA 1535 and TA 100), 9-aminoacridine (TA 97 and TA 1537), 4-nitro-o-phenylenediamine (TA 98) Positive control in the presence of metabolic activation: 2-aminoanthracene STATISTICS: standard error of the mean	
Reliability	:	(2) valid with restrictions	
	:	purity of TS not stated	
Flag 04.03.2003	:	Critical study for SIDS endpoint	(43) (44) (45)
Type	:	Ames test	
System of testing	:	S. typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537	
Test concentration	:	up to 5 mg/plate	
Cycotoxic concentr.	:	no data	
Metabolic activation	:	with and without	
Result	:	negative	
Method	:	other: according to Ames et. al (1975)	
Year	:	1984	
GLP	:	no data	
Test substance	:	other TS: 99.8 %	
Test condition	:	Solvent: DMSO Metabolic activation system: S9-mix from PCB induced Fischer rats In this study 200 different compounds have been tested (pos. and neg. results) No information on positive control. The result was considered positive if the number of colonies found was twice the number in the control. SATISTICS: not done	
Reliability	:	(2) valid with restrictions	
	:	no data for positive controls	
Flag 19.02.2003	:	Critical study for SIDS endpoint	(46)
Type	:	Chromosomal aberration test	

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System of testing : Chinese hamster ovary cells
Test concentration : 100, 150, 200 µg/ml without metabolic activation, 50, 124, 250 µg/ml with metabolic activation
Cycotoxic concentr. : up to toxic or near-toxic levels
Metabolic activation : with and without
Result : negative
Method : other
Year : 1986
GLP : no data
Test substance : other TS: purity: no data

Result : Negative controls: 2% cells with aberrations
 Positive controls: above 30 % cells with aberrations
Test condition : - treatment time: 8 hours without metabolic activation
 2 hours with metabolic activation
 - scoring: 200 cells per dose
 - positive controls: MMC, 0.5 µg/ml (without met. act.)
 CPA, 37.5 µg/ml (with met. act.)

Reliability : (2) valid with restrictions
 limited documentation

Flag : Critical study for SIDS endpoint
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(47) (48) (44)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster ovary cells
Test concentration : up to 167 µg/ml
Cycotoxic concentr. : up to toxic- or near-toxic levels
Metabolic activation : with and without
Result : negative
Method : other
Year : 1986
GLP : no data
Test substance : other TS

Result : Positive controls led to more than 3 times the control levels.
 SCE without activation:
 TS was judged negative in the first SCE trial. Due to a low-level increase at the low dose only, a repeat test was performed. In this 2. trial none of the responses were increased over 20% above the control, but the trial was judged equivocal based on a positive trend.
 SCE with activation: no increase
 Overall, the TS was judged negative in the SCE test.

Test condition : - treatment time: 25 hours without metabolic activation
 2 hours with metabolic activation
 - scoring: 50 cells per dose
 - Testing was performed up to or near-toxic levels as evidenced by the reduction of cell confluence at the highest dose.
 - positive controls: MMC, 0.01 µg/ml (without met. act.)
 CPA, 2 µg/ml (with met. act.)

Reliability : (2) valid with restrictions
 limited documentation

Flag : Critical study for SIDS endpoint
 19.02.2003

(47) (48) (44)

Type : other: alkaline elution/rat hepatocyte assay for DNA damage
System of testing : primary rat hepatocytes
Test concentration : 0.1, 0.3, 0.7, 1.0, 1.3 mM
Cycotoxic concentr. : up to cytotoxic concentrations
Metabolic activation : without

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Result : negative
Method : other
Year : 1996
GLP : no data
Test substance : other TS: > 99 %

Result : Menthol showed clear evidence of a dose-related cytotoxic effect and weakly increased the frequency of DNA-double strand breaks. These genotoxic effects were considered to be a consequence of cytotoxicity.
Test condition : To discriminate between cytotoxic and genotoxic effects of chemicals, additional assays of cytotoxicity were performed.

SYSTEM OF TESTING

- Species/cell type: male Sprague-Dawley rats (150-300 g, Charles River Laboratories, Raleigh, NC) hepatocytes
- Cytotoxicity testing: TBDE-0 (Trypan blue dye exclusion), TBDE-3h (after 3 h recovery), Thiazol blue dye reduction (MTT), intracellular adenosine triphosphate content (ATP), intracellular potassium content (K⁺), cell blebbing
- Alkaline elution assays were performed described by Elia, M.C. et al., Int. J. Radiat. Biol., 63, 7-11 (1993), with several modifications.

ADMINISTRATION:

- Positive and negative control groups and treatment:

positive control: 137-Cs 3 Gy

negative control: DMSO 1%

CRITERIA FOR EVALUATING RESULTS: study was conducted to improve criteria for positivity and to reduce false-positive results.

criteria for positive result old:

>= 3.0-fold increase in elution slope

>= 70% relative viability by TBDE-0 assay

criteria for positive result new:

induced elution slope >= 0.020

>= 70% relative viability by TBDE-3 (TBDE after a further 3-h recovery incubation without test chemicals) assay

>= 50% of control ATP content

Reliability : (2) valid with restrictions
non-validated test system
Flag : Critical study for SIDS endpoint

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(49)

Type : Mouse lymphoma assay
System of testing : L5178Y mouse lymphoma cells
Test concentration : without S9-mix: 12.5, 25, 50, 100, 150, 200 µg/ml, with S9-mix: 25, 50, 75, 100, 150, 200, 300 µg/ml
Cytotoxic concentr. : 0.2 mg/ml; see test conditions
Metabolic activation : with and without
Result : negative
Method : other
Year : 1987
GLP : no data
Test substance : other TS: D/L-menthol, purity not stated

Result : No evidence of mutagenicity was obtained at non-toxic and cytotoxic concentrations.

Test condition : - cytotoxic dose: 0.2 mg/plate (+ or - S9-mix; 10% relative total growth)
- relative total growth 150 µg/plate - S9-mix: 24-27%
- relative total growth 150 µg/plate + S9-mix: 52-94%
negative control and vehicle: Ethanol
positive control - S9-mix: methylmethanesulfonate (MMS, 5 µl/ml)
positive control + S9-mix: 3-methylcholanthrene (MCA, 1.5 µg/ml)

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	Both positive control compounds were functional and led to strongly enhanced mutant frequencies.	
Reliability	: (2) valid with restrictions limited documentation	
Flag 04.03.2003	: Critical study for SIDS endpoint	(50) (51) (52) (48) (44)
Type	: Chromosomal aberration test	
System of testing	: Chinese Hamster lung cells (direct method)	
Test concentration	: 0.1, 0.15, 0.2 mg/ml	
Cycotoxic concentr.	: no data	
Metabolic activation	: without	
Result	: negative	
Method	: other	
Year	: 1982	
GLP	: no data	
Test substance	: other TS: 99.8 %	
Result	: No TS related effects on polyploidy. Gaps were included in the frequency of aberrations. Test was equivocal after 24 hours of treatment: - Vehicle control: 2 % aberrations - 0.1 mg/ml: 5 % aberrations - 0.15 mg/ml: 1 % aberrations - 0.2 mg/ml: 6 % aberrations Test was negative after 48 hours of treatment: - Vehicle control: 3 % aberrations - 0.1 mg/ml: 2 % aberrations - 0.15 mg/ml: 1 % aberrations - 0.2 mg/ml: 4 % aberrations Overall conclusion: negative	
Test condition	: SYSTEM OF TESTING - No. of metaphases analyzed: 100 - chromosomal effects evaluated: chromatid and isochromatid gaps, chromatid breaks, chromatid exchanges, chromosome breaks, chromosome exchanges including dicentric and ring chromosomes. CRITERIA FOR EVALUATING RESULTS: negative: incidence less than 4.9 %, ambiguous: incidence between 5.0 and 9.9 %, positive: incidence more than 10%. Solvent: Ethanol Duration of treatment: 24 and 48 hours. In this study 25 chemicals have been tested. Acrylamide and acrylonitrile gave positive results (up to 15 % and 25 % aberrations, respectively). No further positive controls tested. STATISTICS: not performed	
Reliability	: (2) valid with restrictions limited documentation	
Flag 19.02.2003	: Critical study for SIDS endpoint	(46) (53) (54)
Type	: Chromosomal aberration test	
System of testing	: CHO cells	
Test concentration	: 203-297 µg/ml	
Cycotoxic concentr.	: up to cytotoxic concentrations (see results)	
Metabolic activation	: with and without	
Result	: ambiguous	
Method	: other	
Year	: 1998	
GLP	: no data	

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Test substance	: other TS: purity: commercial grade
Result	: First test - without S9: concentrations: 0.3-1.9 mM (according to 46 to 297 µg/ml) cytotoxicity: viability dropped to 12 % at 297 µg/ml, ATP levels dropped from 85% of controls at 47 µg/ml to 33% of controls at 297 µg/ml - % chromosomal aberrations: first test - without S9: 203 µg/ml (1.3 mM): 1% (80 % cell viability) 234 µg/ml (1.5 mM): 2.5% (56 % cell viability) 250 µg/ml (1.6 mM): 7% (47 % cell viability) 266 µg/ml (1.7 mM): 9.5% (39 % cell viability) 281 µg/ml (1.8 mM): 6% (33 % cell viability) second test - without S9: Chromosome aberrations: reproducible with first test but only at highest scoreable dose of 250 µg/ml: 6% (34 % cell viability) 234 µg/ml: no increase (40 % cell viability) test with S9: only one concentration was scoreable 203 µg/ml: 3.0 % (not statistically significant) Negative control values not listed.
Test condition	: - harvest time: 20 hr - treatment time: 3h - vehicle: DMSO - Criteria for positivity: statistically significant increase over concurrent controls in the percentages of cells with chromosomal aberrations at two separate concentrations of test article, with about 50 % cytotoxicity, or a reproducible increase at one dose level. - Cytotoxicity testing: trypan blue exclusion, ATP levels - scoring: 200 cells with 19-23 chromosomes per point - positive control levels: 0 - 2.25 % cells with aberrations (mean 1.50 %) - statistics: Fisher' exact test, with the P values adjusted for multiple comparisons against a common control by the method of Dunnett (J. Am Stat Assoc 50, 1096-1121, 1955).
Reliability	: (2) valid with restrictions Study well documented. Restrictions: no concurrent positive and negative controls
Flag 19.02.2003	: Critical study for SIDS endpoint
Type	: Chromosomal aberration test
System of testing	: CHO cells
Test concentration	: 203, 219, 234 µg/ml
Cycotoxic concentr.	: 234 µg/ml = 45 % viable cells
Metabolic activation	: no data
Result	:
Method	: other
Year	: 1998
GLP	: no data
Test substance	: other TS: purity: commercial grade
Result	: Menthol had no clastogenic activity at non-toxic concentrations. At 234 µg/ml - 7 % cells with aberrations were observed. Flow cytometric measurements showed that DNA synthesis was inhibited by menthol to 41 % of control level at this concentration and cell viability was reduced to 45%.
Test condition	: - harvest time: 20 hr

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	- treatment time: 3h - vehicle: DMSO - Cytotoxicity testing: two-parameter flow cytometry to assess DNA synthesis inhibition (uptake of BrdUrd) - scoring: 200 cells with 19-23 chromosomes per point - statistics: no data	
Reliability	: (2) valid with restrictions limited documentation	
Flag 19.02.2003	: Critical study for SIDS endpoint	(56)
Type	: Chromosomal aberration test	
System of testing	: TK6 human lymphoblasts	
Test concentration	: 0.8, 1.0 and 1.2 mM	
Cycotoxic concentr.	:	
Metabolic activation	: without	
Result	: positive	
Method	: other	
Year	: 1998	
GLP	: no data	
Test substance	: other TS: purity: commercial grade	
Result	: No increase in aberrations at 0.8 and 1.0 mM (125 and 156 µg/ml), but a significant increase (11% cells with aberrations) was seen at 1.2 mM (187 µg/ml), a highly toxic dose with cell counts reduced to 20 % of control.	
Test condition	: - harvest time: 17-35 h, not further specified - treatment time: 3 h - vehicle: DMSO - Criteria for positivity: statistically significant increase over concurrent controls in the percentages of cells with chromosomal aberrations at two separate concentrations of test article, with about 50 % cytotoxicity, or a reproducible increase at one dose level. - Cytotoxicity testing: trypan blue exclusion, ATP levels - scoring: 100 cells containing 45-49 chromosomes per point - positive control levels: 0 - 4 % cells with aberrations (mean 2 %) - statistics: Fisher' exact test, with the P values adjusted for multiple comparisons against a common control by the method of Dunnett (J. Am Stat Assoc 50, 1096-1121, 1955).	
Reliability	: (2) valid with restrictions Study well documented. Restrictions: no concurrent positive and negative controls	
Flag 19.02.2003	: Critical study for SIDS endpoint	(55)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	: Drosophila SLRL test
Species	: Drosophila melanogaster
Sex	: male
Strain	: other: Canton-S
Route of admin.	: oral feed
Exposure period	: 3 d
Doses	: 50000 ppm
Result	: negative
Method	: other
Year	: 1994
GLP	: no data

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Test substance	: other TS: not further specified	
Method	: s described by Woodruff, R.C. et al., Environ. Mutagen. 6, 189-202 (1984); Zimmering, S. et al., Environ. Mutagen. 7, 87-100 (1985); Valencia, R. et al., Environ. Mutagen. 7, 325-348 (1985).	
Test condition	: Treatment: Males were mated to Basc females using a 2 to 3 day brooding pattern for a total of three broods spanning 7 days. Vehicle: Ethanol Control group: yes Mortality: 1 % Sterility: 4 % Total lethals: 1 (treated group), 1 (control group)	
Reliability	: (2) valid with restrictions limited documentation	
Flag 19.02.2003	: Critical study for SIDS endpoint	(57) (58)
Type	: Drosophila SLRL test	
Species	: Drosophila melanogaster	
Sex	: male	
Strain	: other: Canton-S males	
Route of admin.	: other: injection	
Exposure period	: no data	
Doses	: 10000 ppm	
Result	: negative	
Method	: other	
Year	: 1994	
GLP	: no data	
Test substance	: other TS: not further specified	
Method	: as described by Woodruff, R.C. et al., Environ. Mutagen. 6, 189-202 (1984); Zimmering, S. et al., Environ. Mutagen. 7, 87-100 (1985); Valencia, R. et al., Environ. Mutagen. 7, 325-348 (1985).	
Test condition	: Treatment: Males were mated to Basc females using a 2 to 3 day brooding pattern for a total of three broods spanning 7 days. Vehicle: Ethanol Control group: yes Mortality: 3 % Sterility: 43 % Total lethals: 0 (treated group), 4 (control group)	
Reliability	: (2) valid with restrictions limited documentation	
Flag 19.02.2003	: Critical study for SIDS endpoint	(57) (58)
Type	: Micronucleus assay	
Species	: mouse	
Sex	: male	
Strain	: B6C3F1	
Route of admin.	: i.p.	
Exposure period	: 3 days	
Doses	: 250, 500, 1000 mg/kg	
Result	: negative	
Method	: other	
Year	: 1993	
GLP	: no data	
Test substance	: other TS: not further specified	

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Result	: Micronucleated PCEs per 1000 PCE scored: 0 mg/kg / 2.90 250 mg/kg / 3.60 500 mg/kg / 2.20 1000 mg/kg / 3.67 Positive Control DMBA / 7.93 Positive Control MMC / 6.85 Negative Control / 2.38 Cytotoxicity % of PCEs (No. of PCE/No of PCE + No of NCE) 0 mg/kg / 54.4 250 mg/kg / 64.2 500 mg/kg / 56.7 1000 mg/kg / 51.8 * PCE: polychromatic erythrocytes Survival: 0 mg/kg 5/5 250 mg/kg 5/5 500 mg/kg 5/5 1000 mg/kg 3/6 p value: 0.374
Test condition	: TEST ORGANISMS: - Age: 9-14 weeks - Weight at study initiation: 25-33 g - No. of animals per dose: 5-7 ADMINISTRATION: - Vehicle: corn oil - Frequency of treatment: one injection/day - Control groups and treatment: Positive controls: 7,12-dimethylbenzanthracene (12.5 mg/kg) and mitomycin-C (0.2 mg/kg) Negative control: vehicle Time of deaths: Mice were killed 24 hrs after last injection EXAMINATIONS: Bone marrow smears (two slides per mouse) The slides were evaluated for the number of MN-PCE among 2000 PCD and for the percentage of PCE among 200 erythrocytes. STATISTICS: Data were analysed using the Micronucleus Assay Data Management and Statistical software package (version 1.4, ILS, 1990).
Reliability	: (2) valid with restrictions no information on TS provided
Flag	: Critical study for SIDS endpoint
19.02.2003	

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5.7 CARCINOGENICITY

Species	: mouse
Sex	: male/female
Strain	: B6C3F1
Route of admin.	: oral feed
Exposure period	: 103 w
Frequency of treatm.	: continuously
Post exposure period	: 1 w
Doses	: 2000 or 4000 ppm (about 334 or 667 mg/kg bw/d)

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Result	: negative
Contrl group	: yes, concurrent vehicle
Method	: other
Year	: 1976
GLP	: no
Test substance	: other TS: purity of 100 % is assumed
Result	: TOXIC RESPONSE/EFFECT BY DOSE LEVEL - Survival m: 32/50 (62%), 32/50 (64%), 35/50 (70%) - Survival f: 45/50 (90%), 40/50 (80%), 36/50 (72%) - Clinical signs: no clinical signs of toxicity - Body weight gain: 2000 and 4000 ppm m and f: slight body weight gain suppression: < 10% (estimated from graphic) - Food consumption: no effect - Gross pathology: no effect - Histopathology: hepatocellular carcinomas: m: 8/47, 8/49, 14/48; incidence was not statistically significant, within the range of historical controls; observed occasionally in groups of mice of this age and strain in this laboratory. Alveolar/bronchiolar adenomæ or carcinomas: f: 1/49, 3/47, 5/48; incidence was not statistically significant. No increased incidence of neoplasms compared to controls.
Test condition	: TEST ORGANISMS - Age: 6 weeks - Weight at study initiation: male: 23-25 g, female: 19-21 g (estimated from graphic) - Number of animals/dose group: 50 male, 50 female ACTUAL DOSE RECEIVED BY DOSE LEVEL mg/kg bw/d values calculated for mice with a bw of in average 30 g, food consumption of in average 5 g/d ADMINISTRATION / EXPOSURE - Vehicle: corn oil CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: yes (twice daily) - Mortality: yes (twice daily) - Body weight: yes (recorded every two weeks) - Organ weights: no - Food consumption: yes (recorded every two weeks) - Water consumption: no - Ophthalmoscopic examination: no - Haematology: no - Biochemistry: no - Urinalysis: no - Histopathology: yes ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): Organs examined and preserved as described in OECD guideline 451 STATISTICAL METHODS Probabilities of survival: procedure of Kaplan and Meier. Possible dose-related effect on survival: method of Cox. Dose-related trends: Tarone's extensions of Cox's methods.
Reliability	: (2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: no data on clinical chemistry.
Flag	: Critical study for SIDS endpoint
17.07.2002	

(60) (41)

5. Toxicity

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Species	: rat
Sex	: male/female
Strain	: Fischer 344
Route of admin.	: oral feed
Exposure period	: 103 w
Frequency of treatm.	: continuously
Post exposure period	: 2 w
Doses	: 3750 or 7500 ppm (about 188 or 375 mg/kg bw/d)
Result	: negative
Control group	: yes, concurrent vehicle
Method	: other
Year	: 1976
GLP	: no
Test substance	: other TS: purity of 100 % is assumed
Result	: TOXIC RESPONSE/EFFECT BY DOSE LEVEL - Survival m: 31/50 (62%), 33/50 (66%), 34/50 (68%) - Survival f: 36/50 (72%), 35/50 (70%), 38/50 (76%) - Clinical signs: no clinical signs of toxicity - Body weight gain: 3750 ppm, m and f: slight body weight gain suppression: < 10% (estimated from graphic) 7500 ppm, m: slight body weight gain suppression: < 10% (estimated from graphic), f: < 14% (estimated from graphic) - Food consumption: no effect - Gross pathology: no effect - Histopathology: - Chromophobe adenomas of the pituitary gland: - f: 28/48, 25/42, 19/43 - Mammarygland fibroadenomas: - f*: 20/50, 20/49, 7/49 - Mammary adenocarcinomas: - f: 1/50, 3/49, 0/49). In male rats chronic inflammation of the kidney was found with greater frequency in dosed males than in control males (controls: 29/49; low-dose: 41/50; high-dose 41/50). * = statistically significant (p=0.003; 0.028; 0.004)
Test condition	: TEST ORGANISMS - Age: 9 weeks - Weight at study initiation: males: 165-180 g, females: 120-135 g (estimated from graphic) - Number of animals/dose group: 50 male, 50 female ACTUAL DOSE RECEIVED BY DOSE LEVEL mg/kg bw/d values calculated for rats with a bw of in average 400 g, food consumption of in average 20 g/d ADMINISTRATION / EXPOSURE - Vehicle: corn oil CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: yes (twice daily) - Mortality: yes (twice daily) - Body weight: yes (recorded every two weeks) - Organ weight: no - Food consumption: yes (recorded every two weeks) - Water consumption: no - Ophthalmoscopic examination: no - Haematology: no - Biochemistry: no - Urinalysis: no - Histopathology: yes

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ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):

Organs examined and preserved as described in OECD guideline 451.

STATISTICAL METHODS

Probabilities of survival: procedure of Kaplan and Meier.

Possible dose-related effect on survival: method of Cox.

Dose-related trends: Tarone's extensions of Cox's methods.

Reliability

- : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restriction: no data on clinical chemistry.

Flag

04.03.2003

- : Critical study for SIDS endpoint

(60) (61)

5.8.1 TOXICITY TO FERTILITY

Type : other: Chronic (in detail reported in chapter 5.4 and 5.7)

Species : rat

Sex : male/female

Strain : Fischer 344

Route of admin. : oral feed

Exposure period : 103 w

Frequency of treatm. : continuously

Premating exposure period

Male :

Female :

Duration of test :

No. of generation studies :

Doses : 3750, 7500 ppm (= ca. 188, 375 mg/kg bw/d)

Control group : yes, concurrent vehicle

Result : No pathological changes in reproductive organs

Method : other

Year : 1979

GLP : no

Test substance : other TS: purity of 100% is assumed

Test condition : Microscopic examination of prostate, uterus, testis, ovaries, mammary glands and adrenals.
Details of the study design under 5.7 Carcinogenicity and 5.4 Repeated dose toxicity.

ACTUAL DOSE RECEIVED BY DOSE LEVEL

mg/kg bw/d values calculated for rats with a bw of in average 400 g, food consumption of in average 20 g/d

Reliability : (2) valid with restrictions
see 5.7

Flag : Critical study for SIDS endpoint

04.03.2003

(41)

Type : other: Chronic (in detail reported in chapter 5.4 and 5.7)

Species : mouse

Sex : male/female

Strain : B6C3F1

Route of admin. : oral feed

Exposure period : 103 w

Frequency of treatm. : continuously

Premating exposure period

Male :

Female :

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Duration of test :
No. of generation studies :
Doses : 2000, 4000 ppm (= ca. 334, 667 mg/kg bw/d)
Control group : yes, concurrent vehicle
Result : No pathological changes in reproductive organs
Method : other
Year : 1979
GLP : no
Test substance : other TS: purity of 100% is assumed

Test condition : Microscopic examination of prostate, uterus, testis, ovaries, mammary glands and adrenals.
 Details of the study design under 5.7 Carcinogenicity and 5.4 Repeated dose toxicity.
 ACTUAL DOSE RECEIVED BY DOSE LEVEL
 mg/kg bw/d values calculated for mice with a bw of in average 30 g, food consumption of in average 5 g/d

Reliability : (2) valid with restrictions
 see 5.7

Flag : Critical study for SIDS endpoint
 04.03.2003

(41)

Type : other: sub-chronic (in detail reported in chapter 5.4)
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 13 w
Frequency of treatm. : continuously
Premating exposure period
 Male :
 Female :
Duration of test :
No. of generation studies :
Doses : 930, 1870, 3750, 7500 or 15000 ppm (m: 59, 114, 231, 472, 937 mg/kg bw/d; f: 67, 142, 285, 521, 998 mg/kg bw/d)
Control group : yes, concurrent vehicle
Result : no pathological changes in reproductive organs
Method : other
Year : 1976
GLP : no
Test substance : other TS: purity of 100% is assumed

Test condition : Microscopic examination of prostate, uterus, testis, ovaries, mammary glands and adrenals.
 Details of the study design under 5.4 Repeated dose toxicity.

Reliability : (2) valid with restrictions
 see 5.4

Flag : Critical study for SIDS endpoint
 19.02.2003

(39)

Type : other: sub-chronic (in detail reported in chapter 5.4)
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 13 w

5. Toxicity

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Frequency of treatm. : continuously
Premating exposure period :
Male :
Female :
Duration of test :
No. of generation :
studies :
Doses : 930, 1870, 3750, 7500 or 15000 ppm (m: 243, 488, 978, 1956, 3913 mg/kg bw/d, f: 290, 595, 1193, 2386, 4773 mg/kg bw/d)
Control group : yes, concurrent vehicle
Result : No pathological changes in reproductive organs
Method : other
Year : 1976
GLP : no
Test substance : other TS: purity of 100% is assumed

Test condition : Microscopic examination of prostate, uterus, testis,
Reliability : (2) valid with restrictions
see 5.4
Flag : Critical study for SIDS endpoint
19.02.2003

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5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint : other: Induction of S-phase cells
Study descr. in chapter :
Reference :
Type : other: in vivo-in vitro replicative DNA synthesis test (RDS)
Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : gavage or i.p. injection
No. of animals :
Vehicle : other: corn oil
Exposure period :
Frequency of treatm. : single treatment
Doses : 725, 1450 mg/kg
Control group : yes
Observation period :
Result : positive
Method : other
Year : 1994
GLP : no data
Test substance : other TS: not further specified

Result : RDS incidences:
control: 0.7 %
725 mg/kg:
24 hours: 6.0 % (cell viability: 75%)
39 hours: 0.8 % (cell viability: 74 %)
48 hours: 0.6 % (cell viability: 67 %)

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- 1450 mg/kg
 24 hours: 0.6 % (cell viability: 66 %)
 39 hours: 3.9 % (cell viability: 72 %)
 48 hours: 0.5 % (cell viability: 73 %)
- Test condition** : Examination: Hepatocyte isolation and measurement of RDS incidence 24, 39 and 48 hrs after dosage
 RDS incidences were calculated as the percentage of Tritium thymidine-incorporating cells relative to 2000 hepatocytes.
 Judgement criteria for RDS incidence:
 Maximum RDS incidence was 2.0% or above- positive response
 Incidence less than 1.0% - negative response
 Incidence between 1.0 and 2.0 % - equivocal
 Cell viability - trypan blue exclusion test
- Reliability** : (2) valid with restrictions
 limited documentation

19.02.2003

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5.10 EXPOSURE EXPERIENCE

- Type of experience** : Human
- Result** : 0.3 %: significant decrease in perceived intensity, but not for cooling
- Test condition** : 15 persons (10f, 5m, aged 24-33 years)
 Concentrations of d/l-menthol: 0.03% and 0.3%
 10 menthol solution at one of the two concentrations were given to subjects; the rate of intensity of irritation and coolness in mouth was recorded.
- Reliability** : (2) valid with restrictions
 limited documenta tion

19.02.2003

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5.11 ADDITIONAL REMARKS

- Type** : Metabolism
- Result** : D,l-menthol inhibited metabolic cooperation between 6-thioguanine-sensitive and -resistant V79 Chinese hamster lung cells.
- Reliability** : (4) not assignable
 Documentation insufficient.

17.01.2002

(65)

- Type** : other: antimutagenic effect
- Result** : D/L-Menthol showed no antimutagenic effect.
- Test condition** : The inhibitory effects of flavourings (d/l-menthol among others) on mutagenesis induced by chemical in bacteria is studied.
- Reliability** : (2) valid with restrictions
 limited documentation

19.02.2003

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6. Analyt. Meth. for Detection and Identification

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6.1 ANALYTICAL METHODS**6.2 DETECTION AND IDENTIFICATION**

7. Eff. Against Target Org. and Intended Uses

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7.1 FUNCTION**7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED****7.3 ORGANISMS TO BE PROTECTED****7.4 USER****7.5 RESISTANCE**

8. Meas. Nec. to Prot. Man, Animals, Environment

Id

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8.1 METHODS HANDLING AND STORING**8.2 FIRE GUIDANCE****8.3 EMERGENCY MEASURES****8.4 POSSIB. OF RENDERING SUBST. HARMLESS****8.5 WASTE MANAGEMENT****8.6 SIDE-EFFECTS DETECTION****8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER****8.8 REACTIVITY TOWARDS CONTAINER MATERIAL**

9. References

Id	89-78-1
Date	18.03.2003

- (1) Hopp, R., Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties, Recent Advances Tobacco Sci 19, 3 - 46 (1993)
- (2) Haarmann & Reimer GmbH: Chemical Safety Data Sheet "Menthol Rac.", revision 17.4.2002
- (3) Haarmann & Reimer GmbH: Product Specification (2002)
- (4) Lide, D.R. (Ed.), CRC Handbook of Chemistry and Physics, 76th Edition, CRC Press Inc., Boca Raton (1996)
- (5) Blacow, N.W., (1972), Martindale, The Extra Pharmacopoeia, Ed. 26, p.. 374, The Pharmaceutical Press, London
- (6) Bayer AG (1977), Internal Product Specification,
- (7) Griffin S, Wyllie SG, Markham J (1999) Determination of octanol-water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography. J Chromatography A 864: 221 - 228
- (8) Bayer AG 2002, Calculation of log Pow with SRC-KOWWIN v. 1.66 (2000)
- (9) Bayer AG, Calculation of log Kow for D/L-menthol, UWS-Produktsicherheit (1991)
- (10) Hansch, C. & Leo, A.: Substituent Constants for Correlation Analysis in Chemistry and Biology. New York (1979)
- (11) MITI (1992): Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Compiled under the Supervision of Chemical Products Safety Division, Basic Industries Bureau MITI, Ed. by CITI, October 1992
- (12) Bayer AG (1990), internal report, Determination of the Water Solubility HR Product 131136
- (13) Hazardous Substances Data Bank, print from 09/05/2001, original literature: Yalkowsky SH, Dannenfelser RM; The AQUASOL dATABASE of Aqueous Solubility. Fifth Ed, Tucson, AZ: Univ Az, College of Pharmacy (1992)], also cited in SRC-MPBPWIN v1.40
- (14) Yalkowsky SH, Dannenfelser RM (1992)
- (15) Calculation of OH Rate Constant with SRC-AOP v. 1.90
- (16) Hazardous Substances Data Bank, print from 09/05/2001
- (17) Bayer AG (2003) Calculation of Mackay Distribution Level I
- (18) EC, Technical guidance document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 1488/94 on risk assessment for existing substances. European Chemicals Bureau, Ispra, Italy (1996)
- (19) Pitter, P. und Kozderkova, M., Chem. Prum. 20(6), 279-283 (1970)
- (20) Bayer AG: Toxicity of DL-menthol in the acute fish test according to OECD guideline 203. Internal Report No. 136 A/89 F (1990)
- (21) Bayer AG: Acute toxicity of DL-menthol in the acute daphnia test according to the UBA Verfahrensvorschlag (1984). Internal Report No. 136 A/89 D (1990)

9. References

Id 89-78-1
Date 18.03.2003

- (22) Bayer AG: Toxicity of DL-menthol on the alga *Scenedesmus subspicatus* in the growth inhibition test according to OECD guideline 201. Internal Report No. 959 A/00 (2000)
- (23) Bayer AG: Toxicity of DL-menthol on activated sludge in the respiration inhibition test according to OECD guideline 209. Internal Report No. 136 A/89 B (1989)
- (24) Williams, R.T. (1938), The Conjugation of Isomeric 3-Menthanols with Glucuronic Acid and the Asymmetric Conjugation of dl-Menthol and dl-isoMenthol in the Rabbit, *Biochem. J.* 32, 1849-1855
- (25) Haarmann & Reimer GmbH (1974), short report, menthol - examination of acute oral toxicity, Bayer AG, Steinhoff, D., 17.05.1974
- (26) FAO/WHO, Menthol, WHO food additives series: 42: Safety evaluation of certain food additives, 57-76 (1999)
- (27) Herken, H. (1961): Pharmacological report about the tolerance of naturally (l-) and synthetically (d,l-) menthol (Original title: Pharmakologisches Gutachten ueber die Vertraeglichkeit von natuerlichem (l-) und synthetischem (d,l-) Menthol). Unpublished report from the Director of the pharmacological institute of "Freie Universitaet, Berlin-Dahlem", submitted to the World Health Organization by Schering, A.G.: cited in: WHO Food Additives Series No. 10, 64-69 (1976)
- (28) Wokes, F. (1932), The Antiseptic Value and Toxicity of Menthol Isomers, *Quarterly Journal of Pharmacy & Pharmacology* 5, 233-244
- (29) Flury, F. and Seel, H. (1926), Synthetisches Menthol, *Muenchener Medizinische Wochenschrift* 48, 2011-2012
- (30) Levenstein, I: Report to RIFM, February 16, 1973: cited in Opdyke, D.L.J.: *Fd Cosmet. Toxicol.* 14, 473-474 (1976)
- (31) Hazard, R. and Lechat, P. (1952), Toxicité comparée du menthol naturel et du menthol synthétique racémique, *Annales pharm. franc.* 10, 481-487
- (32) Dmitrieva, N.M. et al. (1962), *Farmatsevtichaii Zhurnal* 17(3), 53-57
- (33) Haarmann & Reimer GmbH (1989), Assessment of the skin irritant effect of HR 89/131136 in rabbits, Scantox - biological laboratory ltd lab no. 11877, 16.08.1989
- (34) Haarmann & Reimer GmbH (1974), menthol - medical report ("Aerztliches Gutachten"), Prof. Hopf, 26.4.1974
- (35) Kligman, A.M. (1973), Report to RIFM, February 12, 1973: cited in Opdyke, D.L.J. (1976), *Fd Cosmet. Toxicol.* 14, 473-474
- (36) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/131136 in rabbits, Scantox - biological laboratory ltd lab no. 11753, 02.05.1989
- (37) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/131136 in rabbits, Scantox - biological laboratory ltd lab no. 11873, 16.08.1989
- (38) Tracor Jitco, Inc. (1976), Subcontract No. 74-28-106002, DL-Menthol "13-week subchronic toxicity-mice", Hazleton Laboratories America, Inc.: Final Report, Project No. 976-223, 21.10.1976
- (39) Tracor Jitco, Inc. (1976), Subcontract No. 4-28-106002, DL-Menthol "13-week subchronic toxicity-rats", Hazleton Laboratories America, Inc., Final Report, Project No. 976-243, 21.10.1976.

9. References

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- (40) Herken, H (1961) : Pharmacological expertise on tolerance to natural and synthetic menthol. Unpublished report from Pharmakologischen Institut der Freien Universität, Berlin, Dahlem. Submitted to WHO by Schering AG, Berlin (in German). As cited in: FAO/WHO (1999) Menthol. In: WHO food additives series: 42: Safety evaluation of certain food additives. Geneva, World Health Organization 57 - 76
- (41) NCI (1979) National Cancer Institute: Bioassay of D/L-menthol for possible carcinogenicity. Technical Report Series No. 98, Bethesda, Maryland: 1 - 112
- (42) Nohmi, T. et al. (1985), Mutagenicity Tests on Organic Chemical Contaminants in City Water and Related Compounds, Bull. Nat. Inst. Hyg. Sci. 0 (103), 60-64
- (43) National Toxicology Program, Annual Plan for Fiscal Year 1985, NTP -85-055, March 1985
- (44) Tennant, R.W. et al. (1987), Prediction of Chemical Carcinogenicity in Rodents from in Vitro Genetic Toxicity Assays, Science 236, 933-941
- (45) Zeiger, E. et al. (1988), Salmonella Mutagenicity Tests: IV. Results from the Testing of 300 Chemicals, Environmental and Molecular Mutagenesis 11, Suppl. 12, 1-18
- (46) Ishidate, M. et al. (1984), Primary Mutagenicity Screening of Food Additives Currently Used in Japan, Fd Chem. Toxic. 22, 623-636
- (47) Ivett, J.L. et al. (1989), Chromosomal Aberrations and Sister Chromatid Exchange Tests in Chinese Hamster Ovary Cells in Vitro.IV.Results with 15 Chemicals, Environmental and Molecular Mutagenesis 14, 165-187
- (48) National Toxicology Program, Annual Plan for Fiscal Year 1987, NTP -87-001, May 1987
- (49) Storer, R.D. et al. (1996), Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds, Mutat. Res. 368, 59-101
- (50) Henry, B. et al. (1998), Induction of forward mutations at the thymidine kinase locus of mouse lymphoma cells: evidence for electrophilic and non-electrophilic mechanisms, Mutat. Res. 397(2), 313-335
- (51) Myhr, B. et al. (1985), Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture, Progress in mutation research Vol. 5, 555-568, (Test conditions)
- (52) Myhr, B.C. and Caspary, W.C. (1991), Chemical Mutagenesis at the Thymidine Kinase Locus in L5178Y Mouse Lymphoma Cells: Results for 31 Coded Compounds in the National Toxicology Program, Environ. Mol. Mutag. 18, 51-83
- (53) Ishidate, M. et al. (1988), A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures, Mutation Research 195, 151-213
- (54) Sofuni, T. et al. (1985), Mutagenicity Tests on Organic Chemical Contaminants in City Water and Related Compounds II. Chromosome Abberation Tests in Cultured Mammalian Cells, Bull. Natl. Inst. Hyg. Sci. (Eisei Shikensho Hokoku) Tokyo, 103, 64-75
- (55) Hilliard, C. (1998), Chromosome Aberrations In Vitro Related to Cytotoxicity of Nonmutagenic Chemicals and Metabolic Poisons, Environ.Mol.Mutagen. 31(4), 316-326
- (56) Galloway, S. et al. (1998), DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens, Mutat.Res. 400(1-2), 169-186

9. References

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- (57) Foureman, P. et al. (1994), Chemical Mutagenesis Testing in Drosophila, Environ. Mol. Mutagen. 23, 51-63
- (58) National Toxicology Program, Annual Plan for Fiscal Year 1988, NTP -88-200, June 1988
- (59) Shelby, M.D. et al. (1993), Evaluation of a Three-Exposure Mouse Bone Marrow Micronucleus Protocol: Results with 49 Chemicals, Environ. Mol. Mutagen. 21, 160-179
- (60) Contrera, J. et al. (1997), Carcinogenicity Testing and the Evaluation of Regulatory Requirements for Pharmaceuticals, Regulat. Toxicol. Pharmacol. 25, 130-145
- (61) NCI (1979) National Cancer Institute: Bioassay of D/L-menthol for possible carcinogenicity. Technical Report Series No. 98, Bethesda, Maryland: 1 - 112 U.S. department of health, education, and welfare (1979), "Bioassay of dl-menthol for possible carcinogenicity NCI C G-TR-98/1979, Hazleton Laboratories America, under direct contract to National Cancer Institute (NCI) and under a subcontract to Tracor Jitco, Inc., prime contractor for the National Carcinogenesis Testing Program, 1-112
- (62) Tracor Jitco, Inc. (1976), Subcontract No. 74-28-106002,
- (63) Uno, Y. et al. (1994), An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens, Mutat. Res. 320, 189-205
- (64) Cliff, M. and Green, B. (1994), Sensory Irritation and Coolness Produced by Menthol: Evidence for Selective Desensitization of Irritation, Physiol. Behav. 56(5), 1021-1029
- (65) Willington, S.E. et al. (1989), Methods and criteria for assessing the activity of chemicals in the V79 metabolic cooperation assay, Mutat. Res. 216, 283 (abstr.)
- (66) Ohta, T. et al (1986), Inhibitory Effects of Flavours on Mutagenesis Induced by Chemicals in Bacteria, Food Chem. Toxicol. 24, 51-54

10. Summary and Evaluation

Id

89-78-1

Date

18.03.2003

10.1 END POINT SUMMARY**10.2 HAZARD SUMMARY****10.3 RISK ASSESSMENT**

I U C L I D Data Set

Existing Chemical : ID: 15356-60-2
CAS No. : 15356-60-2
EINECS Name : (+)-menthol
EC No. : 239-387-8
Molecular Formula : C₁₀H₂₀O

Producer related part
Company : Bayer AG
Creation date : 15.11.2001

Substance related part
Company : Bayer AG
Creation date : 15.11.2001

Status :
Memo : ICCA D-menthol

Printing date : 18.03.2003
Revision date :
Date of last update : 18.03.2003

Number of pages : 1

Chapter (profile) : Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile) : Reliability: without reliability, 1, 2, 3, 4
Flags (profile) : Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE),
Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information

Id	15356-60-2
Date	18.03.2003

1.0.1 APPLICANT AND COMPANY INFORMATION**1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR****1.0.3 IDENTITY OF RECIPIENTS****1.0.4 DETAILS ON CATEGORY/TEMPLATE****1.1.0 SUBSTANCE IDENTIFICATION****1.1.1 GENERAL SUBSTANCE INFORMATION**

Purity type	:	
Substance type	:	organic
Physical status	:	solid
Purity	:	
Colour	:	white
Odour	:	minty
Flag	:	Critical study for SIDS endpoint
17.07.2002		

1.1.2 SPECTRA**1.2 SYNONYMS AND TRADENAMES****5-METHYL-2-(1-ETHYLETHYL)-CYCLOHEXANOL**

Flag	:	Critical study for SIDS endpoint
03.06.2002		

CYCLOHEXANOL, 5-METHYL-2-(1-METHYLETHYL)-(1S-(1ALPHA,2BETA,5ALPHA))

Flag	:	Critical study for SIDS endpoint
03.06.2002		

D-MENTHOL

Flag	:	Critical study for SIDS endpoint
03.06.2002		

MENTHOL D

Flag	:	Critical study for SIDS endpoint
17.07.2002		

1. General Information

Id 15356-60-2
Date 18.03.2003

1.3 IMPURITIES**1.4 ADDITIVES****1.5 TOTAL QUANTITY****1.6.1 LABELLING**

Labelling : provisionally by manufacturer/importer
Specific limits :
Symbols : Xi, , ,
Nota : , ,
R-Phrases : (38) Irritating to skin
S-Phrases : (25) Avoid contact with eyes

Flag : Critical study for SIDS endpoint
 17.07.2002

1.6.2 CLASSIFICATION

Classified : provisionally by manufacturer/importer
Class of danger : irritating
R-Phrases : (38) Irritating to skin
Specific limits :

Flag : Critical study for SIDS endpoint
 17.07.2002

1.6.3 PACKAGING**1.7 USE PATTERN**

Type of use : type
Category : Non dispersive use

Remark : Pure D-menthol is used for scientific purposes only. Industrial D-menthol from separation of L-menthol is used as the starting material for racemization to yield (D- and) L-menthol

Flag : Critical study for SIDS endpoint
 17.07.2002

1.7.1 DETAILED USE PATTERN

1. General Information

Id	15356-60-2
Date	18.03.2003

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type : Production

Remark : D/L-menthol is produced via reaction of m-cresol with propen to thymol, and hydrogenation of thymol, resulting in 4 isomers: D/L-neomenthol, D/L-neoisomenthol, D/L-menthol and D/L-isomenthol. D/L-menthol is isolated by fractional distillation.
 To produce L-menthol, D/L-menthol is transesterificated with methylbenzoate and further manufactured. Resulting products are L- and D-menthol

Flag : Critical study for SIDS endpoint
 03.06.2002

1.8 REGULATORY MEASURES**1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES****1.8.2 ACCEPTABLE RESIDUES LEVELS****1.8.3 WATER POLLUTION****1.8.4 MAJOR ACCIDENT HAZARDS****1.8.5 AIR POLLUTION****1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES****1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS****1.9.2 COMPONENTS****1.10 SOURCE OF EXPOSURE****1.11 ADDITIONAL REMARKS****1.12 LAST LITERATURE SEARCH**

Type of search : Internal and External

1. General Information

Id 15356-60-2
Date 18.03.2003

Chapters covered : 5
Date of search : 01.09.2001

Remark : Human Health: last literature search September 2001: CAS number search in external and internal databases, e.g. Biosis, Embase, Toxline, Scisearch

Flag : Critical study for SIDS endpoint
10.07.2002

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 14.01.2002

Remark : Physico-chemical properties / Environment / Ecotoxicology :
Flag : Critical study for SIDS endpoint
29.07.2002

1.13 REVIEWS

Memo : Its Origins, Chemistry, Physiology and Toxicological Properties

Flag : Critical study for SIDS endpoint
03.06.2002

(1)

2. Physico-Chemical Data

Id 15356-60-2
Date 18.03.2003

2.1 MELTING POINT

Sublimation :
Method :
Year : 2002
GLP : no data
Test substance : other TS

Remark : Freezing temp.: minimum 36.0 degrees C
18.03.2003

(2)

Value : 43 °C
Sublimation :
Method :
Year : 1993
GLP : no data
Test substance : no data

Remark : Review article, no information on data source but excellent compilation of major data

Flag : Critical study for SIDS endpoint
18.03.2003

(1)

2.2 BOILING POINT

Value : ca. 216 °C at 1013 hPa
Decomposition :
Method :
Year : 2002
GLP : no data
Test substance : no data

18.03.2003

(2)

Value : 216.5 °C at
Decomposition :
Method :
Year : 1993
GLP : no data
Test substance : no data

Flag : Critical study for SIDS endpoint
18.03.2003

(1)

2.3 DENSITY**2.3.1 GRANULOMETRY****2.4 VAPOUR PRESSURE**

2. Physico-Chemical Data

Id 15356-60-2
Date 18.03.2003

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : 3.4 at °C
pH value :
Method : other (calculated)
Year : 1999
GLP :
Test substance : other TS: D/L-menthol and L-menthol

Method : D/L-Menthol and L-menthol were both measured by reversed-phase high-performance liquid chromatography. Since they had the same log Kow (3.40), D-menthol also has the log Kow 3.40

Flag : Critical study for SIDS endpoint

11.03.2003

(3)

Partition coefficient : octanol-water
Log pow : 3.38 at °C
pH value :
Method : other (calculated): SRC-KOWWIN v. 1.66
Year : 2002
GLP :
Test substance :

18.03.2003

(4)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 431 mg/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

Remark : for L-Menthol

Flag : Critical study for SIDS endpoint

18.03.2003

(2)

2.6.2 SURFACE TENSION**2.7 FLASH POINT**

Value : 98 °C
Type : closed cup

25.01.2002

(2)

2. Physico-Chemical Data

Id	15356-60-2
Date	18.03.2003

2.8 AUTO FLAMMABILITY**2.9 FLAMMABILITY****2.10 EXPLOSIVE PROPERTIES****2.11 OXIDIZING PROPERTIES****2.12 DISSOCIATION CONSTANT****2.13 VISCOSITY****2.14 ADDITIONAL REMARKS**

Memo : alpha D20 + 50.1 degree C

Flag : Critical study for SIDS endpoint
30.07.2002

(1)

3. Environmental Fate and Pathways

Id 15356-60-2
Date 18.03.2003

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight

Method : structure estimation method
Result : Rate constant: $k = 2.4 \times 10^{-11} \text{ cm}^3/\text{molecule}/\text{sec}$ at 25 degrees C;
considering an atmospheric OH-radical concentration of $5 \times 10^5 \text{ OH-radicals}/\text{cm}^3$, the half-life is about 16 h

Reliability : (2) valid with restrictions
accepted calculation procedure

Flag : Critical study for SIDS endpoint
29.07.2002

(5)

3.1.2 STABILITY IN WATER

Result : volatilization half-lives for a model river (1 m deep, flow-rate 1 m/sec, wind velocity 3 m/sec) and a model lake (1 m deep, flow-rate 0.05 m/sec, wind velocity 0.5 m/sec) are estimated to be 2 and 18 days

Reliability : (2) valid with restrictions
accepted calculation procedure derived from l-menthol cause of structural similarities

Flag : Critical study for SIDS endpoint
26.07.2002

(6)

3.1.3 STABILITY IN SOIL**3.2.1 MONITORING DATA****3.2.2 FIELD STUDIES****3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS**

Type : volatility
Media : water - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year : 2003

Result : Based on a water solubility of 431 mg/l and a vapour pressure of 8.5 Pa (see chapter 2), the Henry's law constant is calculated to be $3.08 \text{ Pa} \times \text{m}^3/\text{mol}$

Reliability : (2) valid with restrictions
Generally accepted calculation method, parameters for calculation from l-menthol

3. Environmental Fate and Pathways

Id 15356-60-2
Date 18.03.2003

Flag : Critical study for SIDS endpoint
14.03.2003 (7)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 2003

Result : Air: 43.2 %
Water: 40.6 %
Soil: 8.0 %
Sediment: 8.1 %
Biota: 0.005 %

Test condition : Base data for calculation:
temperature: 20 °C
molar mass: 156.27 g/mol
vapour pressure: 8.5 Pa
water solubility: 431 g/m³
log Kow: 3.4
environmental compartments:
- air: 6*10⁹ m³, 1.2 kg/m³
- water: 7*10⁶ m³, 1000 kg/m³
- soil: 4.5 *10⁴ m³, 1500 kg/m³, 2 % org. C
- sediment: 2.1*10⁴ m³, 1300 kg/m², 5 % org. C
- susp. sediment: 35 m³, 1500 kg/m³, 16.7 % org. C
- aerosol: 0.12 m³, 1500 kg/m³
- aquatic biota: 7 m³, 1000 kg/m³, 5 % fat

Reliability : (2) valid with restrictions
Generally accepted calculation method, parameters for calculation from L-menthol

Flag : Critical study for SIDS endpoint
14.03.2003 (7)

Media : water - air
Method : other (calculation)
Year :

Result : Using the equation $\log K_{oc} = 0.52 \log K_{ow} + 1.02$ and based on a log Kow of 3.4 (see chapter 2) a Koc value of 614 can be calculated for the distribution between the organic phase of soil and pore water

Reliability : (2) valid with restrictions
Generally accepted calculation method

07.03.2003 (8)

3.4 MODE OF DEGRADATION IN ACTUAL USE**3.5 BIODEGRADATION**

Type : aerobic
Inoculum : activated sludge, domestic
Concentration : .84 mg/l related to Test substance
related to
Contact time : 28 day(s)
Degradation : 92 (±) % after 28 day(s)
Result : readily biodegradable
Kinetic of testsubst. : 0 day(s) 0 %
7 day(s) 64 %

3. Environmental Fate and Pathways

Id 15356-60-2

Date 18.03.2003

	14 day(s) 92 %
	21 day(s) 90 %
	28 day(s) 92 %
Control substance	: Acetic acid, sodium salt
Kinetic	: 7 day(s) 86 %
	14 day(s) 100 %
Deg. product	:
Method	: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	: 2003
GLP	: yes
Test substance	: other TS: purity 99.834 %
Remark	: Measured degradation of sodium acetate was 103 % after 14 d
Result	: The biodegradation in the toxic controls exceeded 25 %. According to the guideline, the test substance is not toxic to the medium
Test condition	: Two concentrations of the test substance (0.84 mg/l, 2.01 mg/l) a control (blank medium), an inoculum activity control (sodium acetate) and a toxicity control (sodium acetate and D-menthol) were prepared with mineral medium, saturated with oxygen, placed in approximately 300 ml BOD bottles, and incubated for 28 d in the dark at about 20 °C, except for the activity control and the toxicity control which were incubated for 14 d. To prevent leakage of gases out of the BOD bottles the bottles were incubated upside down. The O ₂ concentration was determined with an oxygen electrode after 0, 7, 14, 21, and 28 d of incubation
Reliability	: (1) valid without restriction
Flag	: Guideline study in accordance with the OECD principles of GLP
12.02.2003	: Critical study for SIDS endpoint
	(9)
Type	: aerobic
Inoculum	: activated sludge, domestic
Concentration	: 2.01 mg/l related to Test substance
	related to
Contact time	: 28 day(s)
Degradation	: 76 (±) % after 28 day(s)
Result	: readily biodegradable
Kinetic of testsubst.	: 0 day(s) 0 %
	7 day(s) 61 %
	14 day(s) 72 %
	21 day(s) 76 %
	28 day(s) 76 %
Control substance	: Acetic acid, sodium salt
Kinetic	: 7 day(s) 86 %
	14 day(s) 100 %
Deg. product	:
Method	: OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	: 2003
GLP	: yes
Test substance	: other TS: purity 99.834 %
Remark	: Measured degradation of sodium acetate was 103 % after 14 d
Result	: The biodegradation in the toxic controls exceeded 25 %. According to the guideline, the test substance is not toxic to the medium
Test condition	: Two concentrations of the test substance (0.84 mg/l, 2.01 mg/l) a control (blank medium), an inoculum activity control (sodium acetate) and a toxicity control (sodium acetate and D-menthol) were prepared with mineral medium, saturated with oxygen, placed in approximately 300 ml BOD bottles, and incubated for 28 d in the dark at about 20 °C, except for the activity control and the toxicity control which were incubated for 14 d. To

3. Environmental Fate and Pathways

Id 15356-60-2

Date 18.03.2003

	prevent leakage of gases out of the BOD bottles the bottles were incubated upside down. The O ₂ concentration was determined with an oxygen electrode after 0, 7, 14, 21, and 28 d of incubation	
Reliability	: (1) valid without restriction	
Flag	: Guideline study in accordance with the OECD principles of GLP	
12.02.2003	: Critical study for SIDS endpoint	(9)
Type	: anaerobic	
Inoculum	: other: enrichment culture from a forest ditch	
Deg. product	:	
Method	:	
Year	: 1992	
GLP	: no	
Test substance	: other TS: (+)-isomenthol, analytical grade	
Method	: Determination of nitrification under nitrate-reducing conditions	
Result	: Denitrification was stimulated by the presence of TS after 12 weeks.	
Test condition	: Culture tubes contained 100 ml water-mud mixture from the forest ditch, 350 ml anoxic mineral salt medium and 400 mg TS (HMN as carrier), N ₂ /CO ₂ -atmosphere, incubation at 28 degrees C in the dark	
Reliability	: (2) valid with restrictions	
	No standard test procedure, but in accordance with generally accepted scientific standards	
28.07.2002		(10)
Type	: anaerobic	
Inoculum	: other bacteria: <i>Pseudomonas citronellolis</i>	
Deg. product	:	
Method	:	
Year	: 1995	
GLP	: no	
Test substance	: other TS: (+)-isomenthol, analytical grade	
Method	: Determination of growth of <i>P. citronellolis</i> under nitrate-reducing conditions	
Result	: Test organisms did not grow with TS as the sole carbon and energy source.	
Test condition	: Culture tubes contained 15 ml anoxic mineral salt medium and 20 mg TS (HMN as carrier), N ₂ /CO ₂ -atmosphere	
Reliability	: (2) valid with restrictions	
	No standard test procedure, but in accordance with generally accepted scientific standards	
30.07.2002		(10)
Deg. product	:	
Method	:	
Year	:	
GLP	:	
Test substance	: other TS: (+)-isomenthol	
Remark	: Based on the previous literature it can be stated that the bacteria species: <i>Thauera terpenica</i> , strain 21 Mol may degrade (+)-isomenthol	
Reliability	: (4) not assignable	
	Review, no experimental data given	
28.07.2002		(11)

3. Environmental Fate and Pathways

Id

15356-60-2

Date

18.03.2003

3.6 BOD5, COD OR BOD5/COD RATIO**3.7 BIOACCUMULATION****3.8 ADDITIONAL REMARKS**

4. Ecotoxicity

Id

15356-60-2

Date

18.03.2003

4.1 ACUTE/PROLONGED TOXICITY TO FISH**4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES****4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE****4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA****4.5.1 CHRONIC TOXICITY TO FISH****4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES****4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS****4.6.2 TOXICITY TO TERRESTRIAL PLANTS****4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS****4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES****4.7 BIOLOGICAL EFFECTS MONITORING****4.8 BIOTRANSFORMATION AND KINETICS****4.9 ADDITIONAL REMARKS**

5. Toxicity

Id 15356-60-2
Date 18.03.2003

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo :
Type : Metabolism
Species :
Number of animals
 Males :
 Females :
Doses
 Males :
 Females :
Vehicle :

Remark : data are reported in chapter 5.11
01.07.2002

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 2046 mg/kg bw
Species : rat
Strain : Wistar
Sex : female
Number of animals : 10
Vehicle : peanut oil
Doses : 1000, 1500, 2000, 2500, 3000 mg/kg bw
Method : other
Year : 1974
GLP : no
Test substance : other TS: d-Menthol dest.

Result : MORTALITY:
- Time of death: 1-2 days after application
- Number of deaths at each dose:
dose (mg/kg) number of deaths
1000 0/10
1500 1/10
2000 5/10
2500 7/10
3000 10/10
CLINICAL SIGNS: narcotic status (no data available on exposure levels at which the clinical signs were observed)

Test condition : ADMINISTRATION:
- Volume administered or concentration: 10-20 ml/kg
- Post dose observation period: 14 days
EXAMINATIONS:
deaths, clinical signs
No information on statistical methods and confidence limits.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment. Restrictions: No information on statistical methods and confidence limits.

Flag : Critical study for SIDS endpoint
01.07.2002

(12)

5. Toxicity

Id 15356-60-2
Date 18.03.2003

5.1.2 ACUTE INHALATION TOXICITY**5.1.3 ACUTE DERMAL TOXICITY****5.1.4 ACUTE TOXICITY, OTHER ROUTES****5.2.1 SKIN IRRITATION**

Species : rabbit
Concentration : 100 %
Exposure : Semiocclusive
Exposure time : 4 hour(s)
Number of animals : 4
Vehicle : other: diethylphthalate (DEP)
PDII :
Result : moderately irritating
Classification :
Method : OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol-d, HR 89/620005, purity: no data

Result : AVERAGE SCORE
 100%/50%/25%/5%/1%/Vehicle
 2.5/1.9/0.7/0.0/0.0/0.0 (erythema)
 2.4/1.3/0.0/0.0/0.0/0.0 (oedema)
 REVERSIBILITY: yes
 Day 7: 100%: 3/4 - treated sites were covered with a layer of white to white-brown scales, 1/4 - massive layer of white-brown scales
 50%: 4/4 - thin layer of white scales
 25%: 2/4 - thin layer of white scales
 Day 14: 100%: 4/4 - treated sites were covered with white to white-brown scales, underlying skin was intact
 50%: 3/4 - treated sites showed scattered scale formation on intact skin.

Test condition : TEST ANIMALS:
 - Strain: Chbb:HM (C.H.Boehringer/Biberach)
 - Sex: female
 - Source: Dr. Karl Thomae GmbH, Biberach an der Riss
 - Age: no data
 - Weight at study initiation: 2200-2900
 - Number of animals: 4
 - Controls: internal control (one part of skin)
 ADMINISTRATION/EXPOSURE
 - Preparation of test substance: dilutions of substance with DEP, concentrated test substance was moistened with DEP in the ratio 6:1
 - Area of exposure: six different fields on back (two anterior, two centrally located and two posterior treatment sites)
 - Concentration in vehicle: 100, 50, 25, 5 and 1 %, Vehicle
 - Total volume applied: 0.5 ml
 - Postexposure period: up to 14 days
 - Removal of test substance: skin was washed with luke warm water and soap

Reliability : (2) valid with restrictions
 purity of test substance not stated

5. Toxicity

Id 15356-60-2
Date 18.03.2003

Flag 24.02.2003	:	Critical study for SIDS endpoint	(13)
Species	:	guinea pig	
Concentration	:	no data	
Exposure	:	Open	
Exposure time	:	14 day(s)	
Number of animals	:	20	
Vehicle	:	no data	
PDII	:		
Result	:	not irritating	
Classification	:	not irritating	
Method	:	other	
Year	:	1974	
GLP	:	no	
Test substance	:	other TS: d-menthol dest.	
Test condition	:	Substance was rubbed into the skin for 30 s once daily. Substance was applicated 2 x 5 days, results were taken after 14 days.	
Reliability	:	(3) invalid Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.	
17.12.2001			(14)

5.2.2 EYE IRRITATION

Species	:	rabbit	
Concentration	:	29 %	
Dose	:	.1 ml	
Exposure time	:	24 hour(s)	
Comment	:	rinsed after (see exposure time)	
Number of animals	:	4	
Vehicle	:	other: diethylphthalate (DEP)	
Result	:	slightly irritating	
Classification	:		
Method	:	OECD Guide-line 405 "Acute Eye Irritation/Corrosion"	
Year	:	1989	
GLP	:	yes	
Test substance	:	other TS: menthol-d, HR 620005 DEP, purity: no data	
Result	:	AVERAGE SCORE - Cornea: 0.4 - Iris: 0.0 - Conjunctivae (Redness): 1.3 - Conjunctivae (Chemosis): 0.1 REVERSIBILITY: yes, no reaction observed after 7 days	
Test condition	:	TEST ANIMALS: - Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya) - Sex: female - Source: Dr. Karl Thomae GmbH, Biberach an der Riss - Age: no data - Weight at study initiation: 2500-2900 g - Number of animals: 4 - Controls: internal control (right eye)	
Reliability	:	(2) valid with restrictions purity of test substance not stated	
Flag 24.02.2003	:	Critical study for SIDS endpoint	(15)

5. Toxicity

Id 15356-60-2
Date 18.03.2003

Species : rabbit
Concentration : 64 %
Dose : .1 ml
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 4
Vehicle : other: 29 % solution of d-menthol in DEP (HR 89/620005 DEP)
Result : slightly irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1989
GLP : yes
Test substance : other TS: menthol-d, HR 620005

Result : AVERAGE SCORE
 HR 89/620005 64%/Vehicle (29% d-menthol in DEP)
 0.9/0.5 (cornea)
 0.0/0.0 (iris)
 2.1/1.2 (redness of conjunctivae)
 0.3/0.0 (chemosis, conjunctivae)
 The right eyes were treated with the vehicle and the left eyes with the test article solution. Both articles had almost the same eye-irritating potential.
 REVERSIBILITY: yes, no reactions observed after 7 days

Test condition : TEST ANIMALS:
 - Strain: Chbb:HM (C.H.Boehringer, Biberach: Himalaya)
 - Sex: female
 - Source: Dr. Karl Thomae GmbH, Biberach an der Riss
 - Age: no data
 - Weight at study initiation: 2300-3000
 - Number of animals: 4
 - Controls: internal control with vehicle (right eye)
 ADMINISTRATION/EXPOSURE
 - Preparation of test substance: Test article was pulverized in a mortar and then diluted with vehicle (absolute concentration of substance in DEP is 64%)
 - Vehicle: 29% d-menthol in DEP (HR 89/620005 DEP; previously tested by Scantox, lab.no.: 11755)
 EXAMINATIONS
 according guideline

Reliability : (2) valid with restrictions
 purity of test substance not stated, no untreated control

Flag : Critical study for SIDS endpoint
 24.02.2003

Species : rabbit
Concentration : 60 %
Dose : .1 ml
Exposure time : 1 minute(s)
Comment : other: see test conditions
Number of animals : 8
Vehicle : other: olive oil
Result : not irritating
Classification : not irritating
Method : Draize Test
Year : 1974
GLP : no
Test substance : other TS: d-menthol dest.

(16)

5. Toxicity

Id 15356-60-2
Date 18.03.2003

Test condition : Substance was initially applied in 10, 20 and 30 % solution.
The eyes of 4 animals were rinsed 1 minute after application with physiological saline, substance remained in the eyes of 4 animals. In a second step animals were treated with the substance in concentrations of 40, 50 and 60 %.

Reliability : (2) valid with restrictions
limited documentation

24.02.2003

(14)

5.3 SENSITIZATION

Type : other: open repetetive dermal test
Species : guinea pig
Number of animals : 20
Vehicle : no data
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1974
GLP : no
Test substance : other TS: d-menthol dest.

Test condition : Substance was rubbed into shaved skin for 30 sec once daily for 3x5 days.
After 5 days without application the test substance was rubbed into an untreated part of the skin.
Results were taken after 24 h, 2 and 3 days.

Reliability : (3) invalid
Significant methodological deficiencies. e.g. concentration and amount of substance is unclear; lack of control experiment.

17.12.2001

(14)

5.4 REPEATED DOSE TOXICITY

5.5 GENETIC TOXICITY 'IN VITRO'

Type : other: Alkaline single cell gel test (comet assay)
System of testing : V79 Chinese hamster cells
Test concentration : 0; 0.5; 1; 2 mmol/l
Cycotoxic concentr. : >= 1 mmol/l
Metabolic activation : with and without
Result : negative
Method : other: as described by Singh et al. (1988), Exp. Cell Res. 175, 184-191, with the modifications as in: Hartmann and Speit (1995), Mut. Res. 346, 49-56
Year : 1997
GLP : no data
Test substance : other TS: D-Menthol purchased from Sigma

Reliability : (2) valid with restrictions
non-validated test system
Flag : Critical study for SIDS endpoint

04.03.2003

(17) (18)

Type : other: Alkaline single cell gel test (comet assay)

5. Toxicity

Id 15356-60-2
Date 18.03.2003

System of testing : Human lymphocytes
Test concentration : 0; 0.2; 0.5; 1; 2 mmol/l
Cycotoxic concentr. : >= 1 mmol/l
Metabolic activation : with and without
Result : negative
Method : other: as described by Singh et al. (1988), Exp. Cell Res. 175, 184-191, with the modifications as in: Hartmann and Speit (1995), Mut. Res. 346, 49-56
Year : 1997
GLP : no data
Test substance : other TS: D-Menthol purchased from Sigma

Reliability : (2) valid with restrictions
non-validated test system
Flag : Critical study for SIDS endpoint

04.03.2003

(17) (18)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

Type : Metabolism
Result : (-)/(+)-menthol glucuronidation ratio: 2.6/1
Vmax (-)/(+)-menthol glucuronidation ratio: 2.8/1
Data suggest, that monkey UGT2B9 and human UGT2B7 are functionally similar (89 % identity in cDNA library).
Test condition : Enantioselective glucuronidation for (+)- and (-)-menthol was studied using expressed monkey UGT2B9 (UDP-glucuronosyltransferase)
Reliability : (2) valid with restrictions
non-standard in vitro test system

24.02.2003

(19)

6. Analyt. Meth. for Detection and Identification

Id

15356-60-2

Date

18.03.2003

6.1 ANALYTICAL METHODS**6.2 DETECTION AND IDENTIFICATION**

7. Eff. Against Target Org. and Intended Uses

Id

15356-60-2

Date

18.03.2003

7.1 FUNCTION**7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED****7.3 ORGANISMS TO BE PROTECTED****7.4 USER****7.5 RESISTANCE**

8. Meas. Nec. to Prot. Man, Animals, Environment

Id

15356-60-2

Date

18.03.2003

8.1 METHODS HANDLING AND STORING**8.2 FIRE GUIDANCE****8.3 EMERGENCY MEASURES****8.4 POSSIB. OF RENDERING SUBST. HARMLESS****8.5 WASTE MANAGEMENT****8.6 SIDE-EFFECTS DETECTION****8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER****8.8 REACTIVITY TOWARDS CONTAINER MATERIAL**

9. References

Id 15356-60-2
Date 18.03.2003

- (1) Hopp, R., Menthol: Its Origins, Chemistry, Physiology and Toxicological Properties, Recent Advances Tobacco Sci 19, 3-46 (1993)
- (2) Haarmann & Reimer GmbH: Chemical Safety Data Sheet "Menthol D Dist.", revision 17.4.2002
- (3) Griffin S, Wyllie SG, Markham J (1999) Determination of octanol-water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography. J Chromatography A 864: 221 - 228
- (4) Bayer AG 2002, Calculation of log Pow with SRC-KOWWIN v. 1.66 (2000)
- (5) Calculation of the OH Rate Constant with SRC-AOP v. 1.90
- (6) Hazardous Substances Data Bank, print from 09/05/2001
- (7) Bayer AG (2003): Calculation of Mackay Distribution Level I
- (8) EC, Technical guidance document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 1488/94 on risk assessment for existing substances. European Chemicals Bureau, Ispra, Italy (1996)
- (9) TNO Chemistry (2003) Unpublished study V4107/01 Determination of the ready biodegradability of Menthol D Dist. in a Closed Bottle Test (OECD Guideline No. 301D, EU C.4-E)
- (10) Harder, J. & Probian, C.: Appl. Environ. Microbiol. 61, 2804-3808 (1995)
- (11) Hylemon, P.B. et al., Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems, FEMS Microbiology Reviews 22, 475-488 (1999)
- (12) Haarmann & Reimer GmbH (1974), short report, menthol - examination of acute oral toxicity, Bayer AG, Steinhoff, D., 17.05.1974
- (13) Haarmann & Reimer GmbH (1989), Assessment of the skin irritant effect of HR 89/620005 in rabbits, Scantox – biological laboratory ltd lab no. 11875, 16.08.1989
- (14) Haarmann & Reimer GmbH (1974), menthol - medical report ("Aerztliches Gutachten"), Prof. Hopf, 26.4.1974
- (15) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/620005 in rabbits, Scantox – biological laboratory ltd lab no. 11755, 02.05.1989
- (16) Haarmann & Reimer GmbH (1989), Assessment of the eye irritant effect of HR 89/620005 in rabbits, Scantox – biological laboratory ltd lab no. 11871, 16.08.1989
- (17) Anderson, D. et al. (1998), Comet assay responses as indicators of carcinogen exposure, Mutagenesis 13(6), 539-555
- (18) Hartmann, A. and Speit, G. (1997), The contribution of cytotoxicity to DNA-effects in the single cell gel test (comet assay), Toxicol. Lett. 90, 183-188
- (19) Green, M. et al. (1997), Glucuronidation of Opioids, Carboxylic Acid-Containing Drugs, and Hydroxylated Xenobiotics Catalyzed by Expressed Monkey UDP-Glucuronosyltransferase 2B9 Protein, Drug Metab. Dispos. 25(12), 1389-1394

10. Summary and Evaluation

Id

15356-60-2

Date

18.03.2003

10.1 END POINT SUMMARY**10.2 HAZARD SUMMARY****10.3 RISK ASSESSMENT**



**Scientific Committee on Health, Environmental and Emerging Risks
SCHEER**

**Opinion on
Additives used in tobacco products
(Opinion 2)
Tobacco Additives II**



The SCHEER approved this Opinion by written procedure on 16 December 2016.

About the Scientific Committees (2016-2021)

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

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SCHEER

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http://ec.europa.eu/health/scientific_committees/experts/declarations/scheer_wg_en

ABSTRACT

The Commission has established a priority list of 15 additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations, based on a scientific Opinion (Tobacco Additives I) of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). The EU Tobacco Product Directive (TPD) prescribes that Member States shall require manufacturers and importers of tobacco products to carry out comprehensive studies on these additives.

The European Commission requested the SCHEER to provide guidance on the type and criteria for comprehensive studies, and on the most suitable methodologies to be used on the first 15 tobacco additives and for additives on future updated lists. The SCHEER has provided such guidance together with a reporting template in Annex I.

As tobacco additives have no benefits for health, but rather may promote use of and addiction to an extremely toxic product, a risk-benefit analysis is not the appropriate paradigm for assessing the additive. When comprehensive studies confirm that additives have any of the four properties listed in Article 6 of the TPD, regulatory action should be considered in line with Article 7 of the TPD. If uncertainties cannot be solved by comprehensive studies, the SCHEER recommends that the assessors consider the worst-case evaluation.

In the first part, the SCHEER proposes a step-wise strategy as the most pragmatic and efficient way to assess the toxic and addictive effects and the characterising flavour and facilitating inhalation properties as potentially contributing to the attractive effects of tobacco additives. The proposed strategy ensures that testing is minimised, including the possibility to evaluate groups of additives having similar structures and properties. In step 1, the collection and then the evaluation of the available data on toxicity, addictiveness, characterising flavour and facilitating inhalation properties of the additive need to be carried out by applying a Weight of Evidence approach (step 1). In step 2, collection/evaluation of data is extended to the additive's pyrolysis products; if no data are available on the identity of the pyrolysis products, they need to be generated using relevant test conditions. Here, it is important to note that no validated methods exist for the determination of pyrolysis products from tobacco additives, but some indications are given in the Opinion.

In case data retrieved in Step 1 and 2 are not sufficient or robust enough to make the evaluation possible, non-testing methods, such as quantitative structure–activity relationship (QSAR) and read across, are proposed, followed by *in vitro* approaches, addressing the different endpoints to be considered (Step 3).

Regarding types of effects, unless the previous step highlighted some concern for a specific end-point, toxicity should be assessed first, as accepted methods and evaluation frameworks are available, followed by assessing whether a product contains a characterising flavour. Next, addictiveness should be assessed, an effect for which no validated tests are available, although some mechanisms underlying addictiveness are known. The issue related to interaction of the additive with other additives/ingredients is also considered.

In addition to proposing specific steps and tests to be considered by industry, some general criteria were also identified. Most importantly, the test outcomes should be

relevant for tobacco smoking. This implies that they should be related to actual levels of human exposure and to tobacco-induced diseases. Furthermore, comparative toxicity testing strategies, where differences in the effect of the tobacco product with and without the additive are evaluated, are not considered suitable to address the properties outlined in the Terms of Reference with the currently available methodology. Indeed, at present, these studies lack discriminative power due to the high background toxicity of tobacco products and their results cannot be generalised to all products and brands, having a different composition with respect to tobacco type, blend and additives. Here, the effects of the pure additive, and its pyrolysis products, are considered in order to evaluate their contribution to tobacco product toxicity. Comparative studies are also not endorsed to study the effect of additives on addictiveness with animal models, for the same reasons. In human studies, there are two exceptions on this general rule: characterising flavour testing and inhalation facilitation or nicotine uptake. For ethical reasons, the performance of new animal studies is not endorsed to assess the contribution of an additive to the tobacco product toxicity. Therefore, as a principle, only *in silico* and *in vitro* studies should be considered for new testing in Step 3, following the EU policy to ban animal studies for chemicals to be used in voluntary products. Human studies are generally discouraged; they may be used (e.g. in case of flavour assessment), but only if the study subjects are informed and not exposed to the harmful smoke emissions of tobacco products.

The data gaps already identified in the Opinion on Tobacco Additives I for the 15 additives included in the EU Commission priority list have been now analysed and the activities to be performed upfront have been described. In general, important data gaps for the 15 priority additives are information on addictiveness, inhalation facilitation and characterising flavour, as well as on the identity of the pyrolysis products.

Keywords: tobacco, additives, combustion products, cigarettes, roll-your-own, smoking, toxicity, addictiveness, attractiveness, characterising flavour, facilitated inhalation.

Opinion to be cited as:

SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Additives used in tobacco products, Opinion II, 16 December 2016.

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1 BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The new Tobacco Products Directive 2014/40/EU strengthens the rules regarding the reporting and composition of tobacco products. In addition to tightening the obligations of manufacturers to report on ingredients¹ contained in tobacco products, the Directive regulates permissible additives (or levels thereof) in order to improve the functioning of the internal market whilst guaranteeing a high level of public health.

A) Article 7 of Directive 2014/40/EU foresees in particular the prohibition of the following:

- 1) tobacco products with a characterising flavour (Art 7(1))
- 2) tobacco products containing the following additives² (Art 7(6)):
 - a) vitamins or other additives that create the impression that a tobacco product has a health benefit or presents reduced health risks;
 - b) caffeine or taurine or other additives and stimulant compounds that are associated with energy and vitality;
 - c) additives having colouring properties for emissions;
 - d) for tobacco products for smoking, additives that facilitate inhalation or nicotine uptake; and
 - e) additives that have CMR³ properties in unburnt form.
- 3) tobacco products containing flavourings in any of their components such as filters, papers, packages, capsules or any technical features allowing modification of the smell or taste of the tobacco products concerned or their smoke intensity. Filters, papers and capsules shall not contain tobacco or nicotine. (Art 7(7))
- 4) tobacco products containing additives in quantities that increase the toxic or addictive effect, or the CMR properties of a tobacco product at the stage of consumption to a significant or measureable degree. (Art 7(9))

The provisions outlined above shall apply in the first stage to cigarettes and roll-your-own tobacco. The exemption for other product categories may be removed under certain conditions.

B) Moreover, in line with Article 6 the Commission has to develop and update a **priority list of at least 15 additives** contained in cigarettes and roll-your-own tobacco by May 2016. This list shall contain additives

- 1) for which initial indications, research, or regulation in other jurisdictions exist suggesting that they have one of the following properties:
 - a) contributes to the toxicity or addictiveness of the products concerned / increases the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
 - b) results in a characterising flavour⁴;

¹ 'ingredient' means tobacco, an additive, as well as any substance or element present in a finished tobacco product or related products, including paper, filter, ink, capsules and adhesives (TPD 2014/40/EU)

² 'additive' means a substance, other than tobacco, that is added to a tobacco product, a unit packet or to any outside packaging (TPD 2014/40/EU)

³ CMR - carcinogenic, mutagenic or toxic for reproduction

- c) facilitates inhalation or nicotine uptake; or
 - d) leads to the formation of substances that have CMR properties / increases the CMR properties in any of the products concerned to a significant or measurable degree; and
- 2) which are amongst the most commonly used additives by weight or number according to the reporting of ingredients.

For these priority additives, enhanced reporting obligations will apply in the form of comprehensive studies which shall examine for each additive whether it has any of the properties 1 a) to d) specified above. Those studies shall take into account the intended use of the products concerned and examine in particular the emissions resulting from the combustion process involving the additive concerned. The studies shall also examine the interaction of that additive with other ingredients contained in the products concerned. The results of these studies shall assist Member States and the Commission in their enforcement efforts regarding Art. 7.

The SCENIHR published a scientific Opinion on the attractiveness and addictiveness of additives in 2010⁵. In light of the time that has passed since then and the need to address the current regulatory requirements, the SCENIHR has been asked to address the questions outlined in the Terms of Reference below.

⁴ 'characterising flavour' means a clearly noticeable smell or taste other than one of tobacco, resulting from an additive or a combination of additives, including, but not limited to, fruit, spice, herbs, alcohol, candy, menthol or vanilla, which is noticeable before or during the consumption of the tobacco product (TPD 2014/40/EU)

⁵http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_031.pdf

2 TERMS OF REFERENCE

The main purpose of the requested scientific Opinion is to assist the Commission in identifying the additives that should be put on the priority list. The scientific Opinion can, however, also provide useful input for Member States and the Commission in their broader regulatory/enforcement activities (e.g. setting thresholds/banning of additives), in particular in areas where the knowledge base may currently still be limited. In particular, the Committee is asked the following:

Opinion 1:

1. Based on scientific evidence (including a review of relevant scientific data) and other relevant information currently available (initial indications, regulation in other jurisdictions), the Committee is asked to identify - for each category separately - those additives that fall/are suspected to fall within the scope of the following categories:

- a. Contributing to the toxicity or addictiveness of the products concerned / increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b. Resulting in a characterising flavour;
- c. Facilitating inhalation or nicotine uptake;
- d. Leading to the formation of substances that have CMR properties / increasing the CMR properties in any of the products concerned (cigarettes/roll-your-own) to a significant or measurable degree;⁶

The assessment should include for each of the additives identified a comprehensive description of the type of information supporting its identification as well as a description and quantification of the strength of the observed characteristic and the strength of the available evidence supporting this finding⁷. If the Committee identifies more than 20 additives for a category, the Committee is entitled to prioritise in the light of the criteria set out in this section. In this case, the description is limited to the top 20 additives per category, whilst the other additives can be listed without description.

The Committee is asked to also consider in its assessment the interaction with other ingredients contained in the products concerned and the emissions resulting from the combustion process involving the additive concerned as well as the intended use of the products. Relevant knowledge gaps should be identified.

As far as relevant information is available, the Scientific Committee is asked to identify within its assessment the most commonly used additives by weight or number. If additives belong to a single group of substances with identical or very similar properties, both the group of substances and the list of substances falling into that group shall be presented and the most relevant substance(s) within that group identified.

6 If an additive is included in Annex VI of Regulation (EC) No 1272/2008, its CMR-classification should be provided and considered as appropriate. Additives that have CMR properties in unburnt form should be identified/listed, but do not require a comprehensive description.

7 Registrations/assessments of relevant substances under Regulation (EC) No 1907/2006 should be provided and considered as appropriate.

When examining the composition of tobacco products and the use of individual substances, the Scientific Committee is invited to consult the data on additives reported by the tobacco industry under the Tobacco Products Directive 2001/37/EC, but may also consider additional data sources. Furthermore, the Committee is invited to consider during their assessment the lists of additives permitted/prohibited for use in tobacco products as implemented by certain Member States.

2. Based on its assessment in point 1, the Committee is asked to establish a list of a minimum of 20 and maximum of 30 additives that are suitable/recommended to be added to the priority list of additives in line with Article 6 of TPD 2014/40/EU. When establishing the list, the Committee shall consider the public health risks associated with the additives (actual or suspected), strength of the available evidence and to the extent possible, the frequency of use of the additives in tobacco products. The Committee should indicate as far as possible rankings of additives in light of the above and provide an explanation for its ranking⁸.

Opinion 2:

3. Furthermore, the Committee is asked to advise the Commission on the type and criteria for comprehensive studies that should be requested from manufacturers to assess the relevance of the individual additives, considering inter alia the knowledge gaps identified in point 1 above and the interaction of the additive with other additives/ingredients. Advice is also sought on the most suitable methodologies to be used (including a structure of the reports that can be peer reviewed).

⁸Substances belonging to the same group of identical/very similar substances should be considered jointly.

3 SCIENTIFIC RATIONAL

3.1 Introduction

In response to the Commission's requests, the SCENIHR adopted Opinion I (Tobacco Additives I), in which 48 single chemicals were listed as priority additives, which met the 30 entries maximum limit because some chemicals with very similar structures (i.e. aliphatic gamma-lactones, including 8 chemicals) and/or properties (e.g. weak acids, including 8 group members) were grouped together. They were selected on the basis of two initial criteria: the frequency of use in different brands and the amounts used in cigarettes, then further screened based on their hazardous properties, because they have been or are suspected of having one or more of the following properties:

- a. Contributing to the toxicity or addictiveness of the products concerned / increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b. Resulting in a characterising flavour;
- c. Facilitating inhalation or nicotine uptake;
- d. Leading to the formation of substances that have CMR properties / increasing the CMR properties in any of the products concerned (cigarettes/roll-your-own) to a significant or measurable degree.

On the basis of these criteria:

- 17 substances were identified because they fall or are suspected to fall in the category: toxic in unburnt form, among which 6 are suspected of CMR potential, which were ranked highest on the suggested list because the Tobacco Products Directive foresees the prohibition of additives that have CMR properties in unburnt form.
- 20 substances were identified because they are known or suspected of forming irritant, toxic and/or CMR chemicals after combustion including sugars, sugar-containing additives and cellulose.
- 14 substances were identified because they are suspected of facilitating inhalation or of increasing nicotine uptake.
- 19 substances were identified because they show a characterising flavour, a factor potentially contributing to attractiveness.

Since the SCENIHR was asked to prioritise the selected chemicals to the best of its ability, three groups were identified. In addition to the 6 chemicals suspected of CMR potential, menthol was included in the 'highest priority group'.

A second group was identified based on the possibility of forming CMR compounds after combustion.

All the remaining identified additives are categorised in the third group, although it was not possible to rank them on the basis of their specific hazard profile and the only possibility was to use content/frequency ranking as a possible criteria for prioritisation or a combination of more than one of four characteristics provided for in Article 6.

On May 18, 2016, the Commission adopted the Commission Implementing Decision (EU) 2016/787 laying down a priority list of additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations⁹, identifying 15 chemicals among those listed in the SCENIHR Opinion (Additives used in tobacco products; Tobacco Opinion 1) adopted in January 2016.

In this Opinion 2, on the basis of the knowledge gaps mentioned in the next section, and after revising the available open literature and approaches taken by International Agencies, the SCHEER provides advice to the Commission on the type and criteria for comprehensive studies that should be requested by Member States from manufacturers to assess the contribution of the individual additives used in cigarette and roll-your-own tobacco to tobacco product toxicity, addictiveness¹⁰, adding to a characterising flavour or facilitating inhalation.. A step-wise strategy was proposed (Section 3.4)¹¹. The issue related to interaction of the additive with other additives/ingredients is also considered.

It should be noted that, by contrast to adding them to medicines or food, for example, additives in tobacco products have no health benefits for the consumer. On the contrary, by making smoking more attractive, they promote an extremely unhealthy behaviour. Therefore, a risk-benefit analysis is not the appropriate paradigm for assessing the additive. When comprehensive studies confirm that additives have any of the four properties listed in Article 6 of the TPD, regulatory action should be considered in line with Article 7 of the TPD. If uncertainties cannot be solved by comprehensive studies, it is a SCHEER recommendation that the assessors consider the worst-case evaluation.

The same reasoning applies to the addictive effects, inhalation facilitation and characterising flavour of tobacco additives, as they will indirectly lead to adverse health consequences by increasing consumption of the product.

In addition to the general strategy, the major data gaps already identified in Tobacco Opinion 1 have been analysed to determine the most appropriate steps (and end-points) to be carried out and then used for the evaluation (Section 3.5), in order to speed up the process, making possible testing feasible within the 18-month time-frame. To give an example, for the 6 chemicals for which a genotoxic potential could not be ruled out for the unburnt form, the first step will be to evaluate their genotoxicity: if the results were positive, no other testing would be necessary, since according to the TPD (Article 7) they would automatically be banned for use as tobacco additives. In case of negative results, they would enter the general strategy of testing and be considered as would any other compound.

⁹<http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016D0787&from=EN>

¹⁰ [For the concepts of addiction \(or "dependence"\), this Opinion refers to the definitions given in the previous evaluation \(SCENIHR, 2010, 2016\). Addictiveness refers to the pharmacological potential of a substance to cause addiction, in line with the TPD definition as 'the pharmacological potential of a substance to cause addiction, a state that affects an individual's ability to control his or her behaviour, typically by instilling a reward or a relief from withdrawal symptoms, or both'. In addition to the neurobiological characteristics of the substance itself, dependence potential is related to the dose, speed of absorption, metabolism, and the physical and chemical features of the formulation \(WHO, 2007\).](#)

¹¹ [SCHEER was not requested to give detailed protocols for specific studies and whenever possible referred to test guidelines or other approaches already adopted in areas other than tobacco products.](#)

3.2 Knowledge gaps identified in Opinion 1

There was generally scant toxicological information regarding tobacco additives analysed for Opinion 1, and the available information was often limited to the oral route of exposure, especially for flavouring substances that are used by the food industry, or, to a lesser degree, to the dermal route, for substances that are also commonly used in cosmetic products. Data on the effects of additives in tobacco following inhalation is generally scant, although this is the most relevant exposure route. Indeed, the additives are either transferred to inhaled smoke in pure form, or are combusted and converted via pyrolysis into potentially toxic products. Because there was also little data on their kinetic behaviour, it was difficult, if not impossible, to make route-to-route extrapolation for additives.

A general scarcity of information was observed regarding the actual level of exposure to additives both in the unburnt form in tobacco products and resulting from combustion – including data on pyrolysis. This is particularly relevant since toxic combustion products generated upon pyrolysis of additives have the potential to increase the exposure to toxic substances and thus increase the health hazard associated with cigarette smoking (National Institute for Public Health and the Environment, 2012). The fate of the additive depends on its physico-chemical properties such as its volatility and reactivity, the design of the cigarette and the smoking topography of the user. The additive may be distilled from the tobacco rod, and end up in smoke intact, or it may be (partly) combusted. In case of (partial) pyrolysis, not only the unburnt additive is relevant, as the smoker will be exposed to the pyrolysis products as well. In the tobacco matrix, either the intact additive or its pyrolysis products may react with other additives, tobacco- or smoke components (pyrosynthesis). For instance, only minor amounts of the non-volatile sugars in tobacco (approximately 0.5% of glucose and sucrose) are transferred unchanged into the mainstream smoke, whereas the major part will combust, pyrolyse or participate in pyrosynthesis processes (Talhout *et al.*, 2006).

Although for most tobacco additives, direct information about their possible contribution to addictiveness and characterising flavours does not exist, information can be derived from the mode of action of the additive (e.g. addictiveness can be related to increased nicotine bioavailability or to local anaesthetic effects facilitating the inhalation of tobacco smoke).

Generally speaking, the scarcity of information on exposure and on toxic effects as described in Opinion 1 made risk assessment for most additives difficult, if not impossible.

3.3 Methodology

3.3.1 Development of the general approach to assess the effects of tobacco additives

Given the fact that additives in tobacco products have no health benefits for the consumer, but rather promote an extremely risky behaviour, risk-benefit evaluations are not appropriate. Based on evaluation of approaches for regulation of other types of components, the SCHEER concluded that a step-wise approach is the most pragmatic and efficient way to proceed in the assessment of the toxic, addictive and attractive

effects of tobacco additives. The tiered approach proposed by DKFZ (DKFZ, 2010) was used as a starting point, but was then further developed and adapted to include the evaluation of the additives in view of their role in creating a characterising flavour, facilitating inhalation and making tobacco products more addictive. The order of the steps has been proposed in such a way to minimise testing. First, an evaluation of the available data is proposed for both the additive in unburnt form (Step 1) and its pyrolysis products (Step 2); if no data are available on the identity of the pyrolysis products, they need to be generated using relevant test conditions. Next, in Step 3 non-testing methods, such as quantitative structure–activity relationship (QSAR) and read across, are employed, followed by *in vitro* approaches. Each step represents a possible decision point, when data allow a proper evaluation based on a WoE approach: if it could be unequivocally demonstrated that the additive falls in one of the four categories of Article 6 of the TPD, no further testing is needed. Regarding types of effects, toxicity is assessed first, as CMR chemicals are not allowed as additives (Article 7), and accepted methods and evaluation frameworks are available for toxicity testing, followed by characterising flavours, because accepted methods and evaluation frameworks are available. Finally, addictiveness is assessed, and since no validated tests are available here, the assessment can be guided by the available knowledge of the mode of action.

3.3.2 Addressing the data gaps identified in Opinion I for the priority list additives

The data gaps already identified in Tobacco Opinion 1 for the 15 additives included in Commission Implementing Decision (EU) 2016/787 have been analysed. Based on the data gaps described in the 'Rationale for inclusion' in Opinion 1, the activities to be performed upfront have been described. Then on the basis of the obtained results, if the additive does not meet the criteria for exclusion as an additive listed in art. 7 of the TPD, it can be subject to the general evaluation step-wise procedure described in the Opinion.

3.3.3 Information collection

Information on guidance for the data collection and tests to be performed in the different steps of the step-wise approach was collected by the SCHEER on available open literature/websites and from documents by other Committees or International Organisations (e.g. WHO, EPA, EFSA, JECFA)

3.3.4 Information evaluation

For this Opinion on tobacco additives, the available information was analysed by the SCHEER to identify tests and testing structures that are appropriate for the assessment of the toxic and addictive effects, facilitation of inhalation and characterising flavours of tobacco additives.

3.4 Step-wise approach to assess the toxic and addictive effects, inhalation facilitation and characterizing flavour properties of tobacco additives

A pragmatic and efficient step-wise approach is suggested, in order to assess the toxic and addictive effects as well as the additives' properties that facilitate inhalation and lend a characterising flavour to cigarettes and roll-your-own tobacco. The tobacco industry has the burden of proof that an additive does not fall within the scope of the four categories mentioned in the terms of reference and it is tobacco industry's responsibility to deliver data. The data need to be evaluated by independent scientific bodies with expertise in risk assessment of the toxic, addictive, inhalation facilitation and characterising flavour properties of chemicals.

The formation of consortia and joint reports by industry is endorsed in order to limit the financial and administrative burden for industry and authorities, as well as the amount of literature evaluation and testing by industry, and subsequent evaluation of the submitted reports by independent institutes. For the toxicological evaluation of additives in tobacco products, the tiered approach proposed by DKFZ (DKFZ, 2010) has been taken as a starting point. This approach has been adapted to the SCHEER purposes and widened to allow for the evaluation of the addictive effects, inhalation facilitation and characterising flavour of additives (see Figure 1). This is because apart from toxicity, tobacco additives may indirectly increase tobacco-related harm by increasing the consumption rate of tobacco products, either by making the product more attractive to the consumer (e.g. by resulting in a characterising flavour and by facilitating inhalation), or by enhancing its addictiveness (National Institute for Public Health and the Environment, 2012). As far as possible, this possibility has to be considered. Although a standardised methodology is not available, it is possible to derive information from the mode of action of the additive (e.g. addictiveness can be related to increased nicotine bioavailability or to local anaesthetic effects facilitating the inhalation of tobacco smoke; see the SCENIHR Opinion, 2010).

Whenever the evaluation of the additive in the unburnt form gives rise to any concern in relation to art 7 of the TPD (e.g. foreseeing the prohibition of additives having CMR properties) based on data collected in Step 1, the evaluation is stopped, meaning that the additive does not meet the requirement of the TPD. The same rule is applied to Step 2 for the pyrolysis products. In these cases, industry can proceed to step 4 in their reporting.

In case data are not available, or are not sufficient or robust enough to make the evaluation possible, the procedure should go to the next step.

In case of high uncertainty about the evaluation based on available data, there are two possible options:

- Application of the 'worst-case' evaluation
- Delivering of additional data (i.e. via Step 3) by tobacco industry

Step 2 is analogous to Step 1 but related to the pyrolysis products; the two steps can take place concurrently, if this is more efficient and saves time. The collection of available data is mandatory in order to priorities the most appropriate end-point(s) to be assessed in Step 3, to limit useless testing.

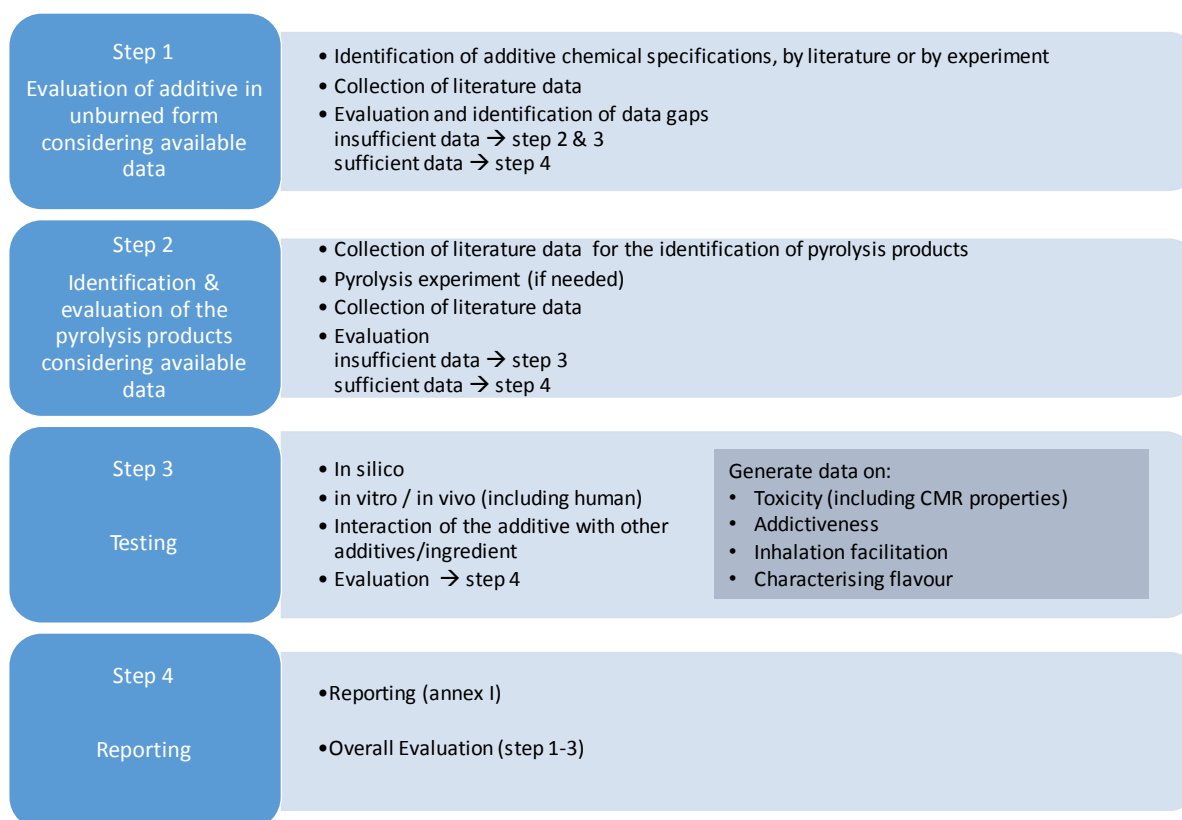


Figure 1. Step-wise approach to be applied to the assessment of the toxic, addictive, inhalation facilitation and characterising flavour properties of tobacco additives. For terminology, please refer to the text. Please note that in vivo tests may only be considered in exceptional cases.

This procedure could be applied either to individual additives or to groups of additives. Additives could be indeed grouped, following rules previously established in other fora to evaluate e.g. groups of food flavouring at EFSA¹² or groups of chemicals in Regulation (EC) No 1907/2006 i.e. REACH (to apply the read-across principles)¹³ in order to limit the use of animal testing (as requested in art. 13). The ECHA provides practical guidance on the issue (available at the ECHA website link); however, to this aim, the approach described in the OECD GUIDANCE ON GROUPING OF CHEMICALS No. 194¹⁴ is recommended.

The approach described in the OECD guidance document (GD) is to consider closely related chemicals as a group, or category, rather than as individual chemicals, for assessing the hazards of chemical substances, increasing efficiency and improving animal welfare. Since the technique of assessing groups of substances is an evolving

¹² <https://www.efsa.europa.eu/en/topics/topic/flavourings>

¹³ <http://echa.europa.eu/support/grouping-of-substances-and-read-across>

¹⁴ GUIDANCE ON GROUPING OF CHEMICALS, SECOND EDITION Series on Testing & Assessment No. 194 (2014) available at [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2014\)4&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en)

science, the GD is revised periodically and it is therefore compulsory that the tobacco industry follows the most updated version when applying it. As it is recommended by the GD itself, early consultations between industry and authorities are recommended to ensure that any regulatory requirements are fulfilled.

The GD outlines a process for grouping chemicals to include the identification of analogues/members of categories, the mechanistic basis for using analogues or chemical categories and the robustness of both approaches. The GD also describes the use of (Q)SARs for data evaluation and data-gap filling (read-across, trend analysis and (Q)SARs).

3.4.1 Step 1: Evaluation of the additive in unburnt form

The first step starts with the identification of the additive chemical specifications, by literature or by experiment (for the physico-chemical characterisation, if not available, data can be obtained following the OECD or ISO test guidelines to this purpose). This initial step is absolutely necessary in order to identify the nature of the additives and comprises also qualifying and quantifying of any impurity present. CAS numbers need to be provided for all relevant chemicals (additives and impurities). The chemistry and specification of a substance (or mixture of substances), in terms of chemical structure(s) and physico-chemical properties is also asked for in other legislations, e.g. for food additives.

It may not always be possible to fully characterise natural extracts, but as much information as possible is required to understand the extent to which variability in composition is controlled during manufacture. Data on the chemical composition of a natural extract additive should be provided by industry with emphasis on the concentrations of constituents of relevance; this includes the concentrations of compounds classified according to their chemical structure (e.g. flavonoids, terpenoids, alkaloids, etc.), constituents being characteristic for tobacco additives (chemical fingerprint, markers). Information on maximum levels for microorganisms and possible contaminants, including e.g. heavy metals, mycotoxins, pesticide residues and polycyclic aromatic hydrocarbon (PAH) residues, should be provided (EFSA, 2012).

Then, all available information on the additive in unburnt form is collected and evaluated. This includes open literature on peer-review journals as well as grey literature (e.g. unpublished reports of studies used for regulatory purposes), including JECFA, EFSA and FEMA documents or data coming from any other regulatory request, in case the additive is used in other contexts.

This step allows the collection of available information on the additive in its unburnt form, useful for its risk assessment. In addition, it allows the identification of the major data gaps (if any) to be addressed in Step 3, with regard to toxicity and addictiveness data, as well as inhalation facilitation or increase nicotine uptake properties or resulting in characterising flavour.

For future reference by the regulator, industry is also asked to indicate which additives are closely related regarding chemical structure, functions, purpose and effects.

3.4.1.1 Collection of available data

Whenever possible, all information already available on the toxicity of the additive (obtained with different experimental models, i.e. *in vivo*, *in vitro*, *in silico* and human data) should be collected, used and evaluated before any testing is initiated (in Step 3). Some knowledge on the toxicity of tobacco additives exists; much less is known on their addictiveness or on how they facilitate inhalation and increase nicotine uptake properties or result in characterising flavour. Open literature as well as grey literature (e.g. unpublished reports of in-house performed studies with regulatory purposes) should be included. If studies have been already performed in view of seeking approval of the same chemical for uses other than tobacco additive by Applicants other than Tobacco Industry, a letter of access should be acquired, in order to avoid repeating the same tests.

Initial electronic literature searches with appropriate key words/dates should be a starting point for data gathering. The databases and search engines used may include for example PubMed, Web of Science, Scopus, Toxline, Chemical and Biological Abstracts, and Google Scholar. The data search methods will identify many papers that potentially could be used. A first screening is then needed in order to focus on those relevant for the specific purposes, using appropriate inclusion/exclusion criteria. Articles that do not appear to meet the inclusion criteria should be excluded from further analysis. To apply a standardised methodology it is recommended that the literature search strategy and selection criteria (inclusion/exclusion) for the review are based on the EFSA Systematic Review Guidance (EFSA Journal 2010).

In order to collect data on addictiveness and on properties that facilitate inhalation or result in characterising flavour, all investigations on possible related mode of action or mechanisms should be considered. In this respect, although never used in this context, an emerging approach is the adverse outcome pathway (AOP) – a framework designed to conceptually link a molecular initiating event to an adverse outcome of relevance to risk assessment (Ankley *et al.*, 2010). The AOP framework allows for a better understanding of the mechanistic linkages between cellular responses and downstream impacts on apical outcomes that are of concern within a regulatory context (Villeneuve and Garcia-Reyero, 2011). Potential practical uses of AOPs also include the above-mentioned grouping of common chemicals for read across (not only based on chemical structures but on biological activity), identification of research and data gaps, serving as a framework for regulatory priority setting, and informing hazard characterization and risk assessment (Becker *et al.*, 2015). AOP methodology may be useful in elucidation of molecular basis for addictiveness of tobacco products e.g. role of pH changes on nicotine absorption, MAO-A inhibition, Dopamine (DA) release and turn over, CYP metabolism and inhibition (for details see paragraph 3.4.3.5). Accordingly, the same applies to investigation on properties facilitating inhalation or resulting in characterising flavour (for details see paragraph 3.4.3.6). OECD developed a guidance document outlining methods and best practices for creating and assessing AOPs, in which it calls for the assessment of an AOP's weight of evidence (OECD, 2013; AOP-Wiki, 2014). AOP wiki represents a joint effort between the European Commission – DG Joint Research Centre (JRC) and the U.S. Environmental Protection Agency (EPA). This serves as one component of a larger OECD-sponsored AOP Knowledge Base effort and represents the central repository for all AOPs developed as part of the OECD AOP Development Effort by the Extended Advisory Group on Molecular Screening and Toxicogenomics.

3.4.1.2 Evaluation

Collected data give information regarding the possibility for the additive used in cigarette/roll-your-own to fall into one or more of the four categories:

- a) Contributing to the toxicity or addictiveness of the products concerned/increasing the toxicity or addictiveness of any of the products concerned to a significant or measurable degree;
- b) Resulting in a characterising flavour;
- c) Facilitating inhalation or nicotine uptake;
- d) Leading to the formation of substances that have CMR properties / increasing the CMR properties in any of the products concerned (cigarettes/RYO) to a significant or measurable degree.

The methodological quality of the selected papers should also be addressed, including the design, execution, analysis and reporting of the study. Expert judgement is vital in the assessment of the quality and the interpretation of data, therefore the appropriate identification and selection of relevant publications/reports is extremely important. When possible (e.g. for toxicity studies) this screening should be based on Klimisch scoring¹⁵.

The acceptance of each publication/report that is considered to be relevant should be based on the quality and relevance criteria summarised in by SCENIHR (2012).

All selected documents of potential importance should be subject to similar treatment in the evaluation process. Positive and negative studies should be evaluated using similar procedures and criteria and considered of similar importance if the quality is judged to be comparable. In positive studies the evaluation needs to consider both causal and non-causal explanations of the results. For example, one key question would be "with what degree of certainty can one rule out the possibility that the observed positive result is produced by bias, e.g. confounding or selection bias, or chance?". In the case of negative studies, it is necessary to assess the certainty with which it can be ruled out that the lack of an observed effect constitutes evidence against a hazard or whether it could result from (masking) bias, e.g., too small exposure contrasts, too crude exposure measurements, too small exposure groups/populations, or chance. Consideration should also be given to the possibility of a publication bias i.e. that positive findings are more likely to be published than negative findings.

It is recommended that the whole data set, judged as relevant, reliable, and of good quality, should be used for the (risk) assessment of the tobacco additive and its pyrolysis products, if any. Different approaches for assessment of whole data sets, referred to as weight of evidence (WoE) evaluation or systematic review (often used interchangeably), have been promoted (Koustas *et al.*, 2014; Rooney *et al.*, 2014; European Food Safety Authority, 2010; IARC, 2006). In general terms, these approaches are processes of summarising, synthesising and interpreting a body of evidence to draw conclusions, e.g. regarding the relationship between a chemical exposure and an adverse health effect. The WoE approach promotes the use and integration of information from all available evidence.

¹⁵H.J. Klimisch, M. Andreae and U. Tillmann (1997): *A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data*, [Regulatory Toxicology and Pharmacology](#) Vol 25, pp 1–5

Unfortunately, formal procedures and consistent terminology for WoE processes are lacking, although a WoE evaluation is mentioned in the REACH regulation, the Biocides directive, the Cosmetics regulation, and the regulation for Classification, Labelling and Packaging (CLP). Some guidance documents are only available for industrial chemicals or contaminants in food (Ågerstrand and Beronius, 2015). However, a number of organisations have established their own frameworks for assessing/evaluating evidence, including the SCENIHR (2012), and the work is still in progress in both the SCHEER and EFSA. Since the approach is rapidly evolving, it is compulsory that in applying it, the Tobacco Industry follows the most updated version.

As indicated above, it is possible to apply substance grouping of read-across principles: this approach uses relevant information from analogous ('source') substances to predict the properties of 'target' substances. The application and reporting of this approach as described in section 3.4 is recommended; if applied correctly, there is no need to have specific information on every additive.

Whenever the evaluation based on the WoE approach of the available data on the additive in its unburnt form unequivocally identifies no concern for any health effect, and it also falls in one of the 4 categories with no data gaps, there is no need to go for further testing in Step 3. On the contrary, whenever the evaluation based on the WoE approach of the available data on the additive in its unburnt form give rise to any concern regarding CMR properties, the evaluation is stopped, meaning that the additive does not meet the requirement of TPD art. 7. In both cases it is possible to directly proceed to Step 4.

In case data are unavailable, insufficient or not robust enough to make any evaluation possible, the procedure should go to Step 3,

3.4.2 Step 2: Evaluation of the pyrolysis products

In the second step, information available on the identification of pyrolysis products of additives must be collected and evaluated. This can be done on the basis of available data (see section 3.4.1.1 for criteria), but in case no sufficient data (in quantitative or qualitative terms) are available, the second step foresees that pyrolysis studies need to be performed in realistic, standardised experimental conditions (see section 3.4.2.2). Then available open and grey literature data on the toxicological profile, addictiveness or properties facilitating inhalation or resulting in characterising flavour on the identified pyrolysis products should be collected, as described in Step 1 for the additive in the unburnt form.

3.4.2.1 Collection of available data

Available data on the pyrolysis products of additives is collected in the same way as described in Step 1 (see 3.4.1.1).

3.4.2.2 Pyrolysis studies(if needed)

To identify the compounds formed during the combustion process of a tobacco additive, tobacco industry in general performs smoke chemistry studies on a comparative basis where a research cigarette is machine smoked with and without the additive present (Talhout *et al.*, 2006). Burning (smoking) the tobacco that contains a specific amount of the additive and subsequent analysis of selected smoke components is described for

many different additives (Baker *et al.*, 2004a; Baker *et al.*, 2004b, c; Carmines, 2002; Rustemeier *et al.*, 2002). However, subtle differences between the selected smoke components will not be noticeable, and it is not feasible to screen the effect on all 6000 known smoke components, hence usually only the so-called Hoffmann analytes are screened. Given the complexity of cigarette smoke, it is difficult to identify individual materials that may result from the pyrolysis of ingredient mixtures, unless radioactively labelled additives are used, but that method is sophisticated and expensive. Furthermore, this method cannot determine whether the additive is a precursor or a catalyst for the formation of a certain smoke component (Torikai *et al.*, 2005).

Pyrolysis, on the other hand, is a useful technique for evaluating materials used at low levels, where it is unlikely that smoke chemistry assays could detect a change. Therefore, combustion processes in a burning cigarette have also been simulated with pyrolysis methods (Baker *et al.*, 2004b; Busch *et al.*, 2012; Lee *et al.*, 2007).

This technique is useful as a first screening of potential pyrolysis products, their thermal stability and the temperature at which they are formed (Baker and Bishop, 2004). However, the pyrolysis conditions only approximate the burning cigarette with regard to temperature and atmosphere and make no allowance for the presence of other tobacco and/or smoke components that may interact with the additives. Pyrosynthesis processes related to the tobacco matrix will not occur when the additive is pyrolysed as a single component outside of the tobacco matrix. When it is suspected that such reactions will occur, one may consider pyrolysing a simple mixture containing the additive together with the component with which reaction is foreseen (either with the component itself or with its pyrolysis products. For instance, micro-vial pyrolysis of a glucose/proline mixture resulted in formation of Amadori intermediates, important in the formation of (Maillard) products that influence the aroma (Mitsui *et al.*, 2015). Pyrolysis was performed at 700 °C, approximating the temperature of the pyrolysis zone of a burning cigarette, for 10 s under atmospheric conditions (headspace gas in vial not replaced by an inert gas).

Pyrolysis studies can be performed under a given set of experimental conditions that need to resemble processes in a burning cigarette in terms of e.g. temperature, rate of temperature change, and atmosphere (amount of oxygen). During the cigarette-burning process, the temperature of the tobacco and the burning cone can range from room temperature up to 900 °C, and the amount of oxygen can range from 0 to 18%. It is important that the design of the pyrolysis study reflects the conditions of burning cigarettes with oxygen levels ranging from 0% to 14% and the temperature in the burning zone ranging from ambient temperature to 900 °C (Baker and Bishop, 2004; Stotesbury *et al.*, 1999; Torikai *et al.*, 2004).

Many studies tried to simulate the processes during smouldering and combustion. Stotesbury performed pyrolysis at 14 sets of pyrolysis conditions: temperatures between 200 °C and 700 °C in 2 % and 10 % oxygen, and at 800 °C and 900 °C in 2 % oxygen. Baker used an atmosphere of 9% oxygen in nitrogen, arguing that this is the average amount throughout the pyrolysis/distillation zone inside the burning cigarette during a puff. From an initial temperature of 300 °C, to simulate the smouldering before taking a puff, the sample is heated at 30 °C s⁻¹ to 900 °C, and kept for 5 seconds, to simulate the maximum duration of the high-burning zone temperature during puff under extreme human smoking conditions. According to Baker, 30 °C s⁻¹ is the approximate mean heating rate throughout the pyrolysis/distillation zone during a puff. This seems rather

slow, as that would imply it would take 20 seconds before the maximum temperature is reached, whereas a human puff only takes one or two second. However, most studies are performed with a similar heating rate (Torikai *et al.*, 2004). Purkis *et al.* programmed the temperature from 300 to 900 at 25 °C per second to reflect cigarette smoking and give an appropriate set of conditions to limit artefact formation (Purkis *et al.*, 2011). It is important that the reaction vial is not closed, so that the additive can distil away at lower temperatures.

Flash pyrolysis is performed when the sample is rapidly inserted in a pre-heated furnace that is already at the highest temperature, for instance at the temperature range of 200–300 °C to simulate cigarette smouldering (Zhou *et al.*, 2011). Time of flight spectroscopy allows for almost real time sampling, enabling identification of reactive compounds before being degraded (Hertz-Schunemann *et al.*, 2015) (Busch *et al.*, 2012).

Taking into account the studies described above, the SCHEER recommends the following experimental design in most cases performed by tobacco industry:

Thermal degradation (pyrolysis, pyrosynthesis and combustion products) of each additive is to be studied under different reaction regimes (inert and 2-14% oxygen) over the temperature range 200–900 °C. The thermal degradation products of two different pyrolysis conditions should be identified:

- (1) upon gradually heating the sample from 200–900 °C and
- (2) conventional pyrolysis, in which a new sample is pyrolysed at minimally 3 different temperatures (~ 300°C, 600°C and 900°C).

Pyrolysis experiments should be carried out at least in triplicate. Chemical analysis of the components in the pyrolysate needs to be performed with state of the art techniques in the field of GC-MS and LC-MS, as appropriate for the specific additive. The World Health Organization in its report to the Sixth Conference of the Parties¹⁶, identified eight non-exhaustive lists of toxicants: Health Canada, RIVM, USA FDA, Counts, Dybing and Fowles, Hoffman analytes, Philip Morris-Australian brands, and Philip Morris-Canadian brands. These toxicants need to be identified and quantified, if present, using analytical reference standards. Tobacco-specific components, such as nitrosamines and alkaloids, are not expected to be present.

For additional components, not on these lists, the following procedure is advised. For identification purposes, library software can be used, such as the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software. Components with a peak-to-peak signal-to-noise ratio below three can be discarded. Also, components with a probability of correct identification below 70% can be excluded.

If components with a toxicological hazard are identified, their identification needs to be confirmed and their amount needs to be quantified using analytical reference standards.

Apart from components that may increase the toxicity, specific attention needs to be given to components that have addictiveness-enhancing properties, flavouring

¹⁶http://apps.who.int/gb/fctc/PDF/cop6/FCTC_COP6_14-en.pdf

properties, or inhalation facilitation properties (e.g. anaesthetic and/or bronchodilating properties) (SCENIHR, 2010).

3.4.2.3 Evaluation of data

The collected data will be evaluated according to a WoE approach (see 3.4.1.2). Again, when the available information is considered reliable and robust enough concerning both the identification of pyrolysis products and on their toxicological and addiction profile or on properties facilitating inhalation, increasing nicotine uptake or resulting in characterising flavour, a solid evaluation can be possible based on the WoE approach, on the basis of which a decision (positive or negative) can be taken. For instance, if the data set unequivocally identifies the additive as not falling into any of the four categories and not posing any concern for any health effect, with no data gaps, there is no need to go for further testing in Step 3. On the contrary, if it is demonstrated that compounds proven to have carcinogenic or mutagenic properties are generated from pyrolysis of an additive, this additive will contribute to the toxicity of the product concerned, considering the stochastic nature of the carcinogenic effect.

When data are unavailable, insufficient or not robust enough to make any evaluation possible, the procedure should go to Step 3.

3.4.3 Step 3: Testing and evaluation of results

The third step is related to the testing of additives or their pyrolysis products, whenever a data gap is identified in Step 1 and 2, or uncertainties have to be reduced, according to methods accepted by other regulations. The outcomes of tests must be related to actual human exposure and tobacco-induced diseases, and be relevant not only for subchronic, but also for chronic exposure in intermittent use sessions (Johnson *et al.*, 2009).

A relevant test design will not only consider methods to investigate toxicity, but also characterising flavour, facilitating inhalation and addictiveness. Therefore information should be collected if it is related to the known mechanisms that contribute to addictiveness or on properties facilitating inhalation, increasing nicotine uptake or resulting in characterising flavour.

Based on expert judgement of the major data gaps with regard to toxicity, characterising flavour, facilitating inhalation properties and addictiveness data identified in Step 1, it must be decided which endpoint to start with. This will generally be the endpoint for which most evidence is available of a potential concern. If no priority concerns have been identified, it is advised to start with toxicity, as in that case, accepted *in vitro* tests are available and there are frameworks for interpreting the results.

This step will also address the possible interactions, at chemical level (e.g. pyrolysis) and for the toxicological part based on the MeA/MoA.

It should be noted that whenever there is a good scientifically-based reason for asking for an exception for presenting data related to a specific end-point, a detailed justification reporting the rationale for the derogation must be provided.

Once data are collected by applying testing in Step 3, they should be evaluated together with data collected in Step 1 and 2 by using a WoE approach. If uncertainties cannot be

reduced by results coming from Step 3 testing, the SCHEER recommends that the assessors consider the 'worst-case' evaluation.

3.4.3.1 Comparative paradigms are not endorsed

In order to provide a relevant outcome to the question of whether an additive contributes to the toxicity, addictiveness and properties facilitating inhalation or resulting in characterising flavour of the products concerned (i.e. cigarette and roll-your-own tobacco; Article 6(2) of the TPD), the study design must adhere to some methodological criteria.

Strictly speaking, the only way to comply with Art.6 TPD2 (A6), to assess whether additives increase "toxicity or addictiveness ... to a significant or measurable degree" should be comparative testing (Kienhuis *et al.*, 2016). However, it must be noted that comparative testing strategies, where differences in effect of the tobacco product with and without the additive are evaluated, at the moment are not considered suitable, given the current toxicity tests and available methodology. Indeed, the emissions of tobacco products are highly toxic, in particular regarding cigarette smoke (Kienhuis *et al.*, 2016). Due to the high intrinsic toxicity of tobacco products, it is challenging to demonstrate any differences, whether they be increases or decreases, induced by an additive.

Due to the high toxicological activity of both the test product (tobacco product with additive) and the control (tobacco product without additive) in comparative testing strategies, the discriminatory power that can be obtained in toxicity assays may not be sufficient (COT/COM/COT, 2009; DKFZ, 2010; Oldham *et al.*, 2012).

Very sensitive tests would be required, with a clear dose-response relationship, in order to show any differences from these high background effects. As such tests are not currently available, no new comparative studies (tobacco product with and without additives) will be considered, since these studies lack discriminative power. Studies that are already available could be presented, but the relevance of their results has to be evaluated in a WoE approach based on these considerations.

In line with this, the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC, 2009) concluded: "*The Committee considered that the available studies used to assess the contribution of individual or mixed ingredients or additives to the overall toxicity of tobacco products are inadequate to assess the risks posed by conventional cigarettes, so it is not possible to assess the modulation of that risk resulting from inclusion of additives. The relationship between effect (an increase in biomarker) and exposure is also poorly understood. Furthermore, it is possible that additives might alter smoker behaviour, such as to increase product use; this increased exposure would be likely to result in an increased risk.*"

Furthermore, an international Working Group on Tobacco Additives (WG), assigned by the Brazilian regulatory agency ANVISA, assessed many industry-sponsored studies addressing the effects of mixtures of commonly used additives on cigarette smoke chemistry and toxicity. Although industry claimed that additives have no effect on the levels of chemical components of cigarette smoke and toxicity, the WG concluded that the available data were insufficient to accept the tobacco industry's claims that additives do not increase the inherent toxicity of tobacco smoke (Ferreira *et al.*, 2015; Working Group on Tobacco Additives, 2014): "*Given the current toxicity tests and test designs, it*

is not yet possible to determine whether or not addition of specific ingredients (tobacco additives) to tobacco products adds to tobacco mainstream smoke's inherent toxicity. This is because tobacco itself is already quite toxic, and any added toxicity is difficult to detect within the current test designs used by tobacco industry, i.e. combinations of in vitro testing and animal testing."

For the future, tests are needed that are sensitive enough to assess the toxicity attributed to additives above the overall toxicity of tobacco products, which can associate assay outcomes to human risk and exposure. In this respect, *in vitro* tests combined with toxicogenomics, using biomarkers of exposure and disease are the most promising (Kienhuis *et al.*, 2016). However, for the time being, no standardised methods have been validated to this purpose.

Another problem with comparative testing is the choice of the product to be tested, since the additional toxicity of the additive would differ between product types and brands. If an additive would be tested in the intended brand, the results (related to toxicity, addictiveness and attractiveness) could not necessarily be generalised to all products and brands, having a different composition. Therefore the obtained results may not lead to general prohibition/acceptance of specific additives but rather to prohibition/acceptance on a product-by-product basis (DKFZ, 2010).

When the addition of sugars is taken as an example, it will be very important whether a reference containing Burley tobacco, that does not contain natural sugars, or Virginia tobacco, with high sugar levels, is selected, or a blend of these tobacco types. This is even more important as cultural differences exist in the preference for Virginia-type cigarettes, American blend, or Burley. According to the TPD, the use of additives necessary for the manufacture of tobacco products should be allowed, as long as they do not result in a characterising flavour or increase the addictiveness, toxicity or CMR properties of the product. Thus, in this particular case, sugar addition to replace what is lost during the curing process, needs to be evaluated against the possibility of toxic and carcinogenic compounds forming following pyrolysis.

For similar reasons, comparative studies are in general also not endorsed for examining the effect of additives on addictiveness. In human studies, there are two exceptions to this general rule. The first is characterising flavour testing. By definition, this is a comparative testing paradigm, as clearly noticeable flavour is a property of a tobacco product, caused by the addition of flavour additives, and not a property of an additive itself (see section 3.4.3.6). In this experimental set-up, strong flavours other than that of tobacco can be distinguished, whereas more subtle flavours caused by an additive will not be consciously discriminated. The second includes additives that facilitate inhalation or nicotine uptake, and perhaps similar effects in humans, as e.g. reported for smokers of mentholated cigarettes vs non-mentholated cigarettes. In this case, large groups of test subjects are needed, and effects on e.g. sensory perception, smoking topography and biomarkers of exposure (e.g. nicotine in blood or cotinine in urine) can be discriminated. In most human studies, any effects of the additive will probably not be picked up in comparative testing designs, or at least be more prominent in non-comparative designs. For instance, when focusing on the dependence capacity of tobacco additives, studies on acute effects done within subject measurements (placebo vs. additive(s) of interest) are regarded as more valuable. This will improve the sensitivity to picking up small changes in neuronal activity caused by administration of the additive.

Instead of using a comparative study design, the effects of the pure additive and its pyrolysis products must be considered in a relevant testing strategy, such as the tiered approach proposed by the SCHEER (Fig.1).

3.4.3.2 The use of animal testing

So far, available inhalation or other animal studies have been used to assess the effect of tobacco additives. Whenever studies using animals are available, they should be evaluated in Step 1 and 2. However, it is ethically questionable to carry out any new animal studies to evaluate the 'safety' of a tobacco additive, as tobacco products are highly harmful with no benefits to individual or public health. Therefore, as a principle, only the use of *in silico* and *in vitro* studies applies to Step 3, following the EU policy recommending implementation of 3R methods for refinement, reduction, and replacement of animal models, leading to the ban of animal studies for chemicals to be used in voluntary products such as cosmetics (EU Regulation no. 1223/2009). Non testing or alternative testing approaches followed for the evaluation of cosmetic ingredients¹⁷, whenever relevant to tobacco products, could be considered.

Human studies are discouraged, although they may be used in specific case (e.g. for characterising flavour assessment or facilitating inhalation properties), but only if the study subjects are informed and not exposed to the harmful smoke emissions of tobacco products. Generally and especially in those specific cases in which animals are proposed, early consultations are recommended between Receiving Competent Authorities at Member State level and tobacco industry, presenting a testing strategy including *in silico*, *in vitro* and only in exceptional cases *in vivo* tests. In order to limit the testing, formation of Consortia is recommended.

In the exceptional cases when animal testing would be deemed necessary, it is compulsory to be compliant with the Animal Welfare EU policy and to respect the Regulation on Animal Testing.

3.4.3.3 Quality system

In line with the provisions of other regulatory contexts, the SCHEER recommends the use of a Quality system (e.g. Good Laboratory Practice or ISO17025) for carrying out the pyrolysis or other physico-chemical studies as well as pre-clinical toxicity studies (including those to assess the mode of action underlying possible contribution to addictiveness and characterising flavour, increasing nicotine uptake or facilitating inhalation).

In case the principle of the Mutual Acceptance of Data is applied (again to limit the testing) the quality system of choice should be the GLP, following the application of the GLP OECD principles, to which all the National Monitoring Authorities in the different OECD Member States make reference. Human studies should follow the appropriate Quality System, since GLP are usually not applied to this kind of testing. For the physico-chemical studies, GLP compliance is not always requested and the ISO17025 could be chosen.

¹⁷http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf

3.4.3.4 Toxicity testing

In silico

If toxicological data on the additives are not available or are limited, they can be produced using *in silico* approaches. As a first step, QSAR methods are encouraged to identify alerts for genotoxicity, carcinogenicity and reproductive toxicity, to get information of potential CMR properties of the additive or of its identified pyrolysis products: this could also take advantage of similarities with other chemicals by applying the read-across methodology.

Non-test information about the biological activity of a substance can be derived in a variety of ways, ranging from simple inspection of the chemical structure through various read-across techniques, the use of expert systems, metabolic simulators, to global or local (Q)SARs. The usefulness of such techniques varies with the amount and nature of information available, as well as with the specific regulatory questions under consideration.

Models for the identification for alert of genotoxicity and carcinogenicity have a long tradition; (Q)SAR models for mutagenicity can apply to a limited set of congeneric substances (local models) or to a wide variety of non-congeneric substances (global models).

Many global models for mutagenicity are commercial and some of the suppliers of these global models consider the data in their modelling sets to be proprietary. Proprietary means that the training set data used to develop the (Q)SAR model is hidden from the user. In other cases it means that it may not be distributed beyond use by regulatory authorities.

There are hundreds of (Q)SAR models available in the literature for predicting test results for genotoxic endpoints for closely related structures (Naven *et al.*, 2012; Bakhtyari *et al.*, 2013). These are known as local (Q)SARs. However, quality of reporting varies from model to model and predictivity must be assessed case-by-case on the basis of clear documentation.

In case of robust data on the identification of structural alert or based on read across indication of CMR properties, it is not necessary to go further, since according to the TPD no CMR substance can be used as a tobacco additive. In case there are doubts, *in vitro* testing can be conducted (see below).

Regarding the other toxicological properties, other QSAR tools are available. Some of them are briefly described in the following:

OECD built an open software application (<http://www.qsartoolbox.org/>), named QSAR Toolbox. The Toolbox and guidance on its use are freely available. The OECD QSAR Toolbox facilitates the practical application of grouping and read-across approaches to fill gaps in (eco-)toxicity data, including but not limited to genotoxicity and carcinogenicity, for chemical hazard assessment. The Toolbox incorporates information and tools from various sources, into a logical workflow. Crucial characteristic of the workflow is the grouping of chemicals into categories (group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties

are likely to be similar or follow a regular pattern, usually as a result of structural similarity). The most important features are:

1. Identification of relevant structural characteristics and potential mechanisms or mode of action of a target chemical.
2. Identification of other chemicals that have the same structural characteristics and/or mechanism or mode of action.
3. Use of existing experimental data to fill the data gap(s).

The Joint Research Centre of the EU provides several tools for modelling for the safety assessment of chemicals. They offer the following computational tools (freely downloadable or accessible from their webpages):

- JRC QSAR Model Database: database hosting structured and peer-reviewed information on QSAR Models (<https://eurl-ecvam.jrc.ec.europa.eu/databases/jrc-qsar-model-database>);
- Toxtree, software tool to generate prediction(s) on mechanisms of action or toxicological effects, the tool is based on a decision-tree approaches (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxtree);
- Dart, (Decision Analysis by Ranking Techniques) a software tool designed to rank chemicals according to environmental and toxicological concerns (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/DART);
- Toxmatch, a flexible application for grouping chemicals based on chemical similarity designed to be helpful in read-across (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxmatch).
- Stat4tox, a tool which carries out concentration-response analysis for *in vitro* experiments (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/stat4tox).

ECHA provides a detailed overview on non-testing methods in sub-section R.7.7.3.1 of Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance (see http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf), in particular with regard to the prediction models for mutagenicity and the OECD QSAR toolbox.

A list of the available (free and commercial) predictive software for ecotoxicological, toxicological and environmental endpoints, including mutagenicity models, has been compiled within the frame of the EU project Antares (<http://www.antares-life.eu/>). For example, the Danish EPA and the Danish QSAR group at DTU Food (National Food Institute at the Technical University of Denmark) have developed a (Q)SAR database that contains predictions from a number of mutagenicity models. The database is freely accessible via <http://qsar.food.dtu.dk>. The online database contains predictions for over 166,000 substances and includes a flexible system for chemical structure and parameter searching. A user manual with information on the individual models including training set

information and validation results is available at the website. The database is also integrated into the OECD (Q)SAR Toolbox.

Another example of a database with predictions on mutagenicity is the Enhanced NCI Database Browser (<http://cactus.nci.nih.gov>) sponsored by the U.S. National Cancer Institute. It contains predictions for over 250,000 substances for mutagenicity as well as other non-mutagenic endpoints, some of which may provide valuable mechanistic information (for example alkylating ability or microtubule formation inhibition). It is also searchable by a wide range of parameters and structure combinations.

Use of harmonised templates, such as the QSAR Model Reporting Format (QMRF) and the QSAR Prediction Reporting Format (QPRF) developed by the Joint Research Centre (JRC) of the European Commission (http://ihcp.jrc.ec.europa.eu/our_labs/predictive_toxicology/qsar_tools/QRF), can help to ensure consistency in summarising and reporting key information on (Q)SAR models and substance specific predictions generated by (Q)SAR models. The JRC website also hosts the JRC (Q)SAR Model Inventory, which is an inventory of information on the validity of (Q)SAR models that have been submitted to the JRC (http://ihcp.jrc.ec.europa.eu/our_databases/jrc-qsarinventory).

If the exposure could be well characterised without uncertainties, the application of the Threshold of Toxicological Concern (TTC) concept could be foreseen providing the applicability domain will be expanded to include sufficient data coming from inhalation studies. Its use has been proposed, but never applied in regulatory context in relation to the inhalation route (Costigan and Meredith, 2015). In addition, the concept of the 'internal TTC', independent on the route of exposure, is a promising approach that is also starting to be considered in the area of cosmetic products (SCCS, 2016).

In vitro

There are a number of *in vitro* tests that can be used to assess many different toxicological end-points. *In vitro* toxicity tests are applicable to a wide variety of test materials including ingredients added to tobacco, tobacco extracts, tobacco smoke condensates, and whole or vapour phase smoke. These assays may also be used to explore interactions between components of cigarette smoke. The test item should be the additive in its unburnt form and relevant pyrolysis products (in case information coming from Step 1 and 2, as well as from non-testing methods in Step 3 give rise to insufficient data or high uncertainties in the evaluations) as well as to tobacco smoke particulate and whole or vapour phase smoke, when relevant.

The first choice has to be given to tests already adopted at international levels (e.g. OECD Test Guidelines¹⁸, or ISO methods) or tests validated by EURL-ECVAM. The OECD test guidelines (TGs) describe the applicability domain, the principles of methods, the test procedure and they also address reporting.

In case a non-TG *in vitro* method is used (e.g. a test validated by EURL-ECVAM), the reporting should be appropriate, following what it is indicated in the OECD Guidance

¹⁸<http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm>

Document for describing Non-Guideline *in vitro* test methods No.211 (2014)¹⁹. The application of integrated approaches on testing and assessment (IATA) is highly recommended, as described in documents adopted by both EURL-ECVAM and OECD²⁰.

Since the acceptance at regulatory levels is evolving, the TG as well as the GDs are periodically revised, therefore it is compulsory that in choosing the appropriate test, the tobacco industry follows the most updated version. Indeed, as requested by EU regulation 1223/2009 on cosmetics, the EU Commission has to report every year to the European Parliament as well as to the EU Council on the progress related to the development, validation and regulatory acceptance of alternative methods as communicated by a Report from EURL-ECVAM²¹.

If information gathered through *in silico* methods are not conclusive and there are doubts related to genotoxicity potential, an *in vitro* genotoxicity test battery or *in vitro* transformation test for carcinogenicity can be applied to clarify these end-points. The *in vitro* genotoxicity testing methodologies are well described in the several adopted OECD TG. Please refer to the OECD Guidance Document on Revisions to OECD Genetic Toxicology Test Guidelines²² or to the OECD web site for the last update on adopted TG. In the battery, it would be necessary to include tests able to identify point mutations in prokaryotic and mammalian cells as well as chromosomal aberrations or *in vitro* micronucleus assay and DNA damage and repair (for details, see also the SCCS Note for Guidance 9th Revision, 2016)²³.

In vitro methods to address local toxicity (i.e. phototoxicity, skin corrosion and irritation, eye irritation, skin sensitisation) are available (see table 1) and should be performed, whenever relevant, using the air-liquid interface.

IT is more difficult, however, to address systemic toxicity by means of *in vitro* testing only, since at the moment no adopted TGs are available.

For carcinogenicity, two cell transformation assays have been included in OECD guidance Documents (table 1), which - using an IATA with *in silico* and read across data - could give sufficiently robust information.

Only a few *in vitro* studies are available to address only very specific reactions possibly leading to reproductive problems, such as *in vitro* binding to estrogen and androgen receptors (see the OECD conceptual framework to evaluate endocrine disrupting chemicals). Nevertheless, they can be included, and evaluation should be carried out also considering the indication coming from QSAR and read across in a WoE approach.

¹⁹GUIDANCE DOCUMENT FOR DESCRIBING NON-GUIDELINE *IN VITRO* TEST METHODSSeries on Testing and Assessment No. 211

²⁰<http://publications.jrc.ec.europa.eu/repository/bitstream/JRC96418/eurl%20ecvam%20toxicokinetics%20strategy.pdf><http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO%282015%2922&doclanguage=en>

²¹ http://ihcp.jrc.ec.europa.eu/our_lab/eurl-ecvam

²²<https://www.oecd.org/chemicalsafety/testing/Genetic%20Toxicology%20Guidance%20Document%20Aug%2031%202015.pdf>

²³http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf

Cytotoxicity testing after repeated exposure (e.g. 14 days) is considered a possible end-point of choice (acute toxicity is not relevant for tobacco products, hence also for additives). Positive and negative controls should be used, cells of human origin have to be preferred; non-specific tests as well as organ specific cells should be used (e.g. cells coming from the lung or intestinal cells, accounting for inhalation and swallowing of smoke, but also cells representing CNS, cardiovascular system, etc. to be evaluated on a case-by-case basis). For a correct interpretation of results, it should be considered that whenever cell lines are used, they are generally characterised by a low and unbalanced metabolic capability, therefore at least one of them should maintain this function over the treatment period (e.g. HepaRG cells) or primary cells should be used. The use of primary cell lines can also account for the interindividual variability that in some cases is translated to differences in susceptibility to toxic effects.

The major endpoints evaluated in *in vitro* cytotoxicity assays include the effect of a substance on cell viability (survival) and growth rates, but other end-points (such as mitochondrial functionality, induction of apoptosis) can be included. Cytotoxicity testing is used in the area of medical devices: methods to be considered can be found within the harmonised European standard ISO 10993-1:2009 "Biological evaluation of medical devices Part 1: Evaluation and testing within a risk management process". The ISO 10993 – 5:2009 describes test methods to assess the *in vitro* cytotoxicity of medical devices.

However, the study design should take into account that the most relevant route of exposure for tobacco additives is inhalation.

There has been significant progress made in recent years in approaches to expose cells *in vitro* to chemicals that pose a toxicological concern via the inhalation route (Aufderheide *et al.*, 2011; Bakand and Hayes, 2010). A number of *in vitro* exposure systems have been developed (although not yet validated) to facilitate the study of the effects of the whole smoke mixture on both mammalian and bacterial cells, and this has been the subject of a recent review (Thorne and Adamson, 2013).

Current cell-based *in vitro* models of the respiratory tract consist mainly of 2D monolayers of primary tracheobronchial epithelial cells or an immortalised cell line cultured on a semipermeable membrane insert at an air-liquid interface to induce cell polarization, differentiation, and mucus production (Forbes *et al.*, 2005). A more sophisticated technique that enables the stable and reproducible exposure of cultivated cells to cigarette smoke at the air-liquid interface such as CULTEXW Radial Flow System (RFS) module has been proposed recently (Rach *et al.*, 2013).

It was documented that air-liquid interface culture played a significant role toward the *in vitro* recapitulation of the *in vivo* environment, presenting the cells with an apical side resembling the lumen of the respiratory tract and a basolateral side representing vascular supply of nutrients (Berube *et al.*, 2010), with increased expression of cilia in primary cells and differences in barrier and mucus-secreting properties of cell lines observed (de Jong *et al.*, 1994, Grainger *et al.*, 2006). However, the absence of an extracellular component with co-cultured cells in a 3D environment can result in an oversimplification of the airway barrier, lacking in physiological relevance. Therefore, more sophisticated models based on 3D human normal and diseased tissue are required to provide *in vitro* models that improve validity of tested compounds in humans. Much of respiratory tissue engineering research has seen a transition from single cell-type culture

on inserts toward co-culture and the inclusion of scaffold material. Accordingly, models, in which the epithelium is cultured at an air-liquid interface over a scaffold substrate embedded with co-cultured cells, are the subject of much interest and are even available now as commercial 3D research products, such as the MatTek EpiAirway-FT technology (Berube *et al.*, 2010). Indeed, there is an overall consensus in the literature that introducing an epithelial cell analogue into the co-culture environment, often through the use of a biomaterial scaffold, could enhance cell culture, cell-cell signalling, and functionality. A triple co-culture system, in which human bronchial epithelial cells A 549, human mesenchymal cells and dendritic cells were cultured in monolayers, has shown promise for studying immunological responses to inhaled particulates (Rothen-Rutishauser *et al.*, 2005, Herzog *et al.*, 2013). Co-culture of Calu-3 cells with Wi38 lung fibroblasts was achieved on the scaffold to create a submucosal tissue analogue of the upper respiratory tract, validating system as a platform to support co-culture and cellular organisation reminiscent of *in vivo* tissue architecture. These scaffolds were validated as a substrate to support functional mucus express from an airway epithelium. Calu-3 cells cultured on CHyA-B scaffolds also expressed the tight junction protein ZO-1 and F-actin, indicating the formation of an epithelial barrier layer on the constructs and differentiation of the Calu-3 cells. Recently, another *in vitro* model system using tissue-engineered constructs has been developed which might improve our understanding of epithelial tissue and disease and use for testing toxicity of different compounds (O'Leary *et al.*, 2016).

Tobacco smoke assessment *in vitro* has traditionally focused on the particulate phase captured on a Cambridge filter pad and eluted in DMSO (Crooks *et al.*, 2013) or bubbled through cell culture media or PBS (Andreoli *et al.*, 2003). Cell cultures are then exposed under submerged conditions to the particulate phase. The gas vapour phase of cigarette smoke has been also considered as described in Health Canada Official Method T-501, T-502, and T-503.

Submerged culture conditions and particulate-based exposures do not represent physiologically that of mainstream tobacco smoke exposure in the human lung. Furthermore, separating smoke fractions in this way could lead to alterations and chemical changes that may not be representative of the whole smoke aerosol. In order to address these challenges, whole smoke exposure systems have been developed. Whole smoke exposure systems offer many technical challenges, but represent a more physiologically relevant test system that captures the full interactions of both the particulate and vapour phases together (Fukano *et al.*, 2004). An additional advantage of these systems is that a multitude of different cell cultures can be exposed at the air-liquid interface (ALI) to whole smoke, better simulating human exposure (CORESTA, 2006).

Whole smoke exposure systems offer the advantage that all phases of smoke can be analysed together or independently depending on the experimental set-up. This has allowed researchers to tailor their experiments to investigate both phases of tobacco smoke, yielding useful information. There is a variety of whole smoke systems available and the majority of these systems can also be used to deliver individual aerosols or other complex aerosol mixtures to cell cultures. However, there are no defined regulatory protocols for tobacco whole smoke exposure systems, and so far there is no recognised approach to the measurement of dose. The vapour phase of cigarette smoke within these systems remains poorly understood. With the variety of exposure options

available to researchers and bespoke systems relatively easy to fabricate or replicate, dosimetry tools may bridge the gap and play an important role, not only in the measurement of actual cellular dose but also in the characterisation and validation of these systems (Thorne and Adamson, 2013).

There are continuous efforts to introduce existing testing methods into regulatory framework of tobacco products risk assessment: product testing protocols are being developed for assays such as Ames bacterial mutagenicity and Neutral Red Uptake (NRU) cytotoxicity (Adamson *et al.*, 2014).

The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA, 2004) *in vitro* Toxicology Task force of industry recommends using a test battery composed of the following assays:

1. A bacterial mutagenicity assay. The Ames Salmonella mutagenicity assay is recommended.
2. A mammalian cell assay for cytogenetics/mutation. The Task Force recommends the micronucleus assay, the chromosome aberration assay or the L5178Y mouse lymphoma assay.
3. A cytotoxicity assay conducted with an appropriate mammalian cell line. The Task Force recommends the neutral red cytotoxicity assay (CORESTA, 2004).

Analysis of the recent publications has shown that these recommendations are used increasingly in the toxicity assessment of different tobacco products (see e.g. Manuppello and Sullivan, 2015).

Considering non-cancer endpoints, a battery of *in vitro* tests have been proposed for assessing CVD risk associated with cigarette smoking (Fearon *et al.*, 2013). The battery is comprised of functional *in vitro* assays to model endothelial damage, angiogenesis, and migration of vascular smooth muscle cells as initial and subsequent events in CVD (Fearon *et al.*, 2013). Other tests, such as for oxidative stress or inflammatory response, may be conducted to assess the adverse effects of cigarette smoke *in vitro*.

However, it is the opinion of the SCHEER that the choice of the test battery should not be fixed *a priori*, and should be rather tailored on the basis of information coming from the *in silico* and read-across analysis (e.g. bridging *in vitro* studies can be necessary to support the read across).

Table 1 summarises the most recent, internationally accepted, validated *in vitro* methods, which may be used for the toxicity assessment of the tobacco products.

Modified after AltTox (<http://alttox.org/mapp/table-of-validated-and-accepted-alternative-methods>) and PISC (<http://www.piscld.org.uk/alternatives-approved-by-regulators/>).

Table 1: *IN VITRO* METHODS ADOPTED FOR REGULATORY USE

Toxicity endpoint	<i>In vitro</i> methods		Recommendations and standard methods (OECD)
ACUTE TOXICITY	3T3 neutral red uptake (NRU) cytotoxicity test to estimate starting doses for oral acute systemic toxicity		OECD GD 129, published in 2010
	NHT neutral red uptake (NRU) cytotoxicity test to estimate starting doses for oral acute systemic toxicity		OECD GD 129, published in 2010
ACUTE PHOTOTOXICITY	3T3 neutral red uptake (NRU) phototoxicity test		OECD TG 432, published in 2004
SKIN IRRITATION	Integrated approach on testing and assessment (IATA)		OECD GD 203, published in 2014
	Reconstructed human epidermis (RhE) test	EpiSkin™ (L'Oréal, France)	OECD TG 439, revised in 2015
		EpiDerm™ (MatTek, US)	OECD TG 439, revised in 2015
		SkinEthic™ (L'Oréal, France)	OECD TG 439, revised in 2015
		LabCyte EPI-Model (J-TEC, Japan)	OECD TG 439, revised in 2015
SKIN ABSORPTION/ PENETRATION	<i>In vitro</i> diffusion method		OECD TG 428, published in 2004
SKIN SENSITISATION	Adverse outcome pathway (AOP) for skin sensitisation		OECD series on testing and assessment 168, published in 2012
	Direct peptide reactivity assay (DPRA)		OECD TG 442C, published in 2015
	ARE-Nrf2 luciferase test method (e.g. KeratinoSens™ assay)		OECD TG 442D, published in 2015
	Human cell line activation test (h-CLAT)		OECD TG 442E, published in 2016
CARCINOGENICITY	Cell transformation assay (CTA) (Syrian hamster embryo cell transformation assay (SHE CTA))		OECD Guidance Document Env/JM/Mono(2015)18, May 2015
	<i>In vitro</i> cell transformation assays (CTA) Bhas 42 cell transformation assay (Bhas 42 CTA)		OECD Guidance Document Env/JM/Mono(2016) 1, January 2016

Besides these internationally validated methods and the ISO 10993 – 5:2009 adopted for medical devices, there is a great number of other methods employing technological innovations, such as reconstituted human tissue cultures, 3-D organotypic cultures comprised of differentiated human cells in co-cultures, air-liquid interface exposure systems, cell transformation assays and high content genomic analysis, that are used for tobacco product analysis.

3.4.3.5 Addictiveness testing

Tobacco comprises of thousands of substances, of which nicotine is the most characterising and most addictive component. Additives, as well as natural tobacco substances other than nicotine, may have addictive capacities themselves or can interact with nicotine and the nicotine receptor system, herewith enhancing the effects of nicotine. For example, these additives can have effects on nicotine bioavailability, duration, and concentration in the blood circulation or nicotine-dependent activation of mesolimbic pathways in the brain. The term 'dependence potential' is commonly used to describe addictive capacity.

Guidelines to assess the impact of tobacco product contents on dependence potential could be similar to those already established for testing the dependence potential of pharmaceutical products (the used methods are known as Abuse Liability Assessment). Special challenges include product complexity and the diverse range of tobacco products (Henningfield *et al.*, 2011; WHO Study Group on Tobacco Product Regulation TobReg, 2012). For example, the US FDA has issued guidance that covers dependence potential assessment for a range of different substances, formulations, and product types in which factors such as additives and product design features may act to either promote or deter dependence potential (U.S. Dept. of Health and Human Services, 2010, 2015).

Experimental testing of the dependence potential of tobacco additives is still limited due to the lack of validated administration models for the examined individual compound itself and in co-administration with other tobacco additives. In the proposed step-wise approach, the possibilities to experimentally quantify the dependence potential of tobacco additives (often) co-administered with nicotine is discussed.

In silico

Nicotinic acetylcholine receptor (nAChR) Computer models. nAChRs are ligand-gated cation channels found throughout the central and peripheral nervous systems (Gotti *et al.*, 2006; Jensen *et al.*, 2005). Neuronal nACh receptors participate in many neurological processes including cognition (Levin and Simon, 1998), pain sensation (Damaj *et al.*, 2000), and nicotine reward mechanisms (Dani and De Biasi, 2001; Pavlovicz *et al.*, 2011; Tapper *et al.*, 2004). In the past years several nAChR *in silico* models have been developed integrating protein (sub-) structures, dynamics and functional relationships. Among those, the most widely expressed nAChR subtype in the brain is the neuronal $\alpha 4\beta 2$ nACh receptor (Haddadian *et al.*, 2008). The $\alpha 4\beta 2$ nAChR comprises high-affinity nicotine-binding sites (Tapper *et al.*, 2004) but the mechanism how ligand binding leads to channel opening remains elusive. The quality of the current $\alpha 4\beta 2$ nAChR model was evaluated using flexible docking of nicotine docking to the closed- and open-channel models. Besides the potential nicotine interactions with surrounding residues that could stabilize nicotine positions, a high degree of involvement of aromatic residues in the nicotine binding sites was also observed (Haddadian *et al.*,

2008). Further development of these models may provide information about how nicotine and other tobacco additives (ligands) regulate nAChR activation in smoking dependence.

Ligand-based Monoamine oxidase (MAO) models. The enzyme MAO plays an important role in the metabolism of several neurotransmitters by oxidative deamination. MAO-A inhibition is associated with enhanced dopamine activity leading to increased reinforcement behaviour. The combustion of, among others, natural or added (poly-)sugars in tobacco products result in acetaldehyde which reacts in the body with tryptophan and tryptamine. This reaction results in the formation of the beta-carbolines, harman and norharmane, which are MAO inhibitors (Herraiz and Chaparro, 2005; Talhout *et al.*, 2007). Other examples of MAO inhibitors isolated from tobacco leaves or present in tobacco smoke are 2,3,6-trimethyl-benzoquinone, 2-naphthylamine and a coffee-extracts and synthesized and modified natural coumarin derivatives (Fowler *et al.*, 2003; Gnerre *et al.*, 2000; He *et al.*, 2014; Herraiz and Chaparro, 2006).

Ligand-based models can provide new insights in enzyme selectivity, mechanisms of action and the relationship between the MAO inhibitory activity and the molecular structure of the different inhibitors (Vilar *et al.*, 2012). There are different types of ligand-based models which can be used, such as Quantitative Structure-Activity Relationship (QSAR) with 2D and 3D descriptors (Johnson, 1976; Vilar *et al.*, 2008; Winkler, 2002), 3D- Comparative Molecular Field Analysis (CoMFA) (Cramer *et al.*, 1988; Zhang *et al.*, 2011), 3D-pharmacophores (Langer and Hoffmann, 2006) or ligand-network models (Keiser *et al.*, 2007; Park and Kim, 2008). QSAR studies have become one of the most popular ligand-based approaches in modern chemistry (Shelke *et al.*, 2011; Vilar *et al.*, 2008; Vilar *et al.*, 2012) and can also be used to model ligand-based selectivity of different tobacco additives and the potency to inhibit MAO activity.

In vitro

Three-dimensional lung tissue constructs (3D lung-on-a chip) and mathematical computer models. These kinds of models are the results of the integration between *in vitro* models (the 3D organ-on-a-chip) and *in silico* models. It has been shown that inhalation during smoking results in a rapid brain increase of nicotine in the brain thereby contributing to nicotine dependence in smokers. Inhalation can be facilitated by certain additives leading to deeper and more frequent inhalation by the cigarette smoker resulting in an increase in lung exposure and nicotine uptake. Additives (e.g. menthol, theobromine and eucalyptol) can achieve this by enhancing sensory properties such as cooling effects or by having local anaesthetic and bronchodilating properties (Usmani *et al.*, 2005). Also, a change in the physical properties of tobacco (e.g. particle size) can be altered by certain additives to allow (nicotine) particles to enter deeper levels of the lungs (SCENIHR, 2010).

The efficiency of nicotine uptake and tobacco additives via the lung in the blood stream is difficult to measure. Engineered 3D lung tissue constructs and mathematical computer models can be used to provide predictive information on lung uptake and particle deposition (Asgharian *et al.*, 2012; Nichols *et al.*, 2014; Nichols *et al.*, 2013). These engineered 3D models of human tissue mimic *in vivo* conditions and allow for more natural and robust human *in vitro* respiratory tract models compared to multi-cell *in vitro* models. These constructs can be used to assess cell-based responses, physiologic functions, pathologic changes and even toxicity or responses to tobacco additives.

The (3D) airway models or lung-on-a-chip models can be also used to measure experimentally effects due to any additive in **altering nicotine uptake** such as alkalizing compounds or that result in easier absorption of nicotine by epithelial.

Capacity to change pH values. Additives that exert capacities to increase the pH values will result in higher amounts of uncharged nicotine (Hurt and Robertson, 1998; Wayne and Carpenter, 2009). This may result in more easily absorption of nicotine by the epithelial cells in the mouth and probably also in the lungs (Tomar and Henningfield, 1997). Opposing positions on smoke pH and its effect on unprotonated nicotine have been published and studies have been designed in an attempt to evaluate this effect empirically, but there is insufficient evidence available to prove or disprove this point. Although the tobacco industry stresses that the buffering capacity of the lung surface liquid (7mval/pH unit) at pH 7.4 is not changed by nicotine concentrations of 0.1 mg per puff (Holma and Hegg, 1989; Klus *et al.*, 2012), it is valuable to check additives or substances for their capacity to change the pH of the tobacco and the smoke.

(Inhibition of) The enzymatic activity of MAO. Additives may influence the dependence potential of nicotine by interacting with the neural responses to the drug. For example, MAO inhibitors that are not leading to dependence on their own slow the breakdown of monoamines such as DA, thereby affecting the overall motivational impact of nicotine. Inhibition of the enzymatic activity of MAO can be measured *in vitro* using peroxidase-linked spectrophotometric assay. Enzymes can be isolated from rat liver microsomes or by recombinant generated enzymes. Using recombinant human MAO-A and MAO-B, IC₅₀ values for enzyme inhibition can be experimentally determined (Lewis *et al.*, 2007). *In vivo* MAO activity can be analysed using PET (see paragraph 'neurobiological effects using imaging techniques' below).

CYP metabolism inhibitor ratio. The metabolism of nicotine is a complex pathway : as first step it is metabolized in the liver by cytochrome CYP2A6 and CYP2B6 enzymes, which can be induced or inhibited by some chemicals (Hukkanen *et al.*, 2005). Inhibition of nicotine metabolism enhances its bioavailability and alters the behavioural effects in mice (Alsharari *et al.*, 2014; Bagdas *et al.*, 2014). Additives modulating the activity of metabolic pathways are therefore likely to affect the dependence potential of nicotine. The effectiveness of an additive in inhibiting nicotine metabolism is expressed as relative CYP inhibitor ratio (Rahnasto *et al.*, 2008). The inhibitory concentration of human CYP2A6 can be tested in an *in vitro* assay using recombinant enzyme or human liver microsomal preparations (Rahnasto *et al.*, 2003). Examples of known tobacco additives that inhibit CYP2A6 enzymes are menthol, benzaldehydes and several lactones added to tobacco (Benowitz *et al.*, 2004; Kabbani, 2013; Kramlinger *et al.*, 2012; Rahnasto *et al.*, 2003).

In vivo (not recommended as the first choice)

Analysis of nicotine as Biomarker of exposure in humans. Nicotine bioavailability is defined by an optimal rate of adsorption and distribution from the lungs into the bloodstream. Upon uptake in the lungs, the bioavailability of nicotine in the body is determined by properties such as its hydrophobicity and solubility. It has been proposed that the use of alkalizing compounds (such as ammonia) as tobacco additive increases the absorption of nicotine in the lungs. A biomarker analysis of nicotine in blood samples from smokers of cigarettes with different ammonia yields was performed to evaluate the effects on nicotine bioavailability. Different ammonia yields in cigarettes did not increase

the rate or amount of nicotine absorption from the lungs to the arterial blood circulation (McKinney *et al.*, 2012; van Amsterdam *et al.*, 2011). It cannot be excluded from these studies that ingredients other than ammonium salts influence nicotine adsorption in a similar way.

Dopamine (DA) release and turn over. Activity of neurons in the mesolimbic DA brain area is not only measured by nACh receptor activation but also by measuring the result of this receptor activation, a change in the release or turnover of DA. DA release and turnover can be measured either *ex vivo* or *in vivo* via isolation of specific brain tissue or microdialysis. A study in mice showed up-regulation of nAChR subtypes in various brain regions upon exposure to nicotine and menthol using western blots. A significant increase in nicotine plasma levels was observed, which was accompanied by an increase of withdrawal intensity (Alsharari, 2015).

Neurobiological effects using imaging techniques. Exerting additive effects on nicotine dependent activation of the mesolimbic pathway can be studied *in vivo* using several neuroimaging techniques like functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) (Jasinska *et al.*, 2014). Each technique can be used to understand only some aspects of processes involved in tobacco consumption like brain structure (MRI), different aspects of brain function (PET, SPECT, fMRI, and ASL), and pharmacokinetics (PET, SPECT) in animals as well as humans (Kober and Deleone, 2011).

The neuronal activity upon exposure to nicotine and other tobacco additives can be measured by the activation of nACh receptors, neurotransmitter release and transcriptional activation of specific mRNAs (van de Nobelen *et al.*, 2016). Labelling and tracing of nicotine, MAO or nAChR can demonstrate nicotine occupancy at nAChRs, nAChR availability and upregulation of nAChRs induced by tobacco smoking (Brody *et al.*, 2014; Jasinska *et al.*, 2014; Volkow *et al.*, 1999). Substances that inhibit the nicotine degradation or metabolism or serve as a carrier for nicotine can be identified by these derivative endpoints.

PET. PET is a common functional neuroimaging technique which uses a short-lived radioactive tracer (incorporated into a biologically active molecule as glucose) infused into a living subject. As the tracer decays, the PET system detects pairs of gamma rays that are indirectly emitted in the process, and uses them to localise the tracer to a particular region in the brain. In this way, the concentration of tracer molecules can be estimated at different locations in the brain or other tissue. Several tracers are discussed below.

Radiotracers:

- **for nicotine.** A PET study with radiolabelled nicotine [¹¹C]nicotine demonstrated that a single puff leads to a rapid rise in brain nicotine concentration with a gradual wash-out period (Berridge *et al.*, 2010). In addition, the nicotine accumulation in the brain during smoking of one full cigarette increases in a linear fashion with successive puffs, rather than in puff-associated spikes and rapid wash-outs. Relating to this finding, the authors reported that dependent smokers showed a slower rate of brain nicotine accumulation than non-dependent smokers (Kober and Deleone, 2011; Rose *et al.*, 2010).

- **for $\alpha 4\beta 2$ nACh receptors.** Researchers have also developed brain-imaging radiotracers for $\alpha 4\beta 2$ nACh receptors with radiolabelled A-85380 compounds (Abreo *et al.*, 1996) or analogues (2-FA /6-FA for PET and 5-IA for SPECT), having the most widespread use (Koren *et al.*, 1998). However, the slow kinetics of these radio ligands restricts the large-scale use in experimental studies (Sabri *et al.*, 2015). New generation $\alpha 4\beta 2$ nAChR specific radioligands are now under development, like [^{18}F]Flubatine, [^{18}F]AZAN and [^{18}F]nifene, demonstrating faster kinetic properties in PET research (Brust *et al.*, 2008; Hillmer *et al.*, 2011; Hockley *et al.*, 2013; Kuwabara *et al.*, 2012; Wong *et al.*, 2013).

PET imaging studies demonstrated the effect of cigarette smoking on $\alpha 4\beta 2$ nAChR occupancy, showing that smoking causes displacement of 2-FA for prolonged time (at least several hours) (Brody *et al.*, 2006a). Dose-dependent reduction in 2-FA displacement was observed by both controlling the number of puffs smoked and the nicotine concentration smoked (Brody *et al.*, 2009). These findings suggest that nicotine mediates 2-FA displacement by occupying $\alpha 4\beta 2$ nAChRs. Several authors, using 5IA-SPECT and 2-FA-PET, have shown that habitual cigarette smoking is associated with up-regulation of $\alpha 4\beta 2$ nAChRs (Cosgrove *et al.*, 2009; Mamede *et al.*, 2007; Mukhin *et al.*, 2008; Staley *et al.*, 2006; Wullner *et al.*, 2008). The nAChR density returns to normal after a prolonged abstinence of weeks to months (Cosgrove *et al.*, 2009; Mamede *et al.*, 2007). In addition, exposure to nicotine from second-hand smoke resulted in substantial brain $\alpha 4\beta 2$ nAChR occupancy in smokers and non-smokers (Brody *et al.*, 2011). Taken together, these results suggest that exposure to cigarette smoke, most likely through the effects of nicotine, influences $\alpha 4\beta 2$ nAChR density in the human brain (Lotfipour *et al.*, 2011).

- **for Dopaminereceptor (DA).** With radiotracers such as [^{11}C]raclopride and [^{11}C]PHNO (PET) or [^{123}I]IBZM (SPECT), striatal DA release has been reliably measured using PET and SPECT imaging (Laruelle, 2000). An increase in DA competes with the radiotracer to bind at the dopamine receptor; resulting in a decrease in radiotracer binding compared to baseline. This allows calculation of the 'occupancy' of the receptors by DA or a change in binding potential and is an indirect measure of DA release based on the 'occupancy model' (Cosgrove *et al.*, 2015; Laruelle, 2000).

Many studies have examined nicotine and tobacco smoking-induced DA release in human subjects. All these studies confirm that smoking elicits ventral striatal dopamine release and is associated with a reduction of craving (Barrett *et al.*, 2004; Brody *et al.*, 2006b; Brody *et al.*, 2004; Montgomery *et al.*, 2007; Scott *et al.*, 2007; Takahashi *et al.*, 2008). In the most recent study, smokers were imaged with [^{11}C] PHNO before and after a cigarette (Le Foll *et al.*, 2014). Binding potential was reduced after smoking by 12 and 15 % in D2-rich and D3-rich regions, respectively.

A major concern with the existing studies is the timing of the dopamine response. The response to smoking a cigarette is a transient increase in DA. The analysis in these studies, however, use an average of all the data collected over 30 min to up to 2 h. This significantly dilutes measurement of a transient dopamine response (Sullivan *et al.*, 2013). Thus, analysis techniques with improved

temporal resolution may be better suited to more transient DA release (Cosgrove *et al.*, 2015).

- **for μ -opioid receptors.** There is strong evidence for a link between nicotine administration and endogenous μ -opioid mechanisms, mediating some of nicotine's addictive properties and distress during withdrawal (Nuechterlein *et al.*, 2016). Acute endogenous opioid release upon nicotine administration was demonstrated in animal and cell culture studies (Boyadjieva and Sarkar, 1997; Davenport *et al.*, 1990). However, in human studies the findings are inconsistent. The indirect measures of neurotransmitter release and μ -opioid receptor activation upon nicotine administration, as measured with PET, have shown both a reduction in binding potential (suggesting activation of neurotransmission) and an increase in binding potential (deactivation) or no significant change in different regions of the brain (Domino *et al.*, 2015; Kuwabara *et al.*, 2014; Ray *et al.*, 2011; Scott *et al.*, 2007). Measures at baseline have also shown either lower or no significant differences between smokers and non-smoking controls (Kuwabara *et al.*, 2014; Scott *et al.*, 2007). The μ -opioid system is suggested to be strongly influenced to placebo treatment (Nuechterlein *et al.*, 2016; Pecina *et al.*, 2015; Scott *et al.*, 2008; Zubieta *et al.*, 2005). Therefore, studying the opioid system does not appear to be the most sensitive and robust way to define tobacco dependence.

How the administration of tobacco additives changes these effects as measured is largely unknown. A recent PET study showed that in brains of female menthol cigarette smokers, nicotine accumulated faster thereby contributing to dependence. However a role of menthol in enhancing brain nicotine accumulation was not supported by this study (Zuo *et al.*, 2015). Another PET study using labelled nAChR subunits showed an upregulation of these receptors in the brain of menthol smokers, indicating a higher nicotine exposure in smokers of menthol cigarettes (Brody *et al.*, 2013). However, other mechanisms for menthol-influencing receptor density are possible. Analysis of nicotinic acetylcholine receptor activity *in vitro* shows that menthol inhibits nAChR subtypes in a non-competitive manner (Ashoor *et al.*, 2013; Hans *et al.*, 2012).

SPECT. In SPECT, the same mechanism is used as in PET, but differs in a way that the radioactive tracer directly emits a single gamma ray during decay. The nature of the signal allows for lower resolution images than PET as the SPECT tracers typically have a longer half-life, but scans are more easily performed. There are differences in the physics and chemistry used in PET versus SPECT, but the outcome measure of receptor availability is the same. Depending on the tracer used, PET and SPECT data can quantify regional brain activity (e.g. via glucose metabolism when the tracer is a modified sugar, as in ^{18}F -fluorodeoxyglucose), receptor occupancy (e.g. with ^{11}C -raclopride and dopamine receptors), and pharmacokinetics when multiple measurements are taken after drug consumption.

Functional Magnetic Resonance Imaging (fMRI). Besides PET, which is already an advanced technique in this field of research, fMRI is a promising and non-invasive upcoming technique. In fMRI, blood oxygen levels (brain activity) can be measured in the brain by use of strong magnetic fields. In the first fMRI study on the effect of acute nicotine administration, active smoking participants were injected with nicotine in

different concentrations. A dose- and time-dependent increased BOLD signal occurred in several cortical and subcortical regions, with prominent signal changes in the cingulate cortex, dorsolateral and medial orbitofrontal regions (Stein *et al.*, 1998) as well as the ventral striatum, amygdala, thalamus and insula (Menossi *et al.*, 2013). fMRI studies assessing tobacco additives with a (passive) inhalation tobacco devices can be used to identify brain areas involved in addiction. As there are no validated administration models for smoking or nicotine administration which can be used during scanning, this is an important limiting factor in fMRI research.

The majority of the above-mentioned imaging studies focus on chronic exposure in a cross sectional design (smokers vs. non-smokers) at a single point in time. When focusing on the dependence capacity of tobacco additives, studies on acute effects using within subject measurements (placebo vs. additive(s) of interest) is regarded as more valuable. This will improve the sensitivity to picking up small changes in neuronal activity caused by administration of the additive. Repeated exposure and repeated measurements can predict dependence capacity. Further improvement of the study protocol and development of even more efficient radio ligands may be beneficial to finding indications for tobacco additives that increase dependence.

Behavioural responses in rodents. Current animal models for tobacco product dependence are based on assessing nicotine dependence rather than dependence of tobacco additives or tobacco products as a whole. These models aim to deliver pure nicotine using an intravenous self-administration paradigm despite the fact that nicotine itself is regarded as a relatively weak reinforcer (Caille *et al.*, 2012). Current tests to analyse dependence potential can monitor self-administration, speed of acquisition, conditioned rewarding effects and drug discrimination (Hoffman and Evans, 2013; Wilkinson and Bevins, 2008; Yararbas *et al.*, 2010). Also severity of withdrawal can be measured (Bagdas *et al.*, 2014). Animal models also allow controlling of factors that can affect study outcome such as environmental factors, genetic background and prior drug exposure. The self-administration paradigm has been widely accepted as a reliable animal model with high predictive value for the dependence potential of a drug and can be used to support findings observed in humans. The current available models can possibly be adapted to assess the effect of (nicotine in combination with) other tobacco-related additives on dependence. A recent animal study showed that the sensory properties of menthol can serve as a conditioned reinforcer for nicotine (Wang *et al.*, 2014).

Behavioural outcome measures in human. Several behavioural measures can be used to assess dependence in human. Dependence for nicotine and smoke(less) tobacco can be self-assessed using the Fagerström Test for Nicotine Dependence (FTND) or the cigarette withdrawal scale (CWS-21) (Etter, 2005; Etter *et al.*, 2003; Fagerstrom, 2012). The FTND uses a twelve-item cigarette dependence scale that covers the main definitions of dependence: compulsion, withdrawal symptoms, loss of control, neglect of other activities, time allocation and persistence despite harm. The FTND can assess the degree or severity of tobacco dependence using a scale indicative for the level of dependence.

The cigarette withdrawal scale (CWS-21) is a 21-item multidimensional self-administered scale that measures withdrawal symptoms and predicts relapse to smoking (Etter, 2005). Recently, a revision for DSM-V was proposed in order to increase the predictive

value of these criteria for tobacco dependence assessment (American Psychiatric Association, 2013; Baker *et al.*, 2012).

Indicators of nicotine dependence were assessed in menthol and non-menthol cigarette smokers using the FTND. Differences were observed in time to first cigarette of the day (TTF) suggesting greater urgency to smoke but not on amount of cigarettes smoked on a day (CPD) (Collins and Moolchan, 2006; Hoffman and Simmons, 2011). An important limitation of these methods is that these tests are a diagnostic instrument for assessing dependence in people and not necessarily the dependence potential of the given substance or product type. However, these questionnaires can be adapted for the assessment of dependence to tobacco-related additives.

Recommendations

To accurately assess tobacco dependence potential for regulatory purposes, it is necessary to use multiple evaluation methods, whereby several factors associated with tobacco dependence are analysed. Combinations of techniques examining neurochemical physiological and behavioural changes in specific brain regions with nicotine dependence may provide sufficient and robust information. Correlations between responses and convergence of studies will lead to evidence-based conclusions. For regulatory purposes, consensus needs to be established on the (combination of) tests that are preferred. The SCHEER therefore proposes to use a step-wise approach of 1) *in silico*, 2) *in vitro*, 3) *ex vivo*, and 4) *in vivo* methods- only in exceptional cases, to be agreed with the Receiving Authority on a case-by-case basis. The use of *in vivo* studies is indeed questionable for ethical reasons, therefore the performance of new studies are only justified under exceptional circumstances. After negative results of testing the tobacco additive on dependence capacity in the first agreed appropriate method (*in silico*), the next step should be considered, and appropriate test(s) should be selected (*in vitro* models), and so on. It is strongly recommended that *in silico* and *in vitro* tests to assess additive-induced addictiveness are developed and validated in the future by independent organisations.

3.4.3.6 Characterising flavour and inhalation facilitation properties

This section will discuss a procedure to assess tobacco products with characterising flavours that are prohibited in the TPD, as well as some other mechanisms such as inhalation facilitation, that may contribute to additive-induced attractiveness.

Animal models do not currently exist for this kind of assessment. In humans, the attractiveness of individual tobacco products can be compared in panel studies, surveys and by experimental measures. To test the response to a specific additive, tobacco products can be produced to exclude or include individual additives. However, this type of research is difficult nowadays due to ethical considerations that will often preclude human testing (SCENIHR, 2010).

Characterising flavours

Over 80% of all Dutch cigarettes contain at least one flavour, and almost half of all additives in any tobacco product is added as a flavour (Pennings *et al.*, 2016). Flavours may be added to tobacco, cigarette paper, the filter or to the foil wrapper, in an attempt to enhance the tobacco flavour, mask unpleasant odour, and deliver a pleasant cigarette-pack aroma (WHO, 2007). Many different additives are used to create a specific taste/flavour in order to attract certain target groups. Regarding flavour, the EU

Tobacco Product Directive (TPD, Article 7) prohibits cigarettes and roll-your-own tobacco having a characterising flavour other than the of tobacco, as they could facilitate initiation of tobacco consumption or affect consumption patterns (European Union, 2014). A characterising flavour is defined as a '*clearly noticeable smell or taste other than one of tobacco, resulting from an additive or a combination of additives, including, but not limited to, fruit, spice, herb, alcohol, candy, menthol or vanilla, which is noticeable before or during the consumption of the tobacco product.*' The prohibition of tobacco products with characterising flavours does not preclude the use of individual additives outright, but it does oblige manufacturers to reduce the additive or the combination of additives to such an extent that the additives no longer result in a characterising flavour.

Talhout *et al.* (2016) published an inventory of methods suitable to assess additive-induced characterising flavours of tobacco products, and concluded that because flavour perception is subjective and requires human assessment, sensory analysis in consumer or expert panel studies is necessitated. They recommend developing validated tests for descriptive sensory analysis in combination with chemical-analytical measurements. Testing a broad range of brands, including those with quite subtle characterizing flavours, will provide the range of concentrations above which an additive will impart a characterising flavour (Talhout *et al.*, 2016).

The Commission has recently adopted two implementing acts establishing the rules and mechanism for determining products with characterising flavours²⁴.

The determination of such flavours can concern products before consumption (e.g. before combustion) as well as emissions resulting from normal use (direct and indirect) of the products. To develop a method for determining characterising flavours and to perform some pilot experiments, the Commission contracted the HETOC Consortium in August 2014²⁵. The HETOC-consortium carried out, as an external contractor, a study on the determination of characterising flavours. Sensory testing complemented by chemical analysis was concluded to be an appropriate method to determine characterising flavours. Their pilot had confirmed that an expert panel is a good approach, but the training phase needs to be more extensive when the real panel is set up. Smelling is the preferred starting point for determining characterising flavours and it was recommended to consider, as a future step, whether a smoking experiment was needed. It was concluded that specific reference spaces for cigarettes and RYO are needed.

Beside the characterising flavour features, other phenomena can contribute to make a tobacco product more 'attractive'. According to the partial guidelines for implementation

²⁴Commission Implementing Regulation (EU) 2016/779 of 18 May 2016 laying down uniform rules as regards the procedures for determining whether a tobacco product has a characterising flavour

http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.131.01.0048.01.ENG&toc=OJ:L:2016:131:TOC

Commission Implementing Decision (EU) 2016/786 of 18 May 2016 laying down the procedure for the establishment and operation of an independent advisory panel assisting Member States and the Commission in determining whether tobacco products have a characterising flavour

http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.131.01.0079.01.ENG&toc=OJ:L:2016:131:TOC

²⁵http://ec.europa.eu/health/tobacco/products/implementation/characterising_flavours_en.htm

of Articles 9 and 10 of the WHO framework convention on tobacco control, “attractiveness” refers to factors such as taste, smell and other sensory attributes, ease of use, flexibility of the dosing system, cost, reputation or image, assumed risks and benefits, and other characteristics of a product designed to stimulate use. Note that not all of these properties are related to additives. WHO-FCTC advises Parties to regulate, by prohibiting or restricting, ingredients that may be used to increase attractiveness of tobacco products (WHO, 2012). The FCTC guidelines in relation to the regulation of the contents of tobacco products and regulation of tobacco product disclosures call in particular for the removal of ingredients that increase palatability, create the impression that tobacco products have health benefits, are associated with energy and vitality or have colouring properties.

The TPD includes two references to attractiveness. In the introductory considerations, point 13, it is mentioned that *“In order to carry out their regulatory tasks, Member States and the Commission require comprehensive information on the ingredients and emissions from tobacco products to assess the attractiveness, addictiveness and toxicity of tobacco products and the health risks associated with the consumption of such products.”* In article 19, Notification of novel tobacco products, it is mentioned that manufacturers and importers of such a product shall provide *‘available scientific studies on toxicity, addictiveness and attractiveness of the novel tobacco product, in particular as regards its ingredients and emissions.’*

In the following, the possibility to assess characteristics other than the characterising flavour as contributors to attractiveness are briefly presented, some of which having the possibility of “Facilitating inhalation or nicotine uptake”, which is a criterion of the mandate (category c) included in the ToR).

Other sensory attributes which can facilitate inhalation or nicotine uptake

Low irritation to mouth, throat and chest and satisfaction can facilitate inhalation and possibly nicotine uptake (Jaffe and Glaros, 1986; Kochhar and Warburton, 1990). Mildness, a combination of improved aftertaste, less bitterness, improved mouth feeling and reduced irritation, is reported to be appreciated, especially by younger and beginner smokers, with their undeveloped tastes and a low tolerance for irritation from tobacco smoke (Carpenter *et al.*, 2007).

Additives that influence these sensory attributes, such as mildness, and a pleasant aftertaste, possibly facilitate smoking initiation. By reducing and changing the harshness of the smoke, special target groups may be reached (Carpenter *et al.*, 2005a, Carpenter *et al.*, 2005b, Cummings *et al.*, 2002, Klein *et al.*, 2008, Wayne and Connolly, 2002). A confidential tobacco industry document describes a class of casing materials referred to as ameliorants used to *“...smooth out harshness and bitterness and/or eliminate pungent aromas from tobaccos”* (Jenkins *et al.*, 1997). Examples of such ameliorants included sugars, cocoa and liquorice. Cocoa, also at levels that do not impart a characterising flavour, can alter cigarette flavour and improve product acceptability (Sokol *et al.*, 2014). Various sugars constitute a large proportion of additives, and the sweetness of the smoke resulting from its caramel combustion products is an important characteristic. Thus, product appeal for starters may be further diminished by regulating trigeminal attributes as well. Smoking panels can be used to assess sensory attributes like irritation, impact, flavour, aftertaste. For irritation, it may also be possible to use *in vitro* models.

Some additives have multiple chemosensory effects. Pyrazines, which are flavours resulting from pyrolysis of amines and sugars, are reported to induce chemosensory effects such as reducing the harshness and irritating effects of nicotine and other tobacco smoke constituents in the airways. In addition, they may reinforce the learned behaviour of smoking, enhance elasticity and help optimise nicotine dosing. Wayne and Henningfield also describe evidence from internal industry documents that “smokers develop a taste for specific flavors or characteristics of tobacco use other than nicotine, and come to associate use with these characteristics” (Wayne and Henningfield, 2008). Vanilla, for example, increases mildness, and smokers will switch to other vanilla-containing brands, but not to brands without vanilla taste. Menthol is also known for its taste, as well as inducing a “cooling” effect which masks the harshness and the taste of raw tobacco (Lawrence *et al.*, 2011).

Harshness and smoothness. According to the tobacco industry definition, harshness is a chemically-induced physical effect associated with a roughness, rawness experience generally localized in the mouth and to a lesser degree in the upper reaches of the throat and the trachea due to inhalation of tobacco smoke. Harshness can also cause a drying, rasping, coarse, astringent sensation usually associated with the smoke flavour of Virginia or air-cured type tobaccos. Harshness is classically measured in four degrees: (i) Free – an absence of harshness; (ii) Touching – a slight awareness of a sensation; (iii) Scratchy – some discomfort, a stinging effect; and (iv) Harsh – rough, raw, raspy, coarse, astringent, painful inhalation. Reducing the harshness of the smoke makes it possible to inhale deeper and increase the number of puffs, as physical barriers will be reduced (Wayne and Henningfield, 2008).

The harshness depends partly on the tar/nicotine ratio, but may also be decreased by certain additives such as propylene glycol or levulinates. Tar provides a strong flavour and mouth sensation, masking the harsher, bitter taste of nicotine which may be unpalatable to new smokers and uncomfortable to established smokers. Certain highly flavoured additives may also have the same properties to “smoothen” or reduce the harsh irritation of nicotine in tobacco smoke. In order to make the smoke less aversive and permit deeper inhalation, additives such as liquorice and menthol are used. Another approach is to use nicotine salts that do not cause the same irritation, but are still delivering nicotine or keeping the nicotine effect by means of a quicker absorption by ensuring larger amounts of free nicotine (SCENIHR 2010, Bates *et al.*, 1999, Keithly *et al.*, 2005). Finally, the addition of humectants such as glycerol, propylene glycol and sorbitol keep the humidity of the tobacco product at a desired level; dry tobacco generates an unpleasant harsh smoke.

Impact and smoothness. The term “impact” is widely used in tobacco industry research and documents, and is a tobacco industry term for smokers’ subjective awareness of the drug effects of nicotine. Organic acids have been used since the 1950s to improve “smoothness” of cigarettes. For example, Philip Morris found that lactic acid decreased subjective ratings of harshness and bitterness, and produced a sweeter flavour. Citric additives have been used not only for reduced harshness and flavour modification, but also to modify smoke pH, to neutralise nicotine “impact” (an industry term denoting the organoleptic sensation caused by nicotine; smokers often describe this as “throat catch” or “throat hit”). Tartaric and lactic acids likewise modify the pH of smoke. All of these organic acids increased smoothness and are associated with a decrease in nicotine “impact” (Philip Morris, 1989) However, it is unclear whether these

effects are due directly to pH modification. Unregulated botanical and chemical additives might have “multiple-use” purposes, such as enhancing flavour and producing “smoother” cigarette smoke, as well as potentially preventing or masking symptoms associated with smoking-related illnesses (Rabinoff *et al.*, 2007).

Facilitate the inhalation of tobacco smoke. Certain ingredients have local anaesthetic effects. As a result, coughing due to inhalation of irritating smoke is dampened and the smoker can inhale the smoke deeper (and more frequently). Examples are components of etheric oils, such as menthol and thymol (SCENIHR 2010).

Appearance, smell and irritation of tobacco smoke. In order to make the smoke more attractive not only to the smoker, but also to other people in the proximity of the smoker, it is important that the smoke is appealing and not annoying. This may be attained with additives that make the smoke whiter and more attractive to people seeing the smoke. The TPD prohibits additives having colouring properties for emissions.

Reduced visibility of side-stream is accomplished by the addition of magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate and calcium carbonate to the wrapper (cigarette paper). This has an effect on particle size; particles become smaller and therefore do not easily scatter light and become less visible. Reducing side-stream emissions is based on encapsulating the smoke in an impermeable cone using different types of additives such as potassium succinate, potassium citrate and magnesium carbonate. By combining the use of additives and the look of the tobacco product, greater acceptance of the smoke may be created. Less resistance may be encountered from persons who do not smoke, and at the same time greater pleasure for the smoker may be created. The same agents may also be used to target the individual product at certain target groups (Carpenter *et al.*, 2005a, Connolly, 2004).

The smell of the smoke may be also changed so that it is also more attractive and less irritating (Connolly *et al.*, 2000, Ling and Glantz, 2005). Connolly *et al.* (2000) examined tobacco industry patents covering the function of environmental tobacco smoke masking. These strategies include reducing smoke odour and reducing side-stream smoke visibility and emissions. Methods to neutralise or reduce lingering smoke odour include addition of acetylpyrazine, anethole and limonene to modify the side-stream odour. These compounds have rather low odour thresholds, and are subsequently easily picked up, while they elicit no trigeminal nerve response. Aroma precursors, e.g. polyanethole provided a noticeable fresher, cleaner and less irritating cigarette side-stream aroma, while others (e.g. cinnamic aldehyde, pinanediol acetal) produce slightly sweet, spicy, clean, fresh, and less cigarette-like aroma. In addition, more “classic” additives (e.g. vanillin, benzaldehyde, bergamot oil, cinnamon/cinnamon extract, coffee extract and nutmeg oil) modify sidestream odour.

Studying sensory effects

Neuro-imaging techniques are used to provide insight into brain processes related to sensory perception. The brain integrates sensory inputs such as taste, touch and smell, and the resulting neural activation can be studied by e.g. fMRI and PET (Small, 2012). Many brain areas are involved, such as brainstem, amygdala, and the orbitofrontal cortex. Odours, tastants, and trigeminal stimuli (intranasal irritants) are processed within the olfactory network, gustatory network, and trigeminal network, which are interacting networks (Lundstrom *et al.*, 2011). The widespread network involved in the processing

of odorants, tastants, and chemical irritants recruits several key cerebral areas, including those responsible for emotions, memories and reward. Reward consists of the psychological components learning, affect, and motivation (Berridge and Robinson, 2003). Physiological, emotional, cognitive and sensory responses caused by flavours and odours can be tested, also in relation to 'reward dose' in the brain (SCENIHR, 2010b). In research projects conducted by Philip Morris from 1982 to 1995, electroencephalography (EEG), pattern reversal evoked potential (PREP), and chemo-sensory event-related potential (CSERP) were used to measure physiological, sensory and cognitive changes related to nicotine and to cigarette additives (Rabinoff *et al.*, 2007).

3.4.3.7 Interaction of the additive with other additives/ingredient

Tobacco smoke is a complex mixture: 9582 chemical components have been identified so far in tobacco and tobacco smoke (Rodgman and Perfetti, 2013), its composition is qualitatively and quantitatively not fully known and may change, depending on the brand. In addition, tobacco being a natural product, its composition is variable over time from batch to batch even within the same brand. One of the major limitations of using *in vitro* and *in vivo* test systems for the toxicological study of inhalational exposures to compounds in tobacco mixtures is the very high number of components in tobacco smoke and the extreme variability of the mixture. The large number of additives (~ 1260 –SCENIHR Tobacco Opinion 1, 2016) present in the Industry's repertoire add further qualitative and quantitative variability to the mixture. The list is open ended, which means that at present there is no restriction on the number of additional additives as alternative chemicals, precursors etc. Moreover, several of these additives are botanical extracts, which, per se are composed of hundreds of components such as flavours, sugars, pH modifiers. These botanical and phytochemical additives are claimed to have a number of properties, including anaesthetic, antibacterial, anticancer, anti-inflammatory, antifungal, and antiviral properties (Rabinoff, 2007), but these 'apparently beneficial' activities cannot justify their use as additives in tobacco products. Indeed, in some cases, they provide for a "smoother" smoking experience by masking adverse symptoms caused by smoking (e.g., cough), preventing awareness in the consumer and reductions in cigarette consumption.

The specific purpose and the required concentration is well researched and optimised by the tobacco industry before any product is marketed; therefore additives included in the composition of each tobacco product represent a mixture itself. This 'optimal' mixture of additives is intentionally added to a known toxic, carcinogenic and addictive product in order to make the product more palatable by masking the bitter taste, improving the flavour and reducing the irritation of inhaled smoke, optimising nicotine uptake.

The possibility that chemical interactions can occur among different tobacco components and additives and among different additives cannot be excluded. These interactions can consist of direct chemical reactions, forming additional different compounds, or being translated in addition, potentiation or antagonism of the effects induced by additives and tobacco components (with the possibility that such an interaction occurs at the level of toxicokinetics and at toxicodynamic level). The number of possible interactions and the number of test combinations increase exponentially with increasing numbers of compounds in a mixture. Moreover, the number of experimental groups will also increase with the number of doses of each compound.

As for the general issue of mixture toxicity, in this specific case it can also not be solved by applying an experimental approach, since to test the thousands of possible mixtures is not feasible, beside the fact that, as previously discussed, their composition is variable. This is an additional reason for considering the approach of using comparative testing strategies insuitable, where differences in effect of the tobacco product with and without the additive are evaluated (see paragraph 3.4.1).

The frameworks for assessing chemical mixtures have been proposed at international and national levels and addressed in several guidance documents.

In most of these documents, the focus is on the mode of action of specific compounds: chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. The approach suggested by the non-food SC on mixture toxicity opinion (SCHER, 2012) as well as the one proposed by EFSA (EFSA, 2012 and EFSA, 2013 on pesticides) can be followed. A case-by-case approach could be useful to define specific testing.

Both the non-food SC and EFSA made use of the WHO/IPCS Framework for Risk Assessment of Combined Exposures to Multiple Chemicals. It is a tiered framework for organising risk assessment tools and data in order to conduct an assessment of combined exposure to multiple chemicals, starting with screening level assessment and proceeding to more complex approaches. The tools and data to be employed are decided on a case-by-case basis by the risk assessor, in order to address the problem at hand, e.g. contaminated site, chemicals in surface water, etc. (Meek *et al.*, 2011).

In the future, the EU project EuroMix (European Test and Risk Assessment Strategies for Mixtures) will provide a test strategy and test instruments using novel techniques for mixture testing based on new and already existing toxicological tests. The tests will result in data needed for refining future risk assessment of mixtures relevant to national food safety authorities, public health institutes, the European Food Safety Authority (EFSA), the European Chemical Agency (ECHA), industry, regulatory bodies and other stakeholders. <https://www.euromixproject.eu/>

The following examples show the interactions of compounds with similar function/activity on the one hand and the camouflaged effect of botanicals on the other hand, revealing the complexity of ascertaining interaction between additives.

Any additives able to interfere with nicotine bioavailability independent of the mechanism can be the cause of addition or synergism of effects. Using at the same time additives altering the pH of tobacco (e.g. alkalisng agents such as ammonium compounds facilitating nicotine passage through the cell membrane in the uncharged volatile form), together with substances such as menthol and thymol, facilitating the inhalation of tobacco smoke (due to local anaesthetic effects) or bronchodilators, such as theobromine (generated from cocoa, caffeine and glycyrrhizine) would, all together, have a non-negligible impact on nicotine bioavailability in the body. Although they present a low concentration singularly and their action can be deemed as non-relevant (SCENIHR 2010), taken together the impact is very different.

To these considerations should be added the use of additives that interfere with nicotine metabolism, additives such as the gamma-aliphatic lactones group (see SCENIHR opinion 1, 2016) are mild to weak inhibitors of CYP2A5 and CYP2A6. As CYP2A6 is

involved in the metabolism of nicotine, the presence of these chemicals could decrease smokers' metabolism of nicotine and maintain higher blood levels (thus increasing smokers' exposure to nicotine by slowing degradation of nicotine in the bloodstream). Furthermore, the inhibitory effect of these chemicals on CYP2A6, although relatively weak in isolation, might be greater when the chemicals act in combination.

Several patents discussed direct "beneficial" physiological actions of botanical additives. In one US patent cited, it was noted that nicotine in cigarettes has a deleterious vasoconstrictive effect on the cardiovascular system, particularly the blood vessels within and surrounding the heart. It was also noted that vaporised niacin in cigarette smoke has a vasodilating action that helps counteract the vasoconstrictive effect of nicotine. Furthermore, additional "beneficial" effects may be obtained when niacin is combined with rutin (a chemical found in many botanicals), "which is considered effective in reducing and preventing capillary fragility." The patent listed 33 botanicals or vegetable materials, or compounds within them, which also appear on the tobacco industry cigarette additive list (Rabinoff, 2007).

Usage of fruit and vegetable extract concentrates/ botanicals can also give an impression of health benefit to the consumer, so could be considered under the TPD Article 7 2 a. This kind of information is very important as more than 100 of 599 documented cigarette additives have pharmacological actions that camouflage the odour of environmental tobacco smoke emitted from cigarettes, enhance or maintain nicotine delivery, could increase the addictiveness of cigarettes and mask symptoms and illnesses associated with smoking behaviours (Rabinoff, 2007).

3.4.4 Step 4: Reporting

In the fourth and last step, a report needs to be drafted on the activities carried out in Steps 1 to 3, to be sent to the relevant authorities. The report should include an overall evaluation of the results from Step 1 to 3. In Annex I a reporting template is provided for this purpose.

The formation of consortia and joint reports by industry is endorsed in order to limit the financial and administrative burden for both industry and authorities, and for the subsequent evaluation of the submitted reports by independent institutes.

3.5 Specific knowledge gaps for the priority list tobacco additives used in cigarettes and roll-your-own tobacco

In addition to the general strategy described in the previous paragraphs, the data gaps already identified in Tobacco Opinion 1 for the 15 additive included in the EU Commission priority list have been analysed. The analysis was based on the 'Rational for inclusion' taken from Opinion I.

Based on that, the activities to be performed upfront will be described, in order to identify the most appropriate steps (and end-points) to be carried out and to speed up the process, making possible testing feasible in the 18 month time-frame. In some cases (e.g. identification of CMR properties of the unburnt form) it would be possible to identify whether or not they should enter the evaluation procedure (e.g. having or not having properties that do not meet the criteria of the TPD, art. 7). Starting at the lowest step, for each of the 15 additives on the priority list, recommendations for experimental

activities to fill the data gaps recognised in Tobacco Opinion 1 are given. If the outcome is negative (i.e. no effect which does not meet the TPD criteria is demonstrated), they will enter the general strategy of testing and be considered as any other compound. It is recommended to address the extensive data search also for the 15 priority list chemicals and to apply the WoE approach, as described in step 1, since the SCENIHR in Opinion 1 was not asked to carry out a risk assessment but a prioritization (based on hazard) of a large number of additives to assist the Commission in the compilation of the priority list.

3.5.1 Carob bean

Synonyms: Locust bean extract, St. Johns bread extract

CAS number: 9000-40-2/84961-45-5

Rational for inclusion

Carob bean extract is a chemically undefined complex additive containing hundreds of chemicals, rich in carbohydrates/sugars. Information on the exact chemical composition of this complex tobacco additive is lacking (e.g. carbohydrate, proteins/amino acids and fats, pH modifiers. It pyrolyses extensively and the combustion of the high carbohydrate/sugars leads to formation of carcinogenic and toxic compounds (e.g. benzene, polycyclic aromatic hydrocarbons, and phenol), aldehydes (acetaldehyde, formaldehyde, furfural and acrolein), organic acids and caramel colour and flavours (Baker & Bishop, 2004, Coggins *et al.*, 2011).

Some of these pyrolysis compounds are reported to reduce nicotine delivery, leading to increased smoking frequency and deeper inhalation of smoke to enable higher absorption of nicotine in the airways. Compounds resulting from sugar combustion, such as acetaldehyde and more complex aldehydes, are MAO inhibitors, may play a crucial role in tobacco addiction. MAO inhibitors increase serotonin extracellular levels and desensitize 5-HT_{1A} autoreceptors, thereby allowing nicotine to activate serotonergic neurons and become addictive.

Moreover, it should be noted that pyrazines (reported as additives to cigarettes to impart flavour in low tar cigarettes; Alpert *et al.*, 2016) may be formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Carob bean extract has a sweet, fruity, chocolaty flavour and contributes to making smoking more attractive by improving flavour, thereby masking its bitter taste and reducing the harshness of smoking.

This information can facilitate the assessment of the influence on the carob bean extract on palatability (a key component of attractiveness), the pro-addictive effect and the interaction with other additives and tobacco chemicals.

Priority activities

Step 1: Data on the chemical composition of the carob bean extract should be provided by industry with emphasis on the concentrations of constituents of relevance;

Step 2: Some information on the effect of pyrolysis of carob bean extract is available, however, it is necessary to:

- further chemically define its pyrolysis products and

- evaluate the CMR properties of its pyrolysis products.

In case of concern for genotoxicity/carcinogenicity of its pyrolysis products, additional testing would be required to continue with **Step 3** in order to generate additional data for a WoE assessment,

If no concerns arise, the additive can enter the tiered procedure for evaluation. The assessment of its pyrolysis product on palatability, pro-addictive effect and the interaction/synergistic effect with other additives and tobacco chemicals should be presented (Step 4).

3.5.2 Cocoa and cocoa products (powder, extracts, shells of cocoa bean etc.)

Complex mixture from Theobroma cocoa beans

CAS Numbers: 95009-22-6 (cocoa powder), 84649-99-0, 84649-99-3 (cocoa extract)

Rational for inclusion

Many forms of cocoa additives such as extracts and powders are used frequently and in relatively high amounts. Added as flavour or casing to tobacco (cocoa extract is the most abundantly used, with 847 counts in NL ingredient lists, none in NTM, total number of brands 4265), average (weight %) 0.105 (0.198). The maximum amount of cocoa as tobacco additive is around 1 % of the total tobacco weight (RIVM, 2012).

Regarding toxicity, the effects of cocoa inhalation through smoking have not been studied. The risk associated with the generation of combustion products produced upon cocoa pyrolysis has not been thoroughly studied and thus, conducting an adequate risk assessment for cocoa or its pyrolysis products is currently not possible.

Regarding addictiveness, several pharmacological effects of cocoa-derived ingredients were reported, including the bronchodilatory effect of theobromine and caffeine, which result in improved bioavailability of nicotine, although data available so far indicate that the content of theobromine per cigarette seems to be too low to have a bronchodilating effect on the lungs (SCENIHR, 2010). Furthermore, reaction products of tryptophan, phenylethylamine, tryptamine and tyramine, are thought to exert monoamine oxidase-inhibiting properties. In general, the pharmacologically active substances present in cocoa do not exclude a psychopharmacological effect in humans, owing to the low exposure concentrations and/or the inability of these substances to cross or reach the blood-brain barrier. Due to a lack of studies specifically on the psychoactive effects of cocoa added to tobacco, there is insufficient evidence that adding cocoa to tobacco makes cigarettes more addictive.

The addition of cocoa to tobacco is intended to enhance flavour. More data are needed on the amount of cocoa needed to impart a noticeable flavour.

Priority activities

Based on the available data, cocoa and cocoa products may result in a characterising flavour and increase inhalation and nicotine uptake. The reported percentage of cocoa

used in cigarettes ranges from 0.2% to 0.66%. Taking this information into account, the content of theobromine and caffeine per cigarette seems too low to have a bronchodilating effect on the lungs and thereby increase the absorption of nicotine. Therefore, there is uncertainty with regard to the direct effect of cocoa additives on the bioavailability of nicotine and more studies are required.

Step 1: Additional data should be sought and evaluated to address the above mentioned questions and all the other available information on the additive in the unburnt form.

Step 2: Pyrolysis of cocoa results in the generation of minor amounts of phenol, o-, m-, p-cresol, xylenols, catechol, palmitic acid and stearic acid (<0.001% (w/w) in tobacco) and nitrous gases, carbon monoxide and dioxide. Tryptophan combustion can generate 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole and 3-amino-1-methyl-5H-pyrido-(4,3-b)indole. Furthermore, tryptophan contains reactive groups and forms reaction products with other compounds during combustion, such as beta-carbolines, including harman (RIVM, 2002). The resulting anti-depressive effects of harman have been suggested to contribute to addiction caused by cigarette smoking. Reaction products of tryptophan, phenylethylamine, tryptamine and tyramine, which are formed during combustion, are thought to exert monoamine oxidase inhibiting properties. Nevertheless, the risk associated with the generation of combustion products produced upon cocoa pyrolysis has not been thoroughly studied and should be carefully evaluated.

Step 3: The exposure to cocoa and cocoa-derived ingredients transferred to cigarette smoke in their pure forms is negligible compared with the exposure to these compounds through food and drinks (RIVM, 2002). However, the consequences of the exposure through inhalation have not been studied. Exposure through smoking should not be neglected as it represents two different types of exposure through inhalation of (1) cocoa itself and (2) combustion products of cocoa and its ingredients (RIVM, 2002).

Several mechanisms of enhancing addictiveness of smoking have been proposed, however, it is unclear whether sufficient amounts of psychoactive compounds are produced to exert psychopharmacological effects that would increase addictiveness. Characterising flavour may make cigarettes more palatable to younger, first-time users and may indirectly facilitate dependence by providing enhanced flavour and mouth sensations, potentially serving as a cue for drug reward. Due to a lack of studies specifically directed to the psychoactive effects of cocoa compounds added to tobacco on addiction, there is insufficient evidence that the addition of cocoa to tobacco contributes to the addictive properties of cigarette smoking.

The addition of cocoa to tobacco is intended to enhance flavour and therefore smoking may result in a characterising flavour. However, although a considerable percentage of cigarette weight could be cocoa additives, it is not known to what degree this influences the flavour of inhaled mainstream or side stream smoke, and especially how this might influence smoking initiation in youths (Fowles, 2001).

3.5.3 Diacetyl

CAS-nr:431-03-8

Synonyms: butanedione, butane-2,3-dione

Rational for inclusion

Diacetyl exposure may lead to serious lung disease after inhalation. For a proper risk assessment, it is necessary to better characterise the concentrations in mainstream smoke. SCOEL accepted that there is uncertainty about the importance of the genotoxicity of diacetyl. There were no data on carcinogenicity. In addition, it can result in a characterising flavour, which can contribute to increasing attractiveness.

Priority activities

Based on the rationale for inclusion, the main open questions concerning diacetyl are:

- Uncertainty concerning the genotoxicity of diacetyl and no data on carcinogenicity.
- No sufficient data was found concerning the burned (pyrolysis) product.
- Exposure may lead to lung disease after inhalation and should be assessed in appropriate tests.
- Due to the typical flavour of diacetyl it is unclear whether the compound can add to the olfactory cue and attractiveness of the smoking product. The question whether this flavour is distinguishable (attractiveness) and/or has a "smoothing" effect on the smoke (attractiveness/addictiveness) remains unclear.

The first activity to be carried out is related to the need to rule out the genotoxicity of the compound. In case of identification of genotoxic potential, the additive will be out according to the TPD provisions; otherwise it should enter the step-wise procedure.

Step 1: Additional data should be sought and evaluated to address the above mentioned questions

Step 2: Pyrolysis products should be studied and evaluated using the available data.

Step 3: In order to fill the gaps identified in Step 1 and 2, toxicity testing for inhalation exposure and then for characterising flavour, inhalation facilitation and addictiveness (considering its potential characterising flavour as well as the "smoothing" effects) for the additive in its unburnt and burnt form.

3.5.4 Fenugreek extract

Synonyms: fenugreek (*trigonella foenum graecum* L.) extract, resin, & absolute
CAS number: 84625-40-1

Physical properties: Complex mixture, dark brown paste

Rationale for inclusion

Natural/botanical concentrates/extracts/resins (e.g. from several fruits - fig, plum, raisins, fenugreek, carob, cocoa, caramel, rum, etc.) form a large number of tobacco additives. They are poorly characterised complexes of several to hundreds of chemicals; the composition further depends upon variable factors influencing botanical source and preparation methods. Although generally recognised as safe as food additives and flavours, this classification is not valid for their inhalation effects and pyrolysis products in tobacco smoke. The combustion/pyrolysis chemistry of each of these additives is not well known in terms of their physiological, toxicological and synergistic additive effects to potentiate the harmful effects of tobacco smoke.

However, many of the botanical extracts have a rich carbohydrate, polysaccharides and sugar content, together with varying amounts of proteins, amino acids and other flavour compounds. Fenugreek seeds contain mucilage (glycoproteins and polysaccharides) and some amount of sugar. The pyrolysis of this class of compounds has been well reported. Upon combustion/pyrolysis at temperatures (up to 900°C) attained during smoking, these compounds, especially the carbohydrates, give rise to a complex mixture of toxic, carcinogenic and mutagenic compounds, as well as aroma/flavour compounds.

Compounds formed include smoothing agents (e.g. organic acids), flavours (e.g. caramel), compounds that facilitate nicotine delivery (e.g. aldehydes) and compounds with CMR properties (e.g. PAHs, formaldehyde). Moreover, pyrazines are important flavour impact compounds that are formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Several pyrazines are also added as additives to cigarettes to impart flavour to low tar cigarette (Alpert *et al.*, 2016). The complex mixtures used as additives cause tremendous harm and contribute to CMR properties, addictiveness and attractiveness of tobacco smoke.

Priority activities

Step 1: Data on the chemical composition and specification of the Fenugreek extract (powder, concentrate) should be provided by industry with emphasis on the concentrations of constituents of relevance, production procedure, maximum levels for microorganisms and possible contaminants. According to available information, *Trigonella foenum-graecum* seeds contain mucilage, trigonelline, 4-hydroxyisoleucine, sotonol, diosgenin, phenolic acids, and protodioscin.

The use of fruit and vegetable extract concentrates are acknowledged to be beneficial to health. Fenugreek extract can thus give an impression of health benefit (*TPD Article 7 6a*) to the consumer. Moreover, as it is also used as a medicinal product, this could also give the impression of health protection.

Step 2: Fenugreek extract does not transfer intact to the mainstream smoke, but undergoes extensive pyrolysis. Based on the available studies (Baker and Bishop 2005), pyrolysis products from fenugreek extract include pyridine, benzene (carcinogen), toluene and furfural. The pyrolysis products, once characterised, should be evaluated according to the procedure.

If the evaluation shows that it is warranted to move on to **Step 3**, the effects which have been considered as matters of concern (e.g. neuropharmacological activities, CNS depressant and stimulant as well as allergic reaction and exacerbation of asthma) should be investigated first. Therefore, the industry should use the proposed step-wise system and the general strategy described to prove that the additive in the unburnt and burnt form does not contribute to toxicity, addictiveness, the characterising flavour or to facilitating inhalation of the tobacco product concerned.

3.5.5 Fig extract

Complex mixture (figus carica l. extract)

CAS number: 90028-74-3 (any other related one if used)

CoE number: 198

Rationale for inclusion

Natural/botanical concentrates/extracts/resins (e.g. from several fruits - fig, plum, raisins, fenugreek, carob, cocoa, caramel, rum, etc.) form a large number of tobacco additives. They are poorly characterised complexes of several to hundreds of chemicals; the composition further depends upon variable factors influencing botanical source and preparation methods. Although generally recognised as safe as food additives and flavours, this classification is not valid for their inhalation effects and pyrolysis products in tobacco smoke. The combustion/pyrolysis chemistry of each of these additives is not well known in terms of their physiological, toxicological and synergistic additive effects to potentiate the harmful effects of tobacco smoke.

However, many of the botanical extracts have a rich carbohydrate/sugar content, together with varying amounts of proteins, amino acids and other flavour compounds. The pyrolysis of this class of compounds has been well reported. Upon combustion/pyrolysis at temperatures (up to 900°C) attained during smoking, these compounds, especially the carbohydrates, give rise to a complex mixture of toxic, carcinogenic and mutagenic compounds, as well as aroma/flavour compounds.

Compounds formed include smoothing agents (e.g. organic acids), flavours (e.g. caramel), facilitating nicotine delivery (e.g. aldehydes) and with CMR properties (e.g. PAHs, formaldehyde). Moreover, pyrazines are important flavour impacting compounds that are formed under pyrolytic conditions via reactions between amines and carbonyl compounds, generally sugars. Several pyrazines are also added as additives to cigarettes to impart flavour to low tar cigarettes (Alpert *et al.*, 2016). The complex mixtures used as additives cause tremendous harm and contribute to CMR properties, addictiveness and palatability (a key component of attractiveness) of tobacco smoke.

Priority activities

Step 1: Data on the chemical composition of the fig extract should be provided by industry with emphasis on the concentrations of constituents of relevance, production procedure, maximum levels for microorganisms and possible contaminants. The use of fruit and vegetable extract concentrates are acknowledged to be beneficial to the health. Fig extract can thus give an impression of providing a health benefit (*TPD Article 7 6 a*) to the consumer.

Step 2: It does not transfer intact to the mainstream smoke, but undergoes extensive pyrolysis. Information available so far indicates that pyrolysis products include formation of benzene, toluene; in addition the combustion of the sugars lead to the formation of carcinogenic polyaromatic hydrocarbons, a variety of aldehydes, such as acetaldehyde (irritant and possible carcinogen), acrolein (irritant), 2-furfural and a mixture of organic acids. Converging data indicate that MAO (monoamine oxidase) inhibitors contained in tobacco and tobacco smoke act synergistically with nicotine to enhance addiction potential. Smokers have reduced levels of MAO in the brain. Among MAO inhibitors, compounds resulting from sugar combustion, such as acetaldehyde and more complex aldehydes, may play a crucial role in tobacco addiction. MAO inhibitors increase serotonin extracellular levels and desensitize 5-HT_{1A} autoreceptors, thereby allowing nicotine to activate serotonergic neurons and become addictive. As yet, data about the role of acetaldehyde are the only ones available. They are inconclusive and further investigation about other aldehydes is needed before the role of sugars as indirectly addictive compounds can be confirmed (SCENIHR 2010 report). Therefore, any information available on these compounds should also be reported.

Step 3: In order to fill the gaps identified in Step 1 and 2, toxicity testing for inhalation exposure and then for characterizing flavour, inhalation facilitation and addictiveness for the additive in its unburnt and burnt form. Overall, the industry should use the proposed step-wise system and the general strategy described, to prove that the additive does not contribute to the tobacco product toxicity and does not fall into the four categories listed in Art. 6 of the TPD.

3.5.6 Geraniol

CAS number 106-24-1

Rational for inclusion

Geraniol is a known flavouring agent for food and is added to tobacco products for flavouring (one of the factors potentially contributing to attractiveness). More data are needed on the amount of geraniol that imparts a noticeable flavour other than tobacco. No data are available regarding addictiveness.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of geraniol through cigarette smoking. Therefore, research is needed to determine the amount of geraniol in mainstream cigarette smoke. However, considering that the toxicological properties of geraniol are mainly linked to a high potential for skin sensitisation (in addition to skin and eye irritation), no levels considered safe for the majority of consumers could be established from the available data. Geraniol oxidation products (e.g. geranial, epoxy-geraniol, epoxy-geranial) are also potent sensitisers in animals. It could be expected that geraniol would be a respiratory sensitiser (although no information is available on this issue).

It is unknown if geraniol combustion products (about 10-15% of the additive) formed upon smoking a cigarette are toxic or not. Additional pyrolysis experiments are recommended.

Priority activities

Step 1: The chemical characterization of the additive is of paramount importance: indeed, geraniol can contain the relevant impurity methyleugenol, which is a genotoxic carcinogen. Only in the absence of that impurity, proven by specific certificate of analysis, could geraniol be considered as a possible candidate as a tobacco additive.

If it could be demonstrated that geraniol does not contain methyleugenol, the additive can enter the procedure for evaluation.

Step 1: The collection of the available data could be useful anyway, although as already indicated in Tobacco Opinion I, this would lead to confirming that data are available to demonstrate that pure geraniol did not induce gene mutations in *Salmonella typhimurium* and mammalian cells and although equivocal response resulted in an *in vitro* clastogenicity test, its genotoxicity can be reasonably considered eligible. In addition, after long-term studies, no carcinogenicity potential was attributed to food grade geranyl acetate (29 % citronellyl acetate and 71% geranyl acetate). Therefore those end-points are already addressed. Since the oral absorption has been demonstrated to be >80%, the systemic toxicity after inhalation (also assuming a total absorption through the lung (100%)) the effects are not expected to be different. Since

the relevant NOAEL are relatively high (558 mg geraniol/kg bw per day for rats and 279 mg geraniol/kg bw/day for mice), systemic general toxicity is not considered relevant at the doses used as tobacco additive. Therefore data are available to address these end-points. In addition the ECHA REACH registration dossier updated on 18 March 2016 reported a general population inhalation DNEL (Derived No Effect Level) of 47.8 mg/m³ for geraniol.

Step 2: Additional standardised pyrolysis experiments are recommended to identify the products formed other than geraniol, to be then evaluated for their toxicological properties plus addictiveness, characterising flavour and facilitating inhalation.

Step 3: In the case of geraniol, the local toxicity is relevant, as it is a known skin and eye irritant. Furthermore, geraniol and many of its oxidation products (by air oxidation and by metabolic transformation) have already been proven to be skin sensitisers in predictive animal tests. Indeed, geraniol is included among the fragrance substances of clinical importance known to be a prehapten as well as a prohaptens. For skin sensitisation, the SCCS considered that 0.01% could be efficient in limiting elicitation. No data are available for irritation of mucosa in the airways as well as for respiratory sensitisation, but since there is a high potential for inducing that kind of effects, also considering that the few pyrolysis studies available indicate that geraniol is mainly (85-90%) transferred intact to smoke, these should be tested first. In case results would indicate a potential for respiratory irritation sensitisation, the use of geraniol as a tobacco additive should be carefully evaluated.

Characterising flavour should be addressed first, since geraniol is a known flavouring agent for food and is added to tobacco products for flavouring. More data are needed on the amount of geraniol that imparts a noticeable flavour other than tobacco. Geraniol has been demonstrated to have the property to activate cold-menthol "transient receptor potential melastatin 8" (TRPM8). TRPM8 mediates a pleasant cooling sensation in the upper airways that can mask the harsh and irritation effects of tobacco smoke. TRPM8 activation is therefore a suitable physiological mechanism to promote inhalation, especially by unexperienced smokers during the initiation and adoption stages (Behrendt *et al.*, 2004; Lübbert *et al.*, 2013).

3.5.7 Glycerol

CAS number: 56-81-5

Rational for inclusion

Glycerol is added as a humectant to tobacco (to help keep it moist). Its addition is mostly during the "casing" of the tobacco. The amount of glycerol present in cigarettes depends on the cigarette brand. The levels of glycerol added to tobacco in the EU is reported to be on average 1.1 %, with a maximum level comprising 4.5 % of the total weight.

Regarding toxicity, it was reported by the tobacco industry that the transfer rate of glycerol to mainstream smoke is 12 %. A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that there are risks of effects on the respiratory tract epithelium from glycerol. No thorough assessment on systemic effects was done.

Pyrolysis studies indicate almost 100 % intact transfer of glycerol (Baker & Bishop, 2004; Purkis *et al.*, 2011). However, it was found that less than 0.1 % of the blend glycerol is converted to acrolein in mainstream smoke for different cigarette designs and smoking regimes tested (Yip *et al.*, 2010). Acrolein is a toxic compound that is highly reactive and causes irritation in the respiratory tract. The relationship between added glycerol and acrolein formation is unclear and further research is needed.

Regarding addictiveness, no data were reported to suggest that glycerol plays a role in smokers' addictiveness to cigarettes.

Humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Glycerol is, therefore, considered to positively influence cigarette smoking, given that humidification improves the palatability of cigarettes. Glycerol does not have a strong flavour, and is, therefore, not expected to impart a noticeable flavour.

Priority activities

Step 1: Additional data should be sought and evaluated to address the issues raised above. This should include evaluating information available on the assessment of the systemic effects of glycerol.

Step 2: Pyrolysis studies have found that a small proportion of glycerol (<0.1%) is converted to acrolein in mainstream smoke and is also generated during the combustion of many other products in tobacco. Both glycerol and acrolein cause irritation to the respiratory tract and acrolein is highly reactive. The relationship between added glycerol and acrolein formation is unclear. If no other information can be retrieved from the available data search, it will be necessary to produce new data (Step 3).

Step 3: Further data is needed to study the additive effects of glycerol or its reactivity with other compounds.

3.5.8 Guaiacol

CAS number 90-05-1

Rational for inclusion

Guaiacol is a known flavouring agent for food and is added to tobacco products for flavouring. More data are needed on the amount of guaiacol that imparts a noticeable flavour other than tobacco.

Its use as a local anaesthetic can enhance smoke inhalation, thus potentially contributing to addictiveness.

To perform a toxicity risk evaluation, it is necessary to know the exposure level of guaiacol through cigarette smoking. Therefore, research is needed to determine the amount of guaiacol in mainstream cigarette smoke.

Guaiacol is a severe eye irritant, a skin irritant and also reported to be a respiratory tract irritant. Other toxicological information on repeated exposure is scant. On the basis of results on structurally related compounds, effects are likely related to the irritation potential at the contact site, generating hyperplasia. Apart from the absence of

mutagenicity tested with the Ames test, the only genotoxicity test on mammalian cells gave positive results (SCE in human lymphocytes). More data are needed for a better evaluation.

Pyrolysis experiments performed with lignin found many guaiacol derivatives besides guaiacol itself and suggest that it transfers largely intact into the smoke.

Priority activities

Since the genotoxic potential of guaiacol is of concern, this is the first issue to be clarified. The SCHEER is aware that negative results have been already published by using the *Ames test*; however, positive results were obtained with human lymphocytes: these data have to be confirmed or denied by means of results coming from appropriate *in silico/in vitro* methods (**Step 3**).

In case of positive results, guaiacol would not meet the TPD requirement (see art.7), no additional testing would be necessary, therefore the procedure can go directly to **Step 4**.

If guaiacol could be proved not to have genotoxic properties, the additive can enter the step-wise procedure for evaluation, starting with:

Step 1: evaluation of available data on the additive in unburnt form

Step 2: Pyrolysis products should be considered

If there are data gaps or uncertainties coming from data collected in Step 1 and 2, further testing would be necessary (proceed to Step 3).

Step 3: Guaiacol is reported to be a respiratory tract irritant: this property should be specifically addressed as a priority. In addition, other toxicological end-point and properties as local anaesthetic, potentially contributing to addictiveness, should be also investigated, together with characterising flavour and inhalation facilitation.

3.5.9 Guar gum

Synonyms: Guaran, Guar Flour, Jaguar

CAS number: 900-30-0 (Guar depolymerised **CAS number:** 68411-94-9) and others

Rational for inclusion

Guar gum is an extract of the seeds of the guar bean plant. Guar gum consists of high molecular weight polysaccharides and some amount of protein. Reconstituted tobacco is made up of mashed tobacco stems and other parts of the tobacco leaf that would otherwise be discarded. Guar gum (and its derivatives) is added to reconstituted tobacco in cigarettes. Guar gum is also used to prepare the cigarette paper that wraps the tobacco.

The amount of guar gum added to bind the tobacco can make up between 0.6-1.8 % of the total weight of the tobacco used in one cigarette. Guar gum is generally regarded as safe for use in food and cosmetics. However, guar gum does not transfer intact to the mainstream smoke, but undergoes pyrolysis, giving rise to toxic/carcinogenic

(e.g. formaldehyde, benzo(a)pyrene and benzene) compounds. Irritating and toxic fumes, gases and acrid smoke can be formed when the additive is heated to decomposition.

Regarding flavours, it is well known that the thermal degradation of sugars and carbohydrates at lower temperatures as in foods contribute to complex aromas. Several flavour compounds were reported due to pyrolysis reactions of guar gum. These flavour compounds singly or in combination with the thousands of other smoke constituents can act synergistically and contribute to the attractiveness of smoking by improving smoke flavour, thereby masking its bitter taste, reducing the harshness of smoking, creating sensory cues, which all could contribute to the optimisation of nicotine dosing and enhance abuse potential.

Guar gum is hazardous when heated to decomposition, emitting acrid smoke and irritating fumes. Although some information on the effect of pyrolysis is available from the internal industry documents, further chemically defining this additive from the point of view as a tobacco additive and its pyrolysis products would help confirm/facilitate the assessment of the influence on the carob bean extract on toxicity/carcinogenicity, palatability, pro-addictive effect and the interaction/synergistic effect with other additives and tobacco chemicals.

Priority activities

Step 1: Data on the chemical composition of the guar gum should be provided by industry with emphasis on the concentrations of constituents of relevance.

Step 2: Some information on the effect of pyrolysis of guar gum is available, however, it is necessary to:

- further chemically define its pyrolysis products and
- evaluate the CMR and other toxicological properties of its pyrolysis products.

In case of unequivocally positive results for genotoxicity/carcinogenicity of its pyrolysis products the use of guar gum as a tobacco additive should be carefully evaluated. Additional testing would be required for a WoE assessment in case of uncertainties.

Step 3: If CMR properties are not proven by available data, the additive can enter the step-wise procedure for evaluation, analysing data other than those related to CMR properties. The assessment of its pyrolysis product on palatability, pro-addictive effect and the interaction/synergistic effect with other additives and tobacco chemicals should be analysed.

3.5.10 Liquorice

CAS numbers: 1405-86-3 (α -D-Glucopyranosiduronic acid), 103000-77-7 (β -D-Glucopyranosiduronic acid)

Rational for inclusion

Liquorice is a natural extract of the root of the liquorice (*Glycyrrhiza glabra*) plant – logically an incompletely defined complex mixture of compounds. More than 400 compounds were isolated from *Glycyrrhiza* species. Liquorice extracts are used to

improve the organoleptic properties of tobacco smoke, making the harsh cigarette smoke palatable, thereby facilitating inhalation and resulting in characterising flavour. The taste and flavour of tobacco with added liquorice/liquorice root are described as sweet, woody and round. The major active principle of liquorice is the sweet tasting triterpene glycoside glycyrrhizin.

Glycyrrhizin has been hypothesised as a bronchodilator, although not confirmed. It is not clear whether the levels present are sufficient for this effect, although a synergistic effect with other compounds in cigarette smoke maybe expected.

It is expected to pyrolyse extensively, but there is a lack of information on the pyrolysis products formed, which would help facilitate the assessment of the influence on toxicity/carcinogenicity. Additionally, the effect of liquorice on bronchodilation, alone or in combination with other additives and/or tobacco constituents, needs to be ascertained to better understand its effect on the ease of inhalation of nicotine and other alkaloids, thereby potentiating addictiveness.

Priority activities

The potential genotoxic effects of liquorice extract have been postulated but this is controversial: glycyrrhizinic acid and its derivatives have previously been reported to give both positive and negative results in mutagenicity tests.

Starting with **Step 1** and if necessary continuing to **Step 3**, this end-point should be addressed first to identify alert for the genotoxicity and carcinogenicity of the additive.

If results are negative, the additive could enter the procedure.

Step 1: Evaluation of available data on properties other than genotoxicity/carcinogenicity for the additive in its unburnt form

Step 2: Additional standardised pyrolysis experiments are recommended to identify the products formed during the combustion process of liquorice, to be then evaluated for their CMR properties

Step 3: All the testing regarding general systemic toxicity in chronic inhalation exposure and the one regarding characterising flavour should be addressed; since liquorice can mask the undesirable characteristics of tobacco smoke and acts as a bronchodilator, the possibility for facilitating nicotine uptake should be analysed. More data are needed on the amount of liquorice and glycyrrhizic acid that imparts a noticeable flavour other than tobacco. No data are available regarding addictiveness.

The evaluation of the effects of long-term inhalation exposure to liquorice with different content of glycyrrhizic acid should consider that both renal and hepatic 11-beta-hydroxysteroid dehydrogenase (converts cortisol to cortisone) as well as hepatic delta-4-5-beta-steroid-reductase (inactivates glucocorticoids and mineralocorticoids) are inhibited by glycyrrhetic acid, which can lead to pseudohyperaldosteronism and elevated blood pressure. The flavonoids licochalcone A and B inhibit the elevation of calcium ions induced by thrombin, in a dose-dependent manner. They also inhibit thrombin-induced platelet aggregation *in vitro*. Licochalcone A and B were tested with human neutrophils and were found to inhibit the formation of leukotrienes B1 and C4,

cyto B-induced lysosomal enzyme, platelet activating factor, n-formyl-methionyl-leucyl-phenylalanine and calcium ionophore A.

Hypokalemia, hypernatremia, and water retention are primary problems associated with chronic liquorice ingestion. Changes in the sodium/potassium ratios may result in pH changes. Cardiomyopathy, pulmonary edema, myoglobinuria, ptosis, myopathy, tetany, cramping, seizures, and rhabdomyolysis have also been reported in patients following chronic, excessive liquorice ingestion. Only if it could be demonstrated that chronic liquorice inhalation with tobacco smoke has no systemic effects could the additive enter the step-wise procedure for evaluation. The safety evaluation of glycyrrhizic acid should be based on the data from humans. Since the oral absorption has been demonstrated to be high, the systemic toxicity after inhalation (also assuming a total absorption through the lung (100%)) the effects are not expected to be different. Since the relevant NOAEL is relatively high (2 mg glycyrrhizic acid / kg bw per day for healthy volunteers) and the blood serum half-life is 5 hours, the risk of systemic general toxicity may not be high at the doses used as tobacco additive.

3.5.11 Maltol

CAS Number: 118-71-8

Synonyms: 3-Hydroxy-2-methyl-4-pyrone, 3-Hydroxy-2-methyl-4H-pyran-4-one, Palatone, Larixinic acid, Talmon.

Rational for inclusion

Following the EFSA report on maltol (FGE19 and FGE213 and FGE213 rev 1 - EFSA, 2008, 2009 and 2014), the concern for genotoxicity could not be excluded. Therefore, maltol will be on the priority list until data on its genotoxicity are clarified. In addition, possible effects on the CNS must be clarified.

Priority activities

The main open questions concerning maltol are therefore uncertainty concerning the genotoxicity.

If the existing information (**Step 1**) does not clarify the uncertainties, some additional testing should be undertaken based on the OECD TG (**Step 3**).

If maltol is proven not to be genotoxic, the additive can enter the step-wise procedure for evaluation.

Step1: Evaluation of available data on the additive in unburnt form

Step 2: Pyrolysis products should be considered

In case there are data gaps or uncertainties coming from data collected in Step 1 and 2, further testing would be necessary (proceed to Step 3).

Step 3: Since an inhibition of the response of the GABAA receptors in the presence of maltol has been reported which may contribute to CNS stimulation/addictiveness, these are the priority effects that should be investigated in Step 3, where all the other end-points should be considered.

In addition to the above, the following issues were identified:

Maltol and other hydroxyl cyclohexanone derivatives (such as ethyl maltol) are used to augment or enhance the taste of consumable materials, with a typical odour of cotton candy and caramel. No information on minimum levels of odour awareness was found. Reports on the health effect of maltol also underline its potential anti-apoptotic effect. It is unclear whether the anti-apoptotic is specific directed to healthy cells, neoplastic cells and/or cells undergoing mutations.

3.5.12 Menthol

CAS numbers: l-Menthol: 2216-51-5; D-Menthol: 15356-70-4; D/L Menthol: 89-78-1; Menthol: 1490-04-6

Rational for inclusion

Menthol is one of the most commonly used tobacco additives worldwide. It is a monocyclic terpene alcohol that is used primarily for its chemosensory effects of creating perceptions of a cooling minty taste and smell. Menthol is added at a continuum of concentrations, from imperceptible amounts to levels imparting different levels of a characterising flavour.

In addition, several additives and formulations are used to simulate menthol effects. Menthol induces anaesthetic and sensory effects, facilitates deeper inhalation and adds to the impact of nicotine.

Menthol is a multifunctional additive. It is an effective anaesthetic, antitussive agent that may increase the sensation of airflow and inhibit respiratory rate, thereby allowing increased lung exposure to nicotine, tar and toxic constituents, while masking reactions like coughing or other early warning signs of respiratory disease. It may increase the absorption and lung permeability of smoke constituents, thereby increasing nicotine and carcinogen uptake. It may also decrease nicotine/cotinine metabolism leading to higher doses of nicotine. It is one of the additives that was originally added to create the impression that a tobacco product has health benefits and/or reduced health risks. It affects multiple sensations including taste, aroma and tactile smoothness, and enhances abuse liability. Its pharmacological actions reduce the harshness of smoke and the irritation from nicotine, and may increase the likelihood of nicotine addiction in adolescents and young adults who experiment with smoking and it may make it more difficult to quit.

In 2011, the FDA Tobacco Products Scientific Advisory Committee (TPSAC, 2011) concluded that menthol 1) impacts youth initiation, 2) contributes to adults continuing to smoke, and 3) has an adverse impact on public health by increasing the numbers of smokers with resulting premature death and avoidable morbidity. Finally, they concluded that the "removal of menthol cigarettes from the marketplace would benefit public health in the United States" (TPSAC, 2011; FDA, 2011).

Independently, the US Food and Drug Administration undertook a thorough review and concluded that the data suggested that menthol use is likely associated with increased smoking initiation by youth and young adults, greater addiction, greater signs of nicotine dependence and less likelihood of successfully quitting smoking. These findings, combined with the evidence indicating that menthol's cooling and anaesthetic properties

may reduce the harshness of cigarette smoke and the evidence indicating that menthol cigarettes are marketed as a smoother alternative to non-menthol cigarettes, make it likely that menthol cigarettes pose a public health risk above that seen with cigarettes without menthol (FDA, 2013). The review concluded that although there is little evidence that menthol cigarettes *per se* are more toxic than menthol-free cigarettes, adequate data indicate that menthol presence is associated with increased smoking initiation and greater addiction, especially among young people, as confirmed later by the studies of Nonnemaker *et al.* (2013) and Brennan *et al.* (2015).

Indeed, smokers usually using menthol cigarettes develop greater nicotine dependence, which is likely associated to the anaesthetic properties that reduce the harshness of smoke. In addition, menthol cigarette smokers are less successful quitting smoking (Smith *et al.*, 2014). Recent perception studies confirm earlier work showing that smokers, especially young adults, perceive menthol cigarettes as less harmful (Brennan *et al.*, 2015; Wackowski and Delnevo, 2016).

With regard to toxicity, Noriyasu *et al.* (2013) exposed cell cultures to menthol and non-menthol smoke and found that cell death was significantly enhanced by mentholated smoke, whereas menthol alone was inert. This suggests a synergistic effect with other smoke-compounds and requires further study. A recent study conducted in mice showed that menthol at low concentration strongly suppressed respiratory irritation due to acrolein and cyclohexane, which are smoke irritants in naïve mice. Additionally, menthol suppressed irritation by tobacco smoke in mice. Menthol increased blood cotinine levels, which is a biomarker of nicotine uptake. Thus, menthol appears to suppress smoke-induced irritation, making it easier to inhale smoke and increasing the dosage of nicotine. Due to the similarities in menthol's pharmacology in humans, experiments in animal models suggest that beginning smokers likely prefer menthol-containing cigarettes because their respiratory tract is less irritated. At the same time, these smokers are exposed to higher levels of nicotine and become addicted faster and are less likely to quit smoking (Ha *et al.*, 2015).

At lower application levels, menthol can be used to increase smoothness and reduce harshness in cigarette smoke. This is likely the main reason for use of menthol as an additive, also in "non" menthol brands. Therefore, research to ascertain the physiological and pharmacological impact of low menthol and its interaction with other chemicals, interaction with nicotine, on palatability and inhalation of smoke/nicotine, etc. is recommended.

Baker and Bishop (2004) and Jenkins (1970) reported that after pyrolysis of menthol, it is transferred intact into smoke (99%). Smoking studies resulted in intact transfer of around 98-99%. There was some formation of menthone, menthene and menthane. In contrast, in earlier pyrolysis experiments (Schmeltz and Schlotzhauer, 1968) 84% of the menthol was pyrolysed and phenol and benzo[a]pyrene were found in the pyrolysate.

Priority activities

Step 1: Additional data should be sought and evaluated to address the above mentioned questions.

Step 2: Based on the available studies, pyrolysis of menthol may result in carcinogenic substances (concern category d). There is uncertainty with regard to the nature of the

pyrolysis products. Pyrolysis studies should be carried out to identify these products and the products should be evaluated.

Step 3: Based on the available data, menthol is concluded to impart a characterising flavour if added in sufficient amounts, facilitate inhalation and addictiveness of tobacco products and increase inhalation and nicotine uptake (concern categories A and C). Uncertainty is low. Further testing to show whether menthol is or is not addictive or does or does not increase attractiveness is not recommended in view of the strength of the data.

Further studies are needed into the suggested synergistic effect of menthol with other smoke compounds. Research to ascertain the physiological and pharmacological impact of low menthol and its interaction with other chemicals, interaction with nicotine, on palatability and inhalation of smoke/nicotine, etc. is recommended.

3.5.13 Propylene glycol

CAS number: 57-55-6

Rational for inclusion

Propylene glycol (PG) is added as humectant to tobacco, rather frequently and in relatively high amounts (1599 counts in NL ingredient lists, 23 in NTM, total number of brand 4265), average (weight %) 1.579 (1.636).

Regarding inhalation facilitation, humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Internal tobacco industry documents reported that adding 3-7 weight percent of PG increased the mildness and reduced irritation (although this is higher than amounts typically present in tobacco cigarettes). Propylene glycol is, therefore, considered to positively influence the attractiveness of cigarette smoking given that humidification improves palatability of expected to impart a noticeable flavour.

Regarding addictiveness, no data were reported to suggest that propylene glycol plays a role in smokers' addictiveness to cigarettes.

Regarding toxicity, it was reported by tobacco industry that the transfer rate of propylene glycol to mainstream smoke is 10 %. A risk assessment procedure using a Margin of Exposure (MOE) analysis concluded that risks of effects on the respiratory tract epithelium from propylene glycol exist. No thorough assessment on systemic effects was made.

Propylene oxide is regarded as possibly carcinogenic to humans and trace amounts are present in propylene glycol. Additionally, pyrolysis of propylene glycol results in formation of small amounts (<10 %) of 1,3-propylene glycol, acetol or acetic anhydride, and pyruvaldehyde.

Finally and importantly, propylene glycol and/or its combustion products is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur.

Priority activities

Step 1: Since propylene oxide, which is regarded as possibly carcinogenic to humans (IARC Group 2B carcinogen), is found in trace amounts in industrially-produced propylene glycol, the specification should be provided.

Data available should be collected to prove or disprove whether propylene glycol increases the risks of effects on the respiratory tract epithelium.

Step 2: Pyrolysis products should be better characterised also considering that Propylene oxide has been reported to be generated during cigarette smoking.

Step 3: The effect of propylene glycol on inhalation facilitation to cigarettes at levels found in European cigarettes (range of 0.2 to 2.4%) warrants investigation. The additive effects of propylene glycol and/or its reactivity with other compounds should be further investigated.

No thorough assessment of the systemic effects of propylene glycol has been done, so this should be looked at further.

3.5.14 Sorbitol

CAS number: 50-70-4

Rational for inclusion

Sorbitol is added as a humectant to tobacco (210 times in NL ingredient lists, 30 in NTM, total no of brands 4265), average (weight %) 0.232 (0.458).

Regarding inhalation facilitation, humectants are added to trap water, thereby keeping the moisture in the tobacco and preventing it from drying out. Sorbitol is, therefore, considered to positively influence attractiveness of cigarette smoking given that humidification improves palatability of cigarettes. Sorbitol gives tobacco smoke a slightly bitter taste and a vague odour of cellulose and is, therefore, not expected to impart a noticeable attractive flavour when used in higher amounts.

Regarding addictiveness, no data were reported to suggest that sorbitol plays a role in smokers' addictiveness to cigarettes. However, its combustion products, such as acetaldehyde and formaldehyde, were proposed to increase the addictive effect of nicotine, although data on acetaldehyde produced by pyrolysis entering the brain through the smoke inhaled are inconclusive (SCENIHR 2010).

Regarding toxicity, sorbitol was reported to pyrolyse at 900°C to compounds, such as 2-furfural (31.4 %, see section on furfural), acetaldehyde (irritant and possible human carcinogen), formaldehyde (irritant, carcinogen). Other pyrolysis products of sorbitol include furan, 2-methyltetrahydrofuran, propionaldehyde, acetone, methanol, and carbon monoxide (Baker and Bishop, 2004). Further research is needed to confirm these effects, especially if sorbitol pyrolysis results in carcinogenic compounds.

Finally, it must be borne in mind that sorbitol (and/or its combustion products) is only one component out of the thousands of compounds contained in cigarette smoke, thus additive effects or reactions with other compounds are likely to occur.

Priority activities

Step 2: The main concern for sorbitol to be addressed, before it can enter the evaluation procedure, is the formation of toxic pyrolysis and CMR products. Pyrolysis experiments must be carried out using conditions relevant for cigarette smoking (see section 3.4.1). In case of positive results, sorbitol as a tobacco additive would fall in one of the four categories listed in art. 6 of the TPD and its use as an additive in tobacco product should be carefully evaluated.

If it can be demonstrated that no toxic or carcinogenic pyrolysis products are formed, the additive can enter the step-wise procedure for evaluation.

Step1: Evaluation of available data on the additive in unburnt form

If there are data gaps or uncertainties coming from data collected in Step 1 and 2, further testing would be necessary (proceed to Step 3).

Step 3: In particular, it needs to be assessed whether it increases inhalation facilitation of cigarette smoking. It is not expected that sorbitol will give a characterising flavour but data regarding addictiveness should be produced.

3.5.15 Titanium Dioxide

CAS numbers: 13463-67-7 (mixture of mainly rutile and anatase); 1317-80-2 (rutile); 1317-70-0 (anatase)

Rational for inclusion

The SCCS evaluated its use as cosmetic ingredient (sunscreen). With regard to inhalation toxicity, it was concluded that in subacute repeated dose inhalation toxicity studies, nano-size TiO₂ induce an acute inflammation in the lungs, that may be reversible depending on the dose and the time after exposure. In view of this, acute inflammation (spray) applications, which may result in inhalation exposure, were not recommended by the SCCS. Titanium dioxide was classified by IARC as a Group 2B carcinogen (i.e. possibly carcinogenic to humans) (IARC, 2010).

To perform a risk evaluation, it is necessary to know the exposure level of titanium dioxide through cigarette smoking. Therefore, research is needed to determine the amount of titanium dioxide in mainstream cigarette smoke. Because inhalation toxicity is also related to the size of the particles, a distinction needs to be made between nano and non-nano size.

Priority activities

Step1: Evaluation of available data on the additive in unburnt form

Step 2: Not applicable because titanium is already in its highest oxidised state

Step 3: In subacute repeated dose inhalation toxicity studies, nano-size TiO₂ induces an acute inflammation in the lungs. Based on the available data, titanium dioxide is classified as a IARC Group 2B carcinogen (concern category d).

Within the scope of the EU CLP Regulation, a proposal for harmonised classification of TiO₂ was submitted (ECHA, CLH report, Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2,

Substance Name: Titanium dioxide, Version 2, May 2016). It is proposed to reclassify TiO₂ (all forms) specifically by inhalation as Carcinogen Cat 1B. It was concluded that no carcinogenic concern was reported by both oral and dermal routes but that there is sufficient evidence of carcinogenicity in experimental animals after inhalation. Indeed, a causal relationship has been established between TiO₂ and the increase of malignant lung tumours in female rats and benign lung tumours in males and female rats in 2 inhalation and 2 instillation studies. Human data do not suggest an association between occupational exposure to TiO₂ and risk for cancer. However, all these studies have methodological limitations and the level of exposure reported is debatable. Although the full mode of action is still unclear, an inflammatory process and indirect genotoxic effect through ROS production seems to be the major mechanism to explain the effects induced by TiO₂. It is considered that this mode of action is principally due to the biopersistence and poor solubility of the TiO₂ particles.

Evidence may be provided to address the uncertainties with regard to the genotoxicity and carcinogenicity of TiO₂ by inhalation for humans.

4 OPINION

As requested by Article 6 of the Tobacco Products Directive 2014/40/EU (TPD), the Commission has established a priority list of 15 additives contained in cigarettes and roll-your-own tobacco subject to enhanced reporting obligations that is to be updated on a regular basis. SCENIHR Tobacco Opinion 1, provided the scientific basis for the establishment of such priority list.

The TPD prescribes that Member States shall require manufacturers and importers of tobacco products to carry out comprehensive studies on these additives. The SCHEER was requested to provide guidance on the type and criteria for the comprehensive studies, and the most suitable methodologies to be used for the first list of 15 priority additives, as well as for additives on future updated lists.

In the first part of the current Opinion, the SCHEER proposed a step-wise strategy (Section 3.4), as the most pragmatic and efficient way to proceed in the assessment of the toxic and addictive effects as well as characterising flavour properties, and inhalation facilitation as factors potentially contributing to attractiveness, of additives used in cigarettes and roll-your-own tobacco. The tiered approach proposed by DKFZ (DKFZ, 2010) was used as a starting point, but the SCHEER developed its own approach, also including the evaluation of addictive effects, characterising flavour properties and the inhalation facilitation of additives. The proposed strategy ensures that testing is minimised. For this reason the application of grouping different additives with similar chemical structures and of the read-across principle are endorsed.

First, the chemical specification of the additive has to be available (Step 1). Then collection/evaluation of the available open and grey literature needs to be carried out by applying a WoE approach, for the additive in its unburnt form (Step 1) and its pyrolysis products (Step 2). If no data are available on the identity of the pyrolysis products, they need to be generated using relevant test conditions (Step 2). Here, it needs to be noted that no validated methods are available for the pyrolysis of tobacco additives.

In case data retrieved in Step 1 and 2 are not sufficient or robust enough to make the evaluation possible, non-testing methods such as QSAR and read across are proposed, followed by *in vitro* approaches addressing the different endpoints to be considered (Step 3). Regarding types of effects, unless the previous step highlighted some concern for a specific end-point, CMR properties and toxicity are assessed first, as accepted methods and evaluation frameworks are available, followed by characterising flavour, because procedures are available for the assessment of these end-points. Next, addictiveness is assessed, an effect for which no validated tests are currently available, although some mechanisms underlying addictiveness are known. It is strongly advised that *in silico* and *in vitro* test to assess additive-induced addictiveness are developed and validated by independent organisations in the near future.

In line with the provisions of other regulatory contexts, the SCHEER recommends the use of a Quality system (e.g. Good Laboratory Practice or ISO17025) for carrying out the pyrolysis or other physico-chemical studies as well as pre-clinical studies. Human studies should follow the appropriate Quality System.

The issue related to interaction of the additive with other additives/ingredients is also considered. The industry is asked to provide all known information on the interaction of

additives and their pyrolysis products leading to the intended formation of flavours / pH modifiers/ smoothing agents and other important compounds.

In addition to proposing specific steps and tests to be considered by industry, some general criteria were also identified. A pre-ambule here is that additives in tobacco products have no health benefits for the consumer, but rather may promote the use of and addiction to an extremely toxic product. Therefore, a risk-benefit analysis is not the appropriate paradigm for assessing additives.

When comprehensive studies confirm that additives have any of the four properties listed in Article 6 of the TPD, regulatory action should be considered in line with Article 7 of the TPD. If uncertainties cannot be solved by comprehensive studies performed in Step 3, it is a SCHEER recommendation that the assessors consider the worst-case evaluation.

In order to provide a relevant outcome to the question whether an additive contributes to the toxicity, characterising flavour, facilitating inhalation or addictiveness of the tobacco product, the study design must adhere to some methodological criteria. Most importantly, the test outcomes should be relevant for tobacco smoking. This implies that they should be related to actual human exposure and tobacco-induced diseases, and be relevant not only for acute or subchronic, but also for chronic exposure in intermittent use sessions (Johnson *et al.*, 2009).

Furthermore, comparative toxicity testing strategies, where differences in effect of the tobacco product with and without the additive are evaluated, are not considered suitable to address the properties outlined in the Terms of Reference with the currently available methodology. Indeed, due to the high intrinsic toxicity of tobacco products, it is challenging to demonstrate any differences, whether they are increases or decreases, induced by an additive with the currently available tests and methodologies (Kienhuis *et al.*, 2016). Very sensitive tests would be required, with a clear dose-response relationship, in order to show any differences from these high background effects, which can associate assay outcomes to human risk and exposure. In this respect, *in vitro* tests combined with toxicogenomics, using biomarkers of exposure and disease, are the most promising (Kienhuis *et al.*, 2016). However, for the time being, no standardised methods have been validated for this purpose.

As adequately sensitive tests are not currently available, no new comparative studies (tobacco product with and without additives) using the available methodologies of toxicity testing will be considered for the moment, since these studies generally lack discriminative power.

Here, the effects of the pure additive, and its pyrolysis products, are considered in a relevant testing strategy, such as the tiered approach proposed by the SCHEER (Section 3.4) in order to evaluate their contribution to tobacco product toxicity. Studies that are already available could be presented, but the relevance of their results has to be evaluated in a WoE approach based on these considerations.

Comparative studies are also not endorsed for studying the effect of additives on addictiveness, for the same reasons. In human studies, there are two exceptions to this general rule: characterising flavour testing, and facilitating inhalation or nicotine uptake. The former is, by definition, a comparative testing paradigm, as clearly noticeable

flavour is a property of a tobacco product, caused by the addition of flavour additives, and not a property of an additive itself. The latter is related to additives that facilitate inhalation or nicotine uptake, and when large groups of subjects are enrolled, effects on e.g. sensory perception, smoking topography and biomarkers of exposure (e.g. nicotine in blood or cotinine in urine) can be discriminated.

Another problem with comparative testing is that the outcomes would only apply to that specific tobacco test product. The results (e.g. related to toxicity or addictiveness) cannot be generalised to all products and brands, having a different composition with respect to tobacco type, blend and additives. Therefore the obtained results may not lead to general prohibition/acceptance of specific additives but rather to prohibition/acceptance on a product-by-product basis (DKFZ, 2010).

It is ethically questionable to carry out any new animal studies to evaluate the contribution of an additive to the tobacco product toxicity, addictiveness, characterising flavour, and properties that facilitate inhalation or nicotine uptake. Similarly to most cosmetics, it is not necessary to use tobacco products. Moreover, apart from being unnecessary, tobacco products are highly harmful with no benefits to individual or public health. As additives are used for product improvement, often contributing to detrimental effects for the consumers (e.g. addictiveness), there is no health benefit in using tobacco additives. For the hazard assessment of tobacco additives, *in silico* and *in vitro* methods exist. QSAR methodology has been successfully used for decades for predicting toxicological and pharmacological properties of chemicals. The same applies to *in vitro* methods, which were validated, adopted as official protocols (e.g. OECD Test Guidelines) and accepted, also for regulatory purposes. Additionally, many promising *in vitro* methods are currently being developed to assess different adverse outcomes.

Therefore, as a principle, only the use of *in silico*, and *in vitro* methods will be considered suitable for new studies, following the EU policy to ban animal studies for chemicals to be used in voluntary products such as cosmetics (EU Regulation no. 1223/2009). Only already available data obtained with animal models will be evaluated. Human studies are generally discouraged. These may be used (e.g. in case of flavour assessment), but only if the study subjects are informed and not exposed to the harmful smoke emissions of tobacco products.

The data gaps already identified in Tobacco Opinion 1 for the 15 additive included in the EU Commission priority list have been analysed (Section 3.5). Based on the data gaps described in the 'Rationale for inclusion' taken from Opinion 1, the activities to be performed upfront have been described, indicating the most appropriate steps (and end-points) to be carried out and then used for the evaluation, in order to speed up the process making possible testing feasible in the 18-month timeframe.

In general, data gaps for the 15 priority additives are information on addictiveness and characterising flavour or inhalation facilitation, as well as on the identity of the pyrolysis products. In the past, major emphasis was put on toxicity, whereas limited research was carried out on addictiveness (and even less on attractiveness). Regarding toxicity, data were often taken from the food sector, where pyrolysis and inhalation are not an issue.

In conclusion, this Opinion provides general guidance to tobacco industry to conduct studies and prepare reports on the evaluation of Tobacco additives to be sent to the competent authorities. To this purpose, a reporting template is provided as well (see

Annex I). In addition, specific advice is given for priority testing activities to fill the data gaps recognised in Tobacco Additives Opinion 1. In order to limit the testing and administrative burden, the formation of consortia and joint reports by industry is endorsed.

It needs to be noted that there is a lack of validated methods for the pyrolysis of tobacco additives. For the assessment of many toxicological end-points animal-free methodologies are still lacking, especially when the inhalation route is concerned. Similarly, no addictiveness and attractiveness tests (apart from a procedure for characterising flavours) are available, a knowledge gap already noted by the SCHENIHR in 2010 in its report 'Addictiveness and attractiveness of tobacco additives', and the situation has not significantly improved since then. It is advised that independent bodies or organisations conduct relevant research in these fields.

5 MINORITY OPINION

None.

6 CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this Opinion was opened on the website of the Scientific Committees from 22 July to 22 September 2016. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

Twenty-two organisations and individuals participated in the public consultation, providing input to different parts of the Opinion, resulting in 214 contributions and nearly 1000 comments.

Most comments, by far, were from the tobacco industry, which disagreed with some aspects they considered too demanding to implement; on the contrary, other organisations and individual researchers expressed their appreciation to the SCHEER, recognising the difficulties in fulfilling such a complex mandate and positively commenting on the rationale followed by the SCHEER and on the indication that no animal testing should be performed *ex novo*.

The most frequent comments were related to comparative testing, which was not endorsed by the SCHEER in the preliminary Opinion. The claim was that this is the only way to answer to TPD Art 6 (6), according to which TI should assess whether a given additive results in a significant or measurable increase in toxicity, addictiveness or CMR. Similar criticisms were directed toward the indication in the preliminary Opinion to carry out a pyrolysis study instead of smoke chemistry.

Tobacco industry also repeatedly criticised the preliminary Opinion for supposedly going beyond the Terms of References by examining properties such as 'attractiveness' as well as asking for the application of the precautionary principle.

The SCHEER provided an individual reply to each contributor. Each submission was carefully considered by the SCHEER and the preliminary Opinion has been revised in response to relevant comments.

More precisely, in the Final Opinion SCHEER clarified its position about comparative testing, specifying when it can be considered appropriate (e.g. in some human studies), but stating that at present, methodologies are not yet sensitive enough to discriminate between the very high background toxicity associated with tobacco with and without the additive. The SCHEER concludes that testing the effects of inhaling the pure additive and its pyrolysis products is the only meaningful way to comply with art. 6(2) of the TPD, i.e. to assess whether additives contribute to the toxicity or addictiveness of the products concerned.

For consistency with the terms of reference, the wording in the Final Opinion has been aligned with the TPD, avoiding the use of the term 'attractiveness' and replacing it with properties such as characterising flavour, facilitating inhalation and nicotine uptake, which fall under the TPD.

The SCHEER agreed that the reader might associate the precautionary principle with a risk measurement measure, although that was not the SCHEER's intention. For that reason, any reference to the precautionary principle has been deleted and indications

were given for the assessor on the evaluation of the collected available and new data on the additive and its pyrolysis products in case uncertainties could not be clarified by new testing.

The many papers provided by the tobacco industry were checked and the literature has been accordingly updated with relevant publications. However, in most cases they were not considered to have provided any additional information or any information relevant enough to require amending the Opinion.

In the final Opinion, some changes were included to address specific comments and editorial changes were made to address comments pointing out possible misunderstandings.

The SCHEER would like to thank all contributors for their comments and for the references provided during the public consultation.

The text of the comments received and the response provided by the SCHEER are available here:

http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scheer_consultation_01_en

7 ABBREVIATIONS AND GLOSSARY OF TERMS

AOP	Adverse outcome pathway
ASL	Arterial Spin Labelling
CAS	Chemical Abstracts Service
CLP	Classification, Labelling and Packaging
CMR	Carcinogenic, mutagenic or toxic for reproduction
CNS	Central nervous system
COC	Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
COM	Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CVD	Cardiovascular disease
CYP	Cytochrome P450 monooxygenase
DA	Dopamine
DKFZ	Deutsches Krebsforschungszentrum (German Cancer Research Center)
EC	European Commission
ECDC	European Centre for Disease prevention and Control
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency
EU	European Union
FDA	(US) Food and Drug Administration
FEMA	(US) Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
fMRI	functional magnetic resonance imaging
FTND	Fagerström Test for Nicotine Dependence
GABA	Gamma (γ)-Aminobutyric acid
GABA	γ -aminobutyric acid
GLP	good laboratory practice
IARC	International Agency for Research on Cancer
IC50	The half-maximal inhibitory concentration
IPCS	(WHO) International Programme on Chemical Safety

JECFA	Joint FAO/WHO Expert Committee on Food Additives
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	(EU) Joint Research Centre
MoE	Mode of Exposure
MRI	Magnetic resonance imaging
nACh	nicotinic acetylcholine
NOAEL	No observed adverse effect level
NRC	(US) National Research Council
NTM	Non-tobacco material
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PAH	Polycyclic aromatic hydrocarbon
PBPK	Physiologically based pharmacokinetic
PET	Positron emission tomography
PG	Propylene glycol
pH	Measure of acidity or basicity of a solution
PITOC	EU project "Public Information Tobacco Control"
ppm	Parts per million
QSAR	Quantitative structure--activity relationships
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (The Netherlands National Institute for Public Health and the Environment)
RYO	Roll-your-own (cigarettes)
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental and Emerging Risks
SCHER	Scientific Committee on Health and Environmental Risks
SCOEL	Scientific Committee on Occupational Exposure Limits
SPECT	Single-photon emission computed tomography
TG	(OECD) Test Guidelines
TPD	Tobacco products directive
UK	United Kingdom

US(A)	United States (of America)
WHO	World Health Organization
WoE	Weight of evidence

8 REFERENCES

Abreo M.A., Lin N.H., Garvey D.S., Gunn D.E., Hettinger A.M., Wasicak J.T., Pavlik P.A., Martin Y.C., Donnelly-Roberts D.L., Anderson D.J., Sullivan J.P., Williams M., Arneric S.P., Holladay M.W. (1996). Novel 3-Pyridyl ethers with subnanomolar affinity for central neuronal nicotinic acetylcholine receptors. *J Med Chem* 39, 817-825.

Ågerstrand M. and Beronius A. Weight of evidence evaluation and systematic review in EU chemical risk assessment: Foundation is laid but guidance is needed. *Environ. Int.* 2015, [Epub ahead of print] doi: 10.1016/j.envint.2015.10.008.

Alpert H.R., Agaku I.T., Connolly G.N. (2016). A study of pyrazines in cigarettes and how additives might be used to enhance tobacco addiction. *Tob Control* 25, 444–50.

Alsharari S.D., Siu E.C., Tyndale R.F., Damaj M.I. (2014). Pharmacokinetic and pharmacodynamics studies of nicotine after oral administration in mice: effects of methoxsalen, a CYP2A5/6 inhibitor. *Nicotine Tob Res* 16, 18-25.

Alsharari S.D., King J.R., Nordman J.C., Muldoon P.P., Jackson A., Zhu A.Z., Tyndale R.F., Kabbani N., Damaj M.I. (2015). Effects of Menthol on Nicotine Pharmacokinetic, Pharmacology and Dependence in Mice. *PLoS ONE* 10(9): e0137070.

American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition. 5th ed.

<http://dx.doi.org/10.1176/appi.books.9780890425596>. accessed on 04/05/2015.

Andreoli C., Gigante D., Nunziata A. (2003). A review of in vitro methods to assess the biological activity of tobacco smoke with the aim of reducing the toxicology of smoke. *Toxicology In Vitro*, 17, 587–94.

Ankley G.T., Bennett R.S., Erickson R.J., Hoff D.J., Hornung M.W., Johnson R.D., Mount D.R., Nichols J.W., Russom C.L., Schmieder P.K., Serrano J.A., Tietge J.E., Villeneuve D.L. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29 (3), 730 -41.

Antanares project <http://www.antares-life.eu>

ANVISA Working Group on Tobacco Additives (2014). Report of the Working Group on Tobacco Additives.

http://portal.anvisa.gov.br/wps/wcm/connect/5c3e698046be14978161fd2e64280806/Report_Working_Group_Tobacco_Additives_FINAL_26-08-14pdf?MOD=AJPERES.

AOP-Wiki (2014). Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs. https://aopkb.org/common/AOP_Handbook.

Arimilli S., Damratoski B.E., Bombick B., Borgerding M.F., Prasad G.L. (2012). Evaluation of cytotoxicity of different tobacco product preparations. *Regul Toxicol Pharm* 64, 350–60.

Asgharian B., Price O., McClellan G., Corley R., Einstein D.R., Jacob R.E., Harkema J., Carey S.A., Schelegle E., Hyde D., Kimbell J.S., Miller F.J. (2012). Development of a rhesus monkey lung geometry model and application to particle deposition in comparison to humans. *Inhal Toxicol* 24, 869-99.

Ashoor A., Nordman J.C., Veltri D., Yang K.H., Al Kury L., Shuba Y., Mahgoub M., Howarth F.C., Sadek B., Shehu A., Kabbani N., Oz M. (2013). Menthol binding and inhibition of $\alpha 7$ -nicotinic acetylcholine receptors. *PLoS One* 8, e67674.

Aufderheide M., Knebel J.W., Ritter D. (2003). An improved in vitro model for testing the pulmonary toxicity of complex mixtures such as cigarette smoke. *Exp Toxicol Pathol* 55, 51–7.

Aufderheide M., Scheffler S., Möhle N., Halter B. & Hochrainer D. (2011). Analytical in vitro approach for studying cyto- and genotoxic effects of particulate airborne material. *Anal. Bioanal. Chem.* 401, 3213–20.

Bagdas D., Muldoon P.P., Zhu A.Z., Tyndale R.F., Damaj M.I. (2014). Effects of methoxsalen, a CYP2A5/6 inhibitor, on nicotine dependence behaviors in mice. *Neuropharmacology* 85, 67–72.

Bakand S., Hayes C. (2010). Troubleshooting methods for toxicity testing of airborne chemicals in vitro. *J Pharmacol Toxicol Methods* 61, 76–85.

Baker R.R., Bishop L.J. (2004). The pyrolysis of tobacco ingredients. *Journal of Analytical and Applied Pyrolysis* 71, 223–311.

Baker R.R., Massey E.D., Smith G. (2004a). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association* 42 Suppl, S53–83.

Baker R.R., Pereira da Silva J.R., Smith G. (2004b). The effect of tobacco ingredients on smoke chemistry. Part I: Flavours and additives. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 42 Suppl, S3–37.

Baker R.R., Pereira da Silva J.R., Smith G. (2004c). The effect of tobacco ingredients on smoke chemistry. Part II: casing ingredients. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 42 Suppl, S39–52.

Baker T.B., Breslau N., Covey L., Shiffman S. (2012). DSM criteria for tobacco use disorder and tobacco withdrawal: a critique and proposed revisions for DSM-5. *Addiction* 107, 263–75.

Bal-Price A., Crofton K.M., Sachana M., Shafer T.J., Behl M., Forsby A., Hargreaves A., Landesmann B., Lein P.J., Louisse J., Monnet-Tschudi F., Paini A., Rolaki A., Schrattenholz A., Suñol C., van Thriel C., Whelan M., Fritsche E. (2015). Putative adverse outcome pathways (AOP) relevant to neurotoxicity. *Crit Rev Toxicol* 45(1), 83–91.

Bal-Price, A., Crofton, K.M., Leist, M., Allen, S., Arand, M., Buetler, T., Delrue, N., FitzGerald, R.E., Hartung, T., Heinonen, T., Hogberg, H., Bennekou, S.H., Lichtensteiger, W., Oggier, D., Paparella, M., Axelstad, M., Piersma, A., Rached, E., Schilter, B., Schmuck, G., Stoppini, L., Tongiorgi, E., Tiramani, M., Monnet-Tschudi, F., Wilks, M.F., Ylikomi, T. & Fritsche, E. (2015). International STakeholder NETwork (ISTNET): creating a developmental neurotoxicity (DNT) testing road map for regulatory purposes. *Archives of Toxicology* 89, 269–287.

Barrett S.P., Boileau I., Okker J., Pihl R.O., Dagher A. (2004). The hedonic response to cigarette smoking is proportional to dopamine release in the human striatum as measured by positron emission tomography and [¹¹C]raclopride. *Synapse* 54, 65-71.

Bates C., Jarvis M., Connolly G. (1999). Tobacco Additives: cigarette engineering and nicotine addiction. London: ASH UK Report; 14 July, 1999.

Becker R.A., Ankley G.T., Edwards S.W., Kennedy S., Linkov I., Meek B., Sachana M., Segner H., Van Der Berg B., Villeneuve D.L., Watanbe H., Barton- Maclaren T.S. (2015). Increasing scientific confidence in adverse outcome pathways: application of tailored Bradford-Hill considerations for evaluating weight of evidence. *Regul.Toxicol.Pharmacol.* 72, 514-37.

Benowitz N.L., Herrera B., Jacob P., (2004). Mentholated cigarette smoking inhibits nicotine metabolism. *J Pharmacol Exp Ther* 310, 1208-15.

Berridge K.C., Robinson T.E. (2003). Parsing reward. *Trends in neurosciences* 26, 507-13.

Berridge M.S., Apana S.M., Nagano K.K., Berridge C.E., Leisure G.P., Boswell M.V. (2010). Smoking produces rapid rise of [¹¹C]nicotine in human brain. *Psychopharmacology (Berl)* 209, 383-94.

Berube K., Pitt A., Hayden P., Prytherch Z., and Job C. (2010). Filter-well technology for advanced three-dimensional cell culture: perspectives for respiratory research. *Altern Lab Anim* 38 Suppl 1, 49.

Behrendt *et al.* (2004). *Br J Pharmacol*; 141, 737-45.

Boyadjieva N.I., Sarkar D.K. (1997). The secretory response of hypothalamic beta-endorphin neurons to acute and chronic nicotine treatments and following nicotine withdrawal. *Life Sci* 61, PL59-66.

Brennan E., Gibson L., Momjian A., Hornik R.C. (2015). Are Young People's Beliefs About Menthol Cigarettes Associated With Smoking-Related Intentions and Behaviors? *Nicotine Tob Res.* 17(1), 81-90.

Brody A.L., Olmstead R.E., London E.D., Farahi J., Meyer J.H., Grossman P., Lee G.S., Huang J., Hahn E.L., Mandelkern M.A. (2004). Smoking-induced ventral striatum dopamine release. *Am J Psychiatry* 161, 1211-8.

Brody A.L., Mandelkern M.A., London E.D., Olmstead R.E., Farahi J., Scheibal D., Jou J., Allen V., Tiongson E., Chefer S.I., Koren A.O., Mukhin A.G. (2006a). Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. *Arch Gen Psychiatry* 63, 907-15.

Brody A.L., Mandelkern M.A., Olmstead R.E., Scheibal D., Hahn E., Shiraga S., Zamora-Paja E., Farahi J., Saxena S., London E.D., McCracken J.T. (2006b). Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. *Arch Gen Psychiatry* 63, 808-16.

Brody A.L., Mandelkern M.A., Costello M.R., Abrams A.L., Scheibal D., Farahi J., London E.D., Olmstead R.E., Rose J.E., Mukhin A.G. (2009). Brain nicotinic acetylcholine receptor occupancy: effect of smoking a denicotinized cigarette. *Int J Neuropsychopharmacol* 12, 305-16.

Brody A.L., Mandelkern M.A., London E.D., Khan A., Kozman D., Costello M.R., Vellios E.E., Archie M.M., Bascom R., Mukhin A.G. (2011). Effect of secondhand smoke on occupancy of nicotinic acetylcholine receptors in brain. *Arch Gen Psychiatry* 68, 953-60.

Brody A.L., Mukhin A.G., La Charite J., Ta K., Farahi J., Sugar C.A., Mamoun M.S., Vellios E., Archie M., Kozman M., Phuong J., Arlorio F., Mandelkern M.A. (2013). Up-regulation of nicotinic acetylcholine receptors in menthol cigarette smokers. *Int J Neuropsychopharmacol* 16, 957-66.

Brody A.L., Mukhin A.G., Mamoun M.S., Luu T., Neary M., Liang L., Shieh J., Sugar C.A., Rose J.E., Mandelkern M.A. (2014). Brain Nicotinic Acetylcholine Receptor Availability and Response to Smoking Cessation Treatment: A Randomized Trial. *JAMA Psychiatry*. 71(7), 797-805.

Brust P., Patt J.T., Deuther-Conrad W., Becker G., Patt M., Schildan A., Sorger D., Kendziorra K., Meyer P., Steinbach J., Sabri O. (2008). In vivo measurement of nicotinic acetylcholine receptors with [18F]norchloro-fluoro-homoepibatidine. *Synapse* 62, 205-18.

Busch C., Streibel T., Liu C., McAdam K.G., Zimmermann R. (2012). Pyrolysis and combustion of tobacco in a cigarette smoking simulator under air and nitrogen atmosphere. *Anal Bioanal Chem*, 419-30.

Caille S., Clemens K., Stinus L., Cador M. (2012). Modelling nicotine addiction in rats. *Methods Mol Biol* 829, 243-56.

Camlin N.J., McLaughlin E.A., Holt J.E. (2014). Through the smoke: use of in vivo and in vitro cigarette smoking models to elucidate its effect on female fertility. *Toxicol Appl Pharmacol*. 281(3), 266-75.

Carmines E.L. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1: cigarette design, testing approach, and review of results. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association* 40, 77-91.

Carpenter C.M., Wayne G.F., Connolly G.N. (2005a). Designing cigarettes for women: new findings from the tobacco industry documents. *Addiction* 100, 837-51.

Carpenter C.M., Wayne G.F., Pauly J.L., Koh H.K., Connolly G.N. (2005b). New cigarette brands with flavors that appeal to youth: Tobacco marketing strategies. *Health Aff (Millwood)* 24, 1601-10.

Carpenter C.M., Wayne G.F., Connolly G.N. (2007). The role of sensory perception in the development and targeting of tobacco products. *Addiction* 102, 136-47.

Cavallo D., Ursini C.L., Freseigna A.M., Maiello R., Ciervo A., Ferrante R., Buresti G., Iavicoli S. (2013). Cyto-genotoxic effects of smoke from commercial filter and non-filter cigarettes on human bronchial and pulmonary cells. *Mutat Res*. 750(1-2), 1-11.

Cervellati F., Muresan X.M., Sticozzi C., Gambari R., Montagner G., Forman H.J., Torricelli C., Maioli E., Valacchi G. (2014). Comparative effects between electronic and cigarette smoke in human keratinocytes and epithelial lung cells. *Toxicology in Vitro* 28, 999-1005.

Chen H., Cui L., Jiang X.Y., Pang Y.Q. Tang, G.L. Hou, H.W. Jiang J.H., Hu, Q.Y. (2012). Evaluation of the cytotoxicity of cigarette smoke condensate by a cellular impedance biosensor. *Food & Chemical Toxicology* 50, 612-18.

COC (2009). Annual report 2009. <https://cotfoodgovuk/sites/default/files/cot/cocsection2009pdf>.

Collins C.C., Moolchan E.T. (2006). Shorter time to first cigarette of the day in menthol adolescent cigarette smokers. *Addict Behav* 31, 1460-4.

Commission Implementing Regulation (EU) 2016/779 of 18 May 2016 laying down uniform rules as regards the procedures for determining whether a tobacco product has a characterising flavour: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.131.01.0048.01.ENG&toc=OJ:L:2016:131:TOC

Commission Implementing Decision (EU) 2016/786 of 18 May 2016 laying down the procedure for the establishment and operation of an independent advisory panel assisting Member States and the Commission in determining whether tobacco products have a characterising flavour: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2016.131.01.0079.01.ENG&toc=OJ:L:2016:131:TOC

Combes R., Scott K., Dillon D., Meredith C., McAdam K., Proctor, C. (2012). The effect of a novel tobacco process on the in vitro cytotoxicity and genotoxicity of cigarette smoke particulate matter. *Toxicology in Vitro* 26, 1022-9.

Combes R., Scott K., Crooks I., Dillon D., Meredith C., McAdam K., Proctor, C. (2013). The in vitro cytotoxicity and genotoxicity of cigarette smoke particulate matter with reduced toxicant yields. *Toxicology in Vitro* 27, 1533-41.

Connolly G.N., Wayne G.D., Lymperis D., Doherty M.C. (2000). How cigarette additives are used to mask environmental tobacco smoke. *Tob Control* 9, 283-91.

Connolly G.N. (2004). Sweet and spicy flavours: new brands for minorities and youth. *Tob Control* 13, 211-2.

CORESTA in vitro toxicology task force. (2004). THE RATIONALE AND STRATEGY FOR CONDUCTING IN VITRO TOXICOLOGY TESTING OF TOBACCO SMOKE (May 2004): https://www.coresta.org/sites/default/files/technical_documents/main/IVT_TF_Rationale-IVT-Testing-Tob.-Smoke_Report_Jun04.pdf.

CORESTA in vitro toxicology task force report. (2006). In vitro exposure of cells to smoke at the air liquid interface: https://www.coresta.org/sites/default/files/technical_documents/main/IVT_TF_Report_Smoke_Air_Liquid_Interface.pdf.

Cosgrove K.P., Batis J., Bois F., Maciejewski P.K., Esterlis I., Kloczynski T., Stiklus S., Krishnan-Sarin S., O'Malley S., Perry E., Tamagnan G., Seibyl J.P., Staley J.K. (2009). beta2-Nicotinic acetylcholine receptor availability during acute and prolonged abstinence from tobacco smoking. *Arch Gen Psychiatry* 66, 666-76.

Cosgrove K.P., Esterlis I., Sandiego C., Petrulli R., Morris E.D. (2015). Imaging Tobacco Smoking with PET and SPECT. *Curr Top Behav Neurosci* 24, 1-17.

COT/COM/COT (2009). Carcinogenicity testing of tobacco products. Annual report. Committees on toxicity (COT), mutagenicity (COM), carcinogenicity (COC) of chemicals in food, consumer products and the environment, London.

Counts M.E., Morton M.J., Laffoon S.W., Cox R.H., Lipowicz P.J. (2005). Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. *Regul Toxicol Pharm*, 41(3), 185-227.

Costigan S., Meredith C. (2015) An approach to ingredient screening and toxicological risk assessment of flavours in e-liquids. *Regul Toxicol Pharmacol.* 72(2), 361-9.

Cramer R.D., Patterson D.E., Bunce J.D. (1988). Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J Am Chem Soc* 110, 5959-67.

Crooks I., Dillon D., Scott K., Ballantyne M., Meredith C. (2013). The effect of long term storage on tobacco smoke particulate matter in in vitro genotoxicity and cytotoxicity assays. *Regul Toxicol Pharm* 65, 196–200.

Crooks, I., Scott, K., Dalrymple, A., Dillon, D., Meredith, C. (2015). The combination of two novel tobacco blends and filter technologies to reduce the in vitro genotoxicity and cytotoxicity of prototype cigarettes. *Regul Toxicol Pharmacol.* 71(3), 507-14.

Cummings KM, Morley CP, Horan JK, Steger C, Leawell NR. (2002). Marketing to America's youth: evidence from corporate document. *Tob Control* 11, 15-17.

Damaj M.I., Meyer E.M., Martin B.R. (2000). The antinociceptive effects of alpha7 nicotinic agonists in an acute pain model. *Neuropharmacology* 39, 2785-91.

Dani J.A., De Biasi M. (2001). Cellular mechanisms of nicotine addiction. *Pharmacol Biochem Behav* 70, 439-46.

Davenport K.E., Houdi A.A., Van Loon G.R. (1990). Nicotine protects against mu-opioid receptor antagonism by beta-funaltrexamine: evidence for nicotine-induced release of endogenous opioids in brain. *Neurosci Lett* 113, 40-6.

de Jong P.M., van Sterkenburg M.A., Hesseling S.C., Kempenaar J.A., Mulder A.A., Mommaas A.M., Dijkman J.H., and Ponc M. (1994). Ciliogenesis in human bronchial epithelial cells cultured at the air-liquid interface. *Am J Respir Cell Mol Biol* 10, 271.

DKFZ (2010). Strategy for Toxicity Evaluation of Tobacco Additives and their Regulation. Deutsches Krebsforschungszentrum (DKFZ), Heidelberg.

Domino E.F., Hirasawa-Fujita M., Ni L., Guthrie S.K., Zubieta J.K. (2015). Regional brain [(11)C]carfentanil binding following tobacco smoking. *Prog Neuropsychopharmacol Biol Psychiatry* 59, 100-4.

EFSA (2010). Application of Systematic Review Methodology to Food and Feed Safety Assessments to Support Decision Making. <http://www.efsa.europa.eu/en/search/doc/1637.pdf>

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (2012). Scientific European Food Safety Authority (EFSA), Parma, Italy. Opinion, Guidance for submission for food additive evaluations, *EFSA Journal* 10(7), 2760.

EFSA (2013). International Frameworks Dealing with Human Risk Assessment of Combined Exposure to Multiple Chemicals, *EFSA Journal* 2013; 11(7):3313 [69 pp.]. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3313.pdf

Egger M., Smith D.G. (1997). Meta-analysis: Potentials and promise. *BMJ* 315, 1371–4. [PubMed: 9432250]

Esfahani A., Wong J.M., Truan J., Villa C.R., Mirrahimi A., Srichaikul K., Kendall C.W. (2011). Health effects of mixed fruit and vegetable concentrates: a systematic review of the clinical interventions. *J Am Coll Nutr.* 30(5), 285-94.

Eskes C., Sullivan K., Aardema M., Spielmann H., Hill E., Schoeters G., Curren R., Vinken M., Blaauboer B. (2012). Comparing the challenges in developing and implementing 3Rs alternative methods in Europe and United States: Industrial and academic perspectives. ALTEX 1/12, WC8 Proceedings, 205–9.

Etter J.F., Le Houezec J., Perneger T.V. (2003). A self-administered questionnaire to measure dependence on cigarettes: the cigarette dependence scale. *Neuropsychopharmacology* 28, 359-70.

Etter J.F. (2005). A self-administered questionnaire to measure cigarette withdrawal symptoms: the Cigarette Withdrawal Scale. *Nicotine Tob Res* 7, 47-57.

EU Scientific Committees on Health and Environmental Risks (SCHER), on Emerging and Newly Identified Health Risks (SCENIHR) and Consumer Safety (SCCS) opinion on Toxicity and Assessment of Chemical Mixtures in 2011, supported the use of a tiered approach, such as that presented in the WHO Framework. http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf

EuroMix project: <https://www.euromixproject.eu>.

European Union (2014). Directive 2014/40/EU of the European Parliament. In: Union, E. (Ed.). Official Journal of the European Union.

Fagerstrom K. (2012). Determinants of tobacco use and renaming the FTND to the Fagerstrom Test for Cigarette Dependence. *Nicotine Tob Res* 14, 75-8.

Farsalinos K.E., Romagna G., Alliffranchini E., Ripamonti E., Bocchietto E., Todeschi S., Tsiapras D., Kyrzopoulos S., Voudris, V. (2013). Comparison of the cytotoxic potential of cigarette smoke and electronic cigarette vapour extract on cultured myocardial cells. *Int J Environ Res Public Health* 10, 5146–62.

Fearon I.M., Gaca M.D., Nordskog B.K. (2013). In vitro models for assessing the potential cardiovascular disease risk associated with cigarette smoking. *Toxicology in vitro* 27, 513-22.

Ferreira C.G., Silveira D., Hatsukami D.K., Paumgartten F.J., Fong G.T., Gloria M.B., Toledo M.C., Talhout R. (2015). The effect of tobacco additives on smoking initiation and maintenance. *Cadernos de saude publica* 31, 223-5.

Forbes, B., and Ehrhardt, C. (2005). Human respiratory epithelial cell culture for drug delivery applications. *Eur J Pharm Biopharm* 60, 193.

Forster M., Liu C., Duke M.G., McAdam K.G., Proctor C.J. (2015). An experimental method to study emissions from heated tobacco between 100-200 degrees C. *Chem Cent J* 9, 20.

Fowler J.S., Logan J., Wang G.J., Volkow N.D. (2003). Monoamine oxidase and cigarette smoking. *Neurotoxicology* 24, 75-82.

Fowler P., Smith K., Young J., Jeffrey L., Kirkland D., Pfuhler S., Carmichael P. (2012) Reduction of misleading ("false") positive results in mammalian cell genotoxicity assays. I. Choice of cell type *Mutation Research* 742, 11–25.

Fowles, J. (2001). Chemical factors influencing the addictiveness and attractiveness of cigarettes in New Zealand. New Zealand Ministry of Health

Fowles J., Dybing E. (2003). Application of toxicological risk assessment principles to the chemical toxicants of cigarette smoke. *Tobacco Control* 12, 424–30.

Fukano Y., Ogura M., Eguchi K., Shibagaki M., Suzuki M. (2004). Modified procedure of a direct in vitro exposure system for mammalian cells to whole cigarette smoke. *Experimental and Toxicologic Pathology* 55, 317–23.

Gao H., Prasad G.L., Zacharias W. (2013). Differential cell-specific cytotoxic responses of oral cavity cells to tobacco preparations. *Toxicology in Vitro* 27, 282–91.

Garcia-Canton C., Errington G., Anadon A., Meredith, C. (2014). Characterisation of an aerosol exposure system to evaluate the genotoxicity of whole mainstream cigarette smoke using the in vitro γ H2AX assay by high content screening. *BMC Pharmacology & Toxicology* 23, 15–41.

Gaworski C.L., Schramke H., Diekmann J., Meisgen T.J., Tewes F.J., Veltel D.J., Vanscheeuwijck P.M., Rajendran N., Muzzio M., Haussmann H.J. (2009). Effect of filtration by activated charcoal on the toxicological activity of cigarette mainstream smoke from experimental cigarettes. *Inhal Toxicol* 21, 688–704.

Gaworski C.L., Wagner K.A., Morton M.J., Oldham M.J. (2011). Insights from a multi-year program designed to test the impact of ingredients on mainstream cigarette smoke toxicity. *Inhal Toxicol Suppl* 1, 172–83.

Gee D. (2008). Establishing evidence for early action: the prevention of reproductive and developmental harm. *Basic Clin Pharmacol Toxicol*. 102, 71–2.

Ghallab A. (2013). In vitro test systems and their limitations *EXCLI J.* 12, 1024–6. Published online 2013 Dec 12. PMID: PMC4803002.

Gnerre C., Catto M., Leonetti F., Weber P., Carrupt P.A., Altomare C., Carotti A., Testa B. (2000). Inhibition of monoamine oxidases by functionalized coumarin derivatives: biological activities, QSARs, and 3D-QSARs. *J Med Chem*. 43, 4747–58.

Godoy P., Hengstler J.G., Ilkavets I., Meyer C., Bachmann A., Müller A., *et al.* (2009). Extracellular matrix modulates sensitivity of hepatocytes to fibroblastoid dedifferentiation and transforming growth factor beta-induced apoptosis. *Hepatology* 49, 2031–43.

Godoy P., Lakkapamu S., Schug M., Bauer A., Stewart J.D., Bedawi E., *et al.* (2010). Dexamethasone-dependent versus -independent markers of epithelial to mesenchymal transition in primary hepatocytes. *Biol Chem*. 391, 73–83.

Godoy P., Schug M., Bauer A., Hengstler J.G. (2010). Reversible manipulation of apoptosis sensitivity in cultured hepatocytes by matrix-mediated manipulation of signaling activities. *Methods Mol Biol*. 640, 139–55.

Gotti C., Zoli M., Clementi F. (2006). Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci*. 27, 482–91.

Grainger C.I., Greenwell L.L., Lockley D.J., Martin G.P., and Forbes B. (2006). Culture of Calu-3 cells at the air interface provides a representative model of the airway epithelial barrier. *Pharm Res*. 23, 1482.

Guo X., Verkler T.L., Chen Y., Richter P.A., Polzin G.M., Moore M.M., Mei N. (2011). Mutagenicity of 11 cigarette smoke condensates in two versions of the mouse lymphoma assay. *Mutagenesis* 26, 273–81.

Ha, M.A., Smith, G.J., Cichocki, J.A., Fan, L., Liu, Y.S., Caceres, A.I., Jordt, S.E., Morris, J.B. (2015). Menthol attenuates respiratory irritation and elevates blood cotinine in cigarette smoke exposed mice. *PLoS One*. 2015 Feb 13; 10(2):e0117128. doi: 10.1371/journal.pone.0117128. PMID: 25679525.

Haddadian E.J., Cheng M.H., Coalson R.D., Xu Y., Tang P. (2008). In silico models for the human alpha4beta2 nicotinic acetylcholine receptor. *J Phys Chem B* 112, 13981-90.

Hans M., Wilhelm M., Swandulla D. (2012). Menthol suppresses nicotinic acetylcholine receptor functioning in sensory neurons via allosteric modulation. *Chem Senses* 37, 463-9.

Hart A., Roelofs W., Hardy A.R., Macleod A. (2007). Comparative review of risk terminology. A comparative review of terminology and expressions used by the three scientific committees established by Commission Decision 2004/210/EC and by their predecessors established by Commission Decision 97/579/EC (repealed by Commission Decision 2004/210/EC). Report No. S12.454739. The Central Science Laboratory, DEFRA, Sand Hutton, York, UK.

He X., Chen Y.Y., Shi J.B., Tang W.J., Pan Z.X., Dong Z.Q., Song B.A., Li J., Liu X.H. (2014). New coumarin derivatives: Design, synthesis and use as inhibitors of hMAO. *Bioorg Med Chem*. 22(14), 3732-8.

Henningfield J.E., Hatsukami D.K., Zeller M., Peters E. (2011). Conference on abuse liability and appeal of tobacco products: conclusions and recommendations. *Drug Alcohol Depend* 116, 1-7.

Herraiz T., Chaparro C. (2005). Human monoamine oxidase is inhibited by tobacco smoke: beta-carboline alkaloids act as potent and reversible inhibitors. *Biochem Biophys Res Commun* 326, 378-86.

Herraiz T., Chaparro C. (2006). Human monoamine oxidase enzyme inhibition by coffee and beta-carbolines norharman and harman isolated from coffee. *Life Sci* 78, 795-802.

Hertz-Schunemann R., Ehlert S., Streibel T., Liu C., McAdam K., Baker R.R., Zimmermann R. (2015). High-resolution time and spatial imaging of tobacco and its pyrolysis products during a cigarette puff by microprobe sampling photoionisation mass spectrometry. *Anal Bioanal Chem*. 407, 2293-9.

Herzog F., Clift M.J., Piccapietra F., Behra R., Schmid O., Petri-Fink A., and Rothen-Rutishauser B. (2013). Exposure of silver-nanoparticles and silver-ions to lung cells in vitro at the air-liquid interface. *Part Fibre Toxicol* 10, 11.

Hillmer A.T., Wooten D.W., Moirano J.M., Slesarev M., Barnhart T.E., Engle J.W., Nickles R.J., Murali D., Schneider M.L., Mukherjee J., Christian B.T. (2011). Specific alpha4beta2 nicotinic acetylcholine receptor binding of [F-18]nifene in the rhesus monkey. *Synapse* 65, 1309-18.

Hockley B.G., Stewart M.N., Sherman P., Quesada C., Kilbourn M.R., Albin R.L., Scott P.J. (2013). (-)-[(18) F]Flubatine: evaluation in rhesus monkeys and a report of the first fully automated radiosynthesis validated for clinical use. *J Labelled Comp Radiopharm* 56, 595-9.

Hoffman A.C., Simmons D. (2011). Menthol cigarette smoking and nicotine dependence. *Tob Induc Dis* 9 Suppl 1, S5.

Hoffman A.C., Evans S.E. (2013). Abuse potential of non-nicotine tobacco smoke components: acetaldehyde, nornicotine, cotinine, and anabasine. *Nicotine Tob Res* 15, 622-32.

Holma B., Hegg P.O. (1989). pH- and protein-dependent buffer capacity and viscosity of respiratory mucus. Their interrelationships and influence on health. *Sci Total Environ* 84, 71-82.

Horvath A.R., Pewsner D. (2004). Systematic reviews in laboratory medicine: principles, processes and practical considerations. *Clinica Chimica Acta* 342, 23-39.

<http://cactus.nci.nih.gov>

http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

http://ihcp.jrc.ec.europa.eu/our_databases/jrc-qsarinventory

http://ihcp.jrc.ec.europa.eu/our_labs/predictive_toxicology/qsar_tools/QRF

<http://qsar.food.dtu.dk>

<http://www.oecd.org/chemicalsafety/risk-assessment/introductiontoquantitativestructureactivityrelationships.htm>

<http://www.qsartoolbox.org>

<https://eurl-ecvam.jrc.ec.europa.eu/databases/jrc-qsar-model-database>

https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/qsar-tools

https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxtree

https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/DART

https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxmatch

https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/stat4tox

[http://www.comcarelink.health.gov.au/internet/main/publishing.nsf/Content/14F3E74C2B8CFB98CA257BF0001DE830/\\$File/pmorris.pdf](http://www.comcarelink.health.gov.au/internet/main/publishing.nsf/Content/14F3E74C2B8CFB98CA257BF0001DE830/$File/pmorris.pdf)

<http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/ucm297786.htm>

http://www.hc-sc.gc.ca/hl-vs/tobac-tabac/legislation/reg/indust/constitu_e.html

<http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/index-eng.php#main>

Hukkanen J., Jacob P. 3rd, Benowitz N.L. (2005). Metabolism and disposition kinetics of nicotine. *Pharmacol Rev* 57, 79-115.

Hurt R.D., Robertson C.R. (1998). Prying open the door to the tobacco industry's secrets about nicotine: the Minnesota Tobacco Trial. *JAMA* 280, 1173-81.

IARC (2006). Monographs on the Evaluation of Carcinogenic Risks to Humans. Preamble (<http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf>).

- Jaffe A.J., Glaros A.G. (1986). Taste dimensions in cigarette discrimination: a multidimensional scaling approach. *Addict Behav* 11, 407-13.
- Jasinska A.J., Zorick T., Brody A.L., Stein E.A. (2014). Dual role of nicotine in addiction and cognition: a review of neuroimaging studies in humans. *Neuropharmacology* 84, 111-22.
- Jenkins C.R., Boham N., Burden A.J., Dixon M., Dowle M., Fiebelkorn R.T., Ferris R.P., Hemsley S.R., Maswood M.R., Mooney P.J., O'Reilly R., Stephenson A.G., Wagner C., Walshe P.W.D., White P.R. (1997). British American Tobacco Company Limited Product Seminar. Tobacco documents 760076123-760076408, 760076123-760076408.
- Jenkins R.W., Newman R.H., Chavis M.K. (1970). Cigarette smoke formation studies. Smoke distribution and mainstream pyrolytic composition of added ¹⁴C-menthol. *Beitrage zur Tabakforschung* 5, December.
- Jennings P. (2015). The future of in vitro toxicology. *Toxicol In Vitro*. 29(6), 1217-21.
- Jensen A.A., Frolund B., Liljefors T., Krogsgaard-Larsen P. (2005). Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. *Journal of Medical Chemistry* 48, 4705-45.
- Jianlin, L., Guohai, C., Guojun, Z., Jian, J., Fangfang, H., Juanjuan, X., Shu, Z., Zhijian, C., Wei, J., Yezhen, L., Xiaoxue, L. & Jiliang, H. (2009). Assessing cytogenotoxicity of cigarette smoke condensates using three in vitro assays. *Mutation Research* 677, 21–26.
- Jianlin, L., Guohai, C., Guojun, Z., Jian, J., Fangfang, H., Juanjuan, X., Shu, Z., Zhijian, C., Wei, J., Yezhen, L., Xiaoxue, L., Jiliang, H. (2009). Studying the impact of S9 on cytogenotoxicity of cigarette smoke in human peripheral blood lymphocytes in vitro. *Environmental Toxicology & Pharmacology* 28, 275–79.
- Jianlin L., Guohai C, Guojun Z, Jian J, Fangfang H, Juanjuan X, Shu Z, Wei J, Yezhen L, Xiaoxue L, Zhijian C, Jiliang H. (2010). Comparison between two kinds of cigarette smoke condensates (CSCs) of the cytogenotoxicity and protein expression in a human B-cell lymphoblastoid cell line using CCK-8 assay, Comet assay and protein microarray. *Mutation Research* 697, 55–9.
- Johnson C.L. (1976). Quantitative structure-activity studies on monoamine oxidase inhibitors. *J Med Chem* 19, 600-5.
- Johnson M.D., Schilz J., Djordjevic M.V., Rice J.R. & Shields P.G. (2009). Evaluation of in vitro assays for assessing the toxicity of cigarette smoke and smokeless tobacco. *Cancer Epidemiol Biomarkers Prev* 18, 3263–304.
- Kabbani N. (2013). Not so Cool? Menthol's discovered actions on the nicotinic receptor and its implications for nicotine addiction. *Front Pharmacol.* 4, 95.
- Kanebratt K.P., Andersson T.B. (2008). HepaRG cells as an in vitro model for evaluation of cytochrome P450 induction in humans. *Drug Metab Dispos.* 36, 137–45.
- Katrak P., Bialocerkowski A., Massy-Westropp N., Kumar V.S., Grimmer K. (2004). A systematic review of the content of critical appraisal tools. *BMC Medical Research Methodology* 4, 22.
- Keiser M.J., Roth B.L., Armbruster B.N., Ernsberger P., Irwin J.J., Shoichet B.K. (2007). Relating protein pharmacology by ligand chemistry. *Nat Biotechnol.* 25, 197-206.

Keithly L, Ferris Wayne G., Cullen D.M., Connolly G.N. (2005). Industry research on the use and effects of levulinic acid: A case study in cigarette additives. *Nicotine Tob Res.* 7, 761-71.

Kienhuis A.S., Soeteman-Hernández L.G., Staal Y.C.M., van de Nobelen S., Talhout R. (2016). A test strategy for the assessment of additive attributed toxicity of tobacco products. Available online. doi:10.1016/j.fct.2016.05.002.

Kilford J., Thorne D., Payne R., Dalrymple A., Clements J., Meredith C., Dillon D. (2014). A method for assessment of the genotoxicity of mainstream cigarette-smoke by use of the bacterial reverse-mutation assay and an aerosol-based exposure system. *Mutat Res.* 769: 20-8.

Klus H., Scherer G., Muller L. (2012). Influence of additives on cigarette related health risks. *Contributions to Tobacco Research* 25 (3), 412–93.

Kober H., Deleone C.M. (2011). Smoking and Neuroimaging: A Review. *Curr Cardiovasc Risk Rep.* 5, 484-91.

Kochhar N., Warburton D.M. (1990). Puff-by-puff sensory evaluation of a low to middle tar medium nicotine cigarette designed to maintain nicotine delivery to the smoker. *Psychopharmacology (Berl)* 102, 343-9.

Koren A.O., Horti A.G., Mukhin A.G., Gundisch D., Kimes A.S., Dannals R.F., London E.D. (1998). 2-, 5-, and 6-Halo-3-(2(S)-azetidinylmethoxy)pyridines: synthesis, affinity for nicotinic acetylcholine receptors, and molecular modeling. *J Med Chem.* 41, 3690-8.

Kosmider B., Messier E.M., Chu H.W., Mason R.J. (2011). Human alveolar epithelial cell injury induced by cigarette smoke. *PLoS One* 6, e26059.

Koustas E., Lam J., Sutton P., Johnson P.I., Atchley D.S., Sen S., Robinson K.A., Axelrad D.A., Woodruff T.J. (2014). The navigation guide—evidence-based medicine meets environmental health: systematic review of nonhuman evidence for PFOA effects on fetal growth. *Environ Health Perspect.* 122 (10), 1015-27.

Kramlinger V.M., von Weymarn L.B., Murphy S.E. (2012). Inhibition and inactivation of cytochrome P450 2A6 and cytochrome P450 2A13 by menthofuran, beta-nicotyrine and menthol. *Chem Biol Interact* 197, 87-92.

Kuwabara H., Wong D.F., Gao Y., Valentine H., Holt D.P., Ravert H.T., Dannals R.F., Horti A.G. (2012). PET Imaging of nicotinic acetylcholine receptors in baboons with 18F-AZAN, a radioligand with improved brain kinetics. *J Nucl Med.* 53, 121-9.

Kuwabara H., Heishman S.J., Brasic J.R., Contoreggi C., Cascella N., Mackowick K.M., Taylor R., Rousset O., Willis W., Huestis M.A., Concheiro M., Wand G., Wong D.F., Volkow N.D. (2014). Mu Opioid Receptor Binding Correlates with Nicotine Dependence and Reward in Smokers. *PLoS One* 9, e113694.

Landesmann, B., Mennecozzi, M., Berggren, E., Whelan, M. (2013). Adverse outcome pathway-based screening strategies for an animal-free safety assessment of chemicals. *ATLA* 41, 461–71.

Langer T., Hoffmann R.D. (2006). Pharmacophore modelling: applications in drug discovery. *Expert Opin Drug Discov.* 1, 261-7.

Laruelle M. (2000). Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab.* 20, 423-51.

Lawrence D., Cadman B., Hoffman A.C. (2011). Sensory properties of menthol and smoking topography. *Tob Induc Dis* 9 Suppl. 1, S3.

Le Foll B., Guranda M., Wilson A.A., Houle S., Rusjan P.M., Wing V.C., Zawertailo L., Busto U., Selby P., Brody A.L., George T.P., Boileau I. (2014). Elevation of dopamine induced by cigarette smoking: novel insights from a [¹¹C]-+PHNO PET study in humans. *Neuropsychopharmacology* 39, 415-24.

Lee J.G., Lee C.G., Kwag J.J., Rhee M.S., Buglass A.J., Lee G.H. (2007). Fast analysis of nicotine in tobacco using double-shot pyrolysis--gas chromatography--mass spectrometry. *J Agric Food Chem.* 55, 1097-102.

Levin E.D., Simon B.B. (1998). Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology (Berl)*. 138, 217-30.

Levin R., Jansson S.O., Rudén C. (2004). Indicators of uncertainty in chemical risk assessments. *Regul Toxicol Pharm.* 39, 33-43.

Lewis A., Miller J.H., Lea R.A. (2007). Monoamine oxidase and tobacco dependence. *Neurotoxicology* 28, 182-95.

Li X., Shang P., Peng B., Nie C., Zhao L., Liu H., Xie J. (2012). Effects of smoking regimens and test material format on the cytotoxicity of mainstream cigarette smoke. *Food Chem Toxicol.* 50(3-4), 545-51.

Li X., Peng B., Nie C., Shang P., Liu H. (2013). Evaluation of the cytotoxicity of cigarette smoke total particulate matter using three in vitro assays and two types of cells. *Toxicol Mech Methods.* 23(4), 240-6.

Li X., Nie C., Shang P., Xie F., Liu H., Xie J. (2014). Evaluation method for the cytotoxicity of cigarette smoke by in vitro whole smoke exposure. *Exp Toxicol Pathol.* 66, 27-33.

Li X., Lin B., Zhang H., Xie F., Ta N., Tian L., Liu H., Xi Z. (2016). Cytotoxicity and mutagenicity of sidestream cigarette smoke particulate matter of different particle sizes. *Environ Sci Pollut Res Int.* 23(3), 2588-94.

Lin B., Li X., Zhang H., Lin Z., Tian L., Nie C., Fang Y., Xi Z. (2014). Comparison of in vitro toxicity of mainstream cigarette smoke particulate matter from nano- to micro-size. *Food Chem Toxicol.* 64, 353-60.

Lin J.C., Roy J.P., Verreault J., Talbot S., Côté F., Couture R., Morin A. (2012). An ex vivo approach to the differential parenchymal responses induced by cigarette whole smoke and its vapor phase. *Toxicology.* 293(1-3), 125-31.

Ling P.M., Glantz S.A. (2005). Tobacco industry consumer research on socially acceptable cigarettes. *Tobacco Control.* 14(5), e3.

Lotfipour S., Mandelkern M., Brody A.L. (2011). Quantitative Molecular Imaging of Neuronal Nicotinic Acetylcholine Receptors in the Human Brain with A-85380 Radiotracers. *Curr Med Imaging Rev* 7, 107-12.

Lou J., Zhou G., Chu G., Jiang J., Huang F., Zheng S., Lu Y., Li X., He, J. (2010). Studying the cyto-genotoxic effects of 12 cigarette smoke condensates on human lymphoblastoid cell line in vitro. *Mutation Research* 696, 48-54.

Lübbert M., Kyereme J., Schöbel N., Beltrán L., Wetzel C.H., Hatt H. (2013). Transient receptor potential channels encode volatile chemicals sensed by rat trigeminal ganglion neurons. *PLoS One* 8, e77998.

Lundstrom J.N., Boesveldt S., Albrecht J. (2011). Central Processing of the Chemical Senses: an Overview. *ACS chemical neuroscience* 2, 5-16.

Mamede M., Ishizu K., Ueda M., Mukai T., Iida Y., Kawashima H., Fukuyama H., Togashi K., Saji H. (2007). Temporal change in human nicotinic acetylcholine receptor after smoking cessation: 5IA SPECT study. *J Nucl Med.* 48, 1829-35.

Manupallo J.R., Sullivan K.M. (2015). Toxicity assessment of tobacco products in vitro. *ATLA* 43, 39-67.

Mathis C., Poussin C., Weisensee D., Gebel S., Hengstermann A., Sewer A., Belcastro V., Xiang Y., Ansari S., Wagner S., Hoeng J., Peitsch M.C. (2013). Human bronchial epithelial cells exposed in vitro to cigarette smoke at the air-liquid interface resemble bronchial epithelium from human smokers. *American Journal of Physiology: Lung Cellular & Molecular Physiology.* 304, L489-L503.

McAdam K.G., Gregg E.O., Liu C., Dittrich D.J., Duke M.G., Proctor C.J. (2011). The use of a novel tobacco-substitute sheet and smoke dilution to reduce toxicant yields in cigarette smoke. *Food & Chemical Toxicology.* 49, 1684-96.

McKinney D.L., Gogova M., Davies B.D., Ramakrishnan V., Fisher K., Carter W.H., Karnes H.T., Garnett W.R., Iyer S.S., Somani A.A., Kobal G., Barr W.H. (2012). Evaluation of the effect of ammonia on nicotine pharmacokinetics using rapid arterial sampling. *Nicotine Tob Res.* 14, 586-95.

Meek (Bette) M.E., Boobis A.R., Crofton K.M., Heinemeyer G., Van Raaij M., Vickers C. (2011). Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. *Regul Toxicol Pharm.* 60, S1-S14.

Menossi H.S., Goudriaan A.E., de Azevedo-Marques Perico C., Nicastri S., de Andrade A.G., D'Elia G., Li C.S., Castaldelli-Maia J.M. (2013). Neural bases of pharmacological treatment of nicotine dependence - insights from functional brain imaging: a systematic review. *CNS Drugs* 27, 921-41.

Messner, B., Frotschnig, S., Steinacher-Nigisch, A., Winter, B., Eichmair, E., Gebetsberger, J., Schwaiger, S., Ploner, C., Laufer, G. & Bernhard, D. (2012). Apoptosis and necrosis: Two different outcomes of cigarette smoke condensate-induced endothelial cell death. *Cell Death & Disease.* 3, e424.

Mitsui K., David F., Tienpont B., Sandra K., Ochiai N., Tamura H., Sandra P. (2015). Analysis of the reaction products from micro-vial pyrolysis of the mixture glucose/proline and of a tobacco leaf extract: Search for Amadori intermediates. *Journal of chromatography A* 1422, 27-33.

Montgomery A.J., Lingford-Hughes A.R., Egerton A., Nutt D.J., Grasby P.M. (2007). The effect of nicotine on striatal dopamine release in man: A [¹¹C]raclopride PET study. *Synapse* 61, 637-45.

Mukhin A.G., Kimes A.S., Chefer S.I., Matochik J.A., Contoreggi C.S., Horti A.G., Vaupel D.B., Pavlova O., Stein E.A. (2008). Greater nicotinic acetylcholine receptor density in smokers than in nonsmokers: a PET study with 2-18F-FA-85380. *J Nucl Med.* 49, 1628-35.

Nara H., Fukano Y., Nishino T., Aufderheide M. (2013). Detection of the cytotoxicity of water-insoluble fraction of cigarette smoke by direct exposure to cultured cells at an air-liquid interface. *Exp Toxicol Pathol.* 65(5), 683-8.

National Institute for Public Health and the Environment (2012). Tobacco additives; Information for Professionals. <http://www.rivm.nl/dsresource?objectid=rivmp:185755&type=org&disposition=inline>.

Naven R.T., Greene N., Williams R.V. (2012). Latest advances in computational 1 genotoxicity prediction. *Expert Opin Drug Metab Toxicol.* 8, 1579-87.

Nichols J.E., Niles J.A., Vega S.P., Cortiella J. (2013). Novel in vitro respiratory models to study lung development, physiology, pathology and toxicology. *Stem Cell Res Ther.* 4 Suppl 1, S7.

Nichols J.E., Niles J.A., Vega S.P., Argueta L.B., Eastaway A., Cortiella J. (2014). Modelling the lung: Design and development of tissue engineered macro- and micro-physiologic lung models for research use. *Exp Biol Med* (Maywood). 239, 1135-69.

NIEHS (National Institute of Environmental Health Sciences). 2012. Tox21: Transforming Environmental Health. Available: http://www.niehs.nih.gov/health/assets/docs_p_z/tox21_transforming_environmental_health_508.pdf

NIST Standard Reference Database 1A: NIST/EPA/NIH Mass Spectral Library with Search Program. Including a tool for GC/MS deconvolution: AMDIS version 2.72. Data Version: NIST 14. Software Version: 2.2g. The NIST Mass Spectrometry Data Center, National Institute of Standards and Technology. Gaithersburg. Retrieved from <http://www.nist.gov/srd/nist1a.cfm>.

Nonnemaker J., Hersey J., Homsy G., Busey A., Allen J., Vallone D. (2013). Initiation with menthol cigarettes and youth smoking uptake. *Addiction* 108, 171-8.

Noriyasu A., Konishi T., Mochizuki S., Sakurai K., Tanaike Y., Matsuyama K., Uezu K., Kawano T. (2013). Menthol-enhanced cytotoxicity of cigarette smoke demonstrated in two bioassay models. *Tob Induc Dis.* 11(1), 18.

NRC (National Research Council). 2007 Toxicity Testing in the 21st Century: A Vision and A Strategy. Washington, DC: National Academies Press. Available: http://www.nap.edu/openbook.php?record_id=11970.

Nuechterlein E.B., Ni L., Domino E.F., Zubieta J.K. (2016). Nicotine-specific and non-specific effects of cigarette smoking on endogenous opioid mechanisms. *Prog Neuropsychopharmacol Biol Psychiatry* 69, 69-77.

OECD (2013). Guidance Document on Developing and Assessing Adverse Outcome Pathways. OECD Environment, Health and Safety Publications Series on Testing and Assessment. No. 184, ENV/JM/MONO(2013)6.

Okuwa K., Tanaka M., Fukano Y., Nara H., Nishijima Y., Nishino, T. (2010). In vitro micronucleus assay for cigarette smoke using a whole smoke exposure system: A comparison of smoking regimens. *Experimental & Toxicologic Pathology* 62, 433-40.

Oldham M.J., Haussmann H.-J., Gomm W., Rimmer L.T., Morton M.J., McKinney W.J., Jr. (2012). Discriminatory power of standard toxicity assays used to evaluate ingredients added to cigarettes. *Regul Toxicol Pharmacol* 62, 49-61.

O'Leary C., Gilbert J.L., O'Dea S., O'Brien F.J., Cryan S.-A. (2015). Respiratory Tissue Engineering: Current Status and Opportunities for the Future. *Tissue Engineering Part B: Reviews*, 4, 323-44.

O'Leary C., Cavanagh B., Unger R.E., Kirkpatrick C.J., O'Dea S., O'Brien F.J., Cryan S.-A. (2016). The development of a tissue-engineered tracheobronchial epithelial model using a bilayered collagen-hyaluronate scaffold *Biomaterials* 85, 111127.

Park K., Kim D. (2008). Binding similarity network of ligand. *Proteins* 71, 960-71.

Pavlovicz R.E., Henderson B.J., Bonnell A.B., Boyd R.T., McKay D.B., Li C. (2011). Identification of a negative allosteric site on human $\alpha 4\beta 2$ and $\alpha 3\beta 4$ neuronal nicotinic acetylcholine receptors. *PLoS One* 6, e24949.

Pecina, M., Bohnert, A.S., Sikora, M., Avery, E.T., Langenecker, S.A., Mickey, B.J., Zubieta, J.K. (2015). Association Between Placebo-Activated Neural Systems and Antidepressant Responses: Neurochemistry of Placebo Effects in Major Depression. *JAMA Psychiatry* 72, 1087-1094.

Pennings J., Kienhuis A., Schenk E., van de Nobelen S., Visser W., Talhout R. (2016). Additives in Dutch tobacco products; RIVM letter rapport 2015-0201 (in Dutch). http://www.rivm.nl/Documenten_en_publicaties/Wetenschappelijk/Rapporten/2016/maart/Additieven_in_Nederlandse_tabaksproducten_Trendanalyse_gegevens_2010_2014.

Philip Morris. Citric acid. Tobacco Industry Documents: Philip Morris; Bates Number: 2028670128-0129, 1989. Available from: URL: http://tobaccodocuments.org/product_design/2028670128-0129.html.

Purkis S.W., Mueller C., Intorp M. (2011). The fate of ingredients in and impact on cigarette smoke. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 49, 3238-48.

Rabinoff M.D., Caskey N., Rissling A., Park C. (2007). Pharmacological and Chemical Effects of Cigarette Additives. *American Journal of Public Health* 97(11), 1981-91.

Rach J., Budde J., Möhle N., Aufderheide M. (2014). Direct exposure at the air-liquid interface: evaluation of an in vitro approach for simulating inhalation of airborne substances. *J Appl Toxicol.* 34(5), 506-15.

Rahnasto M., Raunio H., Poso A., Juvonen R.O. (2003). More potent inhibition of human CYP2A6 than mouse CYP2A5 enzyme activities by derivatives of phenylethylamine and benzaldehyde. *Xenobiotica* 33, 529-39.

Rahnasto M., Wittekindt C., Juvonen R.O., Turpeinen M., Petsalo A., Pelkonen O., Poso A., Stahl G., Holtje H.D., Raunio H. (2008). Identification of inhibitors of the nicotine metabolising CYP2A6 enzyme--an in silico approach. *Pharmacogenomics J* 8, 328-38.

Ray R., Ruparel K., Newberg A., Wileyto E.P., Loughhead J.W., Divgi C., Blendy J.A., Logan J., Zubieta J.K., Lerman C. (2011). Human Mu Opioid Receptor (OPRM1 A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers. *Proc Natl Acad Sci U S A* 108, 9268-73.

Reichl F.X., Schwenk M., (Herausgeber) (2004). *Regulatorische Toxikologie*. Springer Verlag, Heidelberg.

Renne R. (1992). 2-week and 13-week inhalation studies of aerosolized glycerol in rats, *Inhal Toxicol* 4, 95-111.

Richter P.A., Li A.P., Polzin G., Roy S.K. (2010). Cytotoxicity of eight cigarette smoke condensates in three test systems: comparisons between assays and condensates. *Regul Toxicol Pharmacol.* 58(3), 428-36.

Rodgman A., Perfetti T.A. (2013). *The Chemical Components of Tobacco and Tobacco Smoke*, Second Edition, 2013 by CRC Press Reference - 2332 Pages - 3091 B/W Illustrations ISBN 9781466515482 - CAT# K15040.

Rooney A.A., Boyles A.L., Wolfe M.S., Bucher J.R., Thayer K.A. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. *Environ. Health Perspect.* <http://dx.doi.org/10.1289/ehp.1307972>.

Rose J.E., Mukhin A.G., Lokitz S.J., Turkington T.G., Herskovic J., Behm F.M., Garg S., Garg P.K. (2010). Kinetics of brain nicotine accumulation in dependent and nondependent smokers assessed with PET and cigarettes containing ¹¹C-nicotine. *Proc Natl Acad Sci U S A* 107, 5190-5.

Rothen-Rutishauser B.M., Kiama S.G., Gehr, P. (2005). A three-dimensional cellular model of the human respiratory tract to study the interaction with particles. *Am J Respir Cell Mol Biol* 32, 28.

Rustemeier K., Stabbert R., Haussmann H.J., Roemer E., Carmines E.L. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2: chemical composition of mainstream smoke. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 40, 93-104.

Sabri O., Becker G.A., Meyer P.M., Hesse S., Wilke S., Graef S., Patt M., Luthardt J., Wagenknecht G., Hoepping A., Smits R., Franke A., Sattler B., Habermann B., Neuhaus P., Fischer S., Tiepolt S., Deuther-Conrad W., Barthel H., Schonknecht P., Brust P. (2015). First-in-human PET quantification study of cerebral $\alpha 4 \beta 2^*$ nicotinic acetylcholine receptors using the novel specific radioligand (-)-[(18)F]Flubatine. *Neuroimage* 118, 199-208.

SCENIHR (2010a). Addictiveness and attractiveness of tobacco additives. http://ec.europa.eu/health/scientific_committees/emerging/docs/scenih_r_o_029.pdf. accessed on 10/09/2015.

SCENIHR (2010b). Addictiveness and Attractiveness of Tobacco Additives. http://ec.europa.eu/health/scientific_committees/emerging/docs/scenih_r_o_031.pdf. accessed on .

SCENIHR (2012). Memorandum on the use of the scientific literature for human health risk assessment purposes – weighing of evidence and expression of uncertainty. http://ec.europa.eu/health/scientific_committees/emerging/docs/scenih_r_s_001.pdf.

SCHER, SSCS, SCENIHR (2012). Opinion on the Toxicity Assessment of Chemical Mixtures.

http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf.

Schmeltz I. Schlotzhauer W.S. (1968). Benzo[a]pyrene, phenols and other products from pyrolysis of the cigarette additive, (d,l)-Menthol. *Nature* 219, 370–1.

Scott D.J., Domino E.F., Heitzeg M.M., Koeppe R.A., Ni, L., Guthrie S., Zubieta J.K. (2007). Smoking modulation of mu-opioid and dopamine D2 receptor-mediated neurotransmission in humans. *Neuropsychopharmacology* 32, 450-7.

Scott D.J., Stohler C.S., Egnatuk C.M., Wang H., Koeppe R.A., Zubieta J.K. (2008). Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. *Arch Gen Psychiatry* 65, 220-31.

Shelke S.M., Bhosale S.H., Dash R.C., Suryawanshi M.R., Mahadik K.R. (2011). Exploration of new scaffolds as potential MAO-A inhibitors using pharmacophore and 3D-QSAR based in silico screening. *Bioorg Med Chem Lett* 21, 2419-24.

Small D.M. (2012). Flavor is in the brain. *Physiology & behavior* 107, 540-52.

Smith, S.S., Fiore, M.C., Baker, T.B. (2014). Smoking cessation in smokers who smoke menthol and non-menthol cigarettes. *Addiction*. 109(12), 2107-17.

Sokol N.A., Kennedy R.D., Connolly G.N. (2014). The role of cocoa as a cigarette additive: opportunities for product regulation. *Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco* 16, 984-91.

Staley J.K., Krishnan-Sarin S., Cosgrove K.P., Krantzler E., Frohlich E., Perry E., Dubin J.A., Estok K., Brenner E., Baldwin R.M., Tamagnan G.D., Seibyl J.P., Jatlow P., Picciotto M.R., London E.D., O'Malley S., van Dyck C.H. (2006). Human tobacco smokers in early abstinence have higher levels of beta2* nicotinic acetylcholine receptors than nonsmokers. *J Neurosci* 26, 8707-14.

Stein E.A., Pankiewicz J., Harsch H.H., Cho J.K., Fuller S.A., Hoffmann R.G., Hawkins M., Rao S.M., Bandettini P.A., Bloom A.S. (1998). Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. *Am J Psychiatry* 155, 1009-15.

Stotesbury S., Digard H., Willoughby L., Couch A. (1999). The pyrolysis of tobacco additives as a means of predicting their behaviour in a burning cigarette. *Beitr Tabakforsch Int* 18, 147-63.

Sullivan J.M., Kim S.J., Cosgrove K.P., Morris E.D. (2013). Limitations of SRTM, Logan graphical method, and equilibrium analysis for measuring transient dopamine release with [(11)C]raclopride PET. *Am J Nucl Med Mol Imaging* 3, 247-60.

Takahashi H., Fujimura Y., Hayashi M., Takano H., Kato M., Okubo Y., Kanno I., Ito H., Suhara T. (2008). Enhanced dopamine release by nicotine in cigarette smokers: a double-blind, randomized, placebo-controlled pilot study. *Int J Neuropsychopharmacol* 11, 413-7.

Talhout R., Schulz T., Florek E., van Benthem J., Wester P., Opperhuizen A. (2011). Hazardous Compounds in Tobacco Smoke. *Int J Environ Res Public Health*. 8(2), 613-28.

Talhout R., Opperhuizen A., van Amsterdam J.G. (2006). Sugars as tobacco ingredient: Effects on mainstream smoke composition. *Food Chem Toxicol* 44, 1789-98.

Talhout R., Opperhuizen A., van Amsterdam J.G. (2007). Role of acetaldehyde in tobacco smoke addiction. *Eur Neuropsychopharmacol* 17, 627-36.

Talhout R., van de Nobelen S., Kienhuis A.S. (2016). An inventory of methods suitable to assess additive-induced characterising flavours of tobacco products. *Drug Alcohol Depend* 161, 9-14.

Tapper A.R., McKinney S.L., Nashmi R., Schwarz J., Deshpande P., Labarca C., Whiteaker P., Marks M.J., Collins A.C., Lester H.A. (2004). Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science* 306, 1029-32.

Thielen A., Klus H., Lutz Müller L. (2008). Tobacco smoke: Unraveling a controversial subject. *Experimental and Toxicologic Pathology* 60 (2–3), 141–56.

Thorne D., Adamson J. (2013). A review of in vitro cigarette smoke exposure systems *Experimental and Toxicologic Pathology* 65, 1183 – 93.

Thorne D., Payne R., Haswell L., Dalrymple A., Meredith C., Dillon, D. (2014). Development of a BALB/c 3T3 Neutral Red Uptake cytotoxicity test using a mainstream cigarette smoke exposure system. *BMC Research Notes* 7, 367.

Thorne D., Dalrymple A., Dillon D., Duke M., Meredith, C. (2015). A comparative assessment of cigarette smoke aerosols using an in vitro air-liquid interface cytotoxicity test. *Inhal. Toxicol.* 27(12), 629-40.

Tice R.R., Austin C.P., Kavlock R.J., Bucher J.R. (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect.* 121, 756–65.

Tomar S.L., Henningfield J.E. (1997). Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco. *Tob Control* 6, 219-25.

Torikai K., Yoshida S., Takahashi H. (2004). Effects of temperature, atmosphere and pH on the generation of smoke compounds during tobacco pyrolysis. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association* 42, 1409-17.

Torikai K., Uwano Y., Nakamori T., Tarora W., Takahashi H. (2005). Study on tobacco components involved in the pyrolytic generation of selected smoke constituents. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association* 43, 559-68.

U.S. Dept. of Health and Human Services, F.a.D.A., Center for Drug Evaluation and Research (CDER). (2010). Guidance for Industry Assessment of Abuse Potential of Drugs. <http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm198650.pdf>. accessed on 18/12/2015.

U.S. Dept. of Health and Human Services, F.a.D.A., Center for Drug Evaluation and Research (CDER). (2015). Abuse-Deterrent Opioids — Evaluation and Labeling Guidance for Industry.

US EPA (2000, 2007): <https://www.epa.gov/risk/guidelines-health-risk-assessment-chemical-mixtures>.

Usmani O.S., Belvisi M.G., Patel H.J., Crispino N., Birrell M.A., Korbonits M., Korbonits D., Barnes P.J. (2005). Theobromine inhibits sensory nerve activation and cough. *Faseb j* 19, 231-3.

van Amsterdam J., Sleijffers A., van Spiegel P., Blom R., Witte M., van de Kassteele J., Blokland M., Steerenberg P., Opperhuizen A. (2011). Effect of ammonia in cigarette tobacco on nicotine absorption in human smokers. *Food Chem Toxicol* 49, 3025-30.

van de Nobelen S., Kienhuis A.S., Talhout R. (2016). An Inventory of Methods for the Assessment of Additive Increased Addictiveness of Tobacco Products. *Nicotine Tob Res.* 1–10.

Vilar S., Cozza G., Moro S. (2008). Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery. *Curr Top Med Chem* 8, 1555-72.

- Vilar S., Ferino G., Quezada E., Santana L., Friedman C. (2012). Predicting monoamine oxidase inhibitory activity through ligand-based models. *Curr Top Med Chem* 12, 2258-74.
- Villeneuve D.L., Garcia-Reyero N. (2011). Vision & strategy: predictive ecotoxicology in the 21st century. *Environ. Toxicol. Chem.* 30 (1), 1e8.
- Volkow N.D., Fowler J.S., Ding Y.S., Wang G.J., Gatley S.J. (1999). Imaging the neurochemistry of nicotine actions: studies with positron emission tomography. *Nicotine Tob Res* 1 Suppl 2, S127-132; discussion S139-140.
- Wackowski, O.A., Delnevo, C.D. (2016). Young Adults' Risk Perceptions of Various Tobacco Products Relative to Cigarettes: Results From the National Young Adult Health Survey. *Health Educ. Behav.* 43(3), 328-36.
- Wang H., Word B., Lyn-Cook L. Jr, Yang M., Hammons G., Lyn-Cook B. (2015). Cytotoxicity of chronic exposure to 4 cigarette smoke condensates in 2 cell lines. *Int J Toxicol.* 34(2), 182-94.
- Wang T., Wang B., Chen H. (2014). Menthol facilitates the intravenous self-administration of nicotine in rats. *Front Behav Neurosci* 8, 437.
- Wayne G., Connolly G. (2002). How cigarette design can affect youth initiation into smoking: Camel cigarettes 1983-93. *Tob Control* 11 (Suppl 1), i32-39.
- Wayne G.F., Henningfield J.E. (2008). Tobacco product attractiveness as a contributor to tobacco addiction and disease. Report to Health Canada, 1-37.
- Wayne G.F., Carpenter, C.M. (2009). Tobacco industry manipulation of nicotine dosing. *Handb Exp Pharmacol*, 457-85.
- Weber S., Hebestreit M., Wilms T., Conroy L.L., Rodrigo G. (2013). Comet assay and air-liquid interface exposure system: A new combination to evaluate genotoxic effects of cigarette whole smoke in human lung cell lines. *Toxicology in Vitro* 27, 1987-91.
- Weed D.L. (2005). Weight of evidence: a review of concept and methods. *Risk Anal.* 25(6), 1545-57.
- Weisensee D., Poth A., Roemer E., Conroy L.L., Schlage W.K. (2013). Cigarette smoke-induced morphological transformation of Bhas 42 cells in vitro. *Altern Lab Anim.* 41(2), 181-9.
- WHO (2007). The Scientific Basis of Tobacco Product Regulation. Geneva, Switzerland. pp. 1 -112.
- WHO OECD ILSI/HESI (2011). International Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals Workshop Report <http://www.oecd.org/env/ehs/testing/seriesontestingandassessmentworkshopreportsmeetingreports.htm>.
- WHO (2012). Partial guidelines for implementation of Articles 9 and 10. http://www.who.int/fctc/guidelines/adopted/article_9and10/en/. Accessed on 06/08/2015
- WHO Study Group on Tobacco Product Regulation (2012). Report on the Scientific Basis of Tobacco Product Regulation, WHO Technical Report Series, no. 967. http://www.who.int/tobacco/publications/prod_regulation/trs_967/en/

Wilkinson J.L., Bevins R.A. (2008). Intravenous nicotine conditions a place preference in rats using an unbiased design. *Pharmacol Biochem Behav* 88, 256-64.

Winkler D.A. (2002). The role of quantitative structure--activity relationships (QSAR) in biomolecular discovery. *Brief Bioinform* 3, 73-86.

Wong D.F., Kuwabara H., Kim J., Brasic J.R., Chamroonrat W., Gao Y., Valentine H., Willis W., Mathur A., McCaul M.E., Wand G., Gean E.G., Dannals R.F., Horti A.G. (2013). PET imaging of high-affinity alpha4beta2 nicotinic acetylcholine receptors in humans with 18F-AZAN, a radioligand with optimal brain kinetics. *J Nucl Med* 54, 1308-14.

Working Group on Tobacco Additives 2014. Report of the Working Group on Tobacco Additives.

http://portal.anvis.gov.br/wps/wcm/connect/5c3e698046be14978161fd2e64280806/Report_Working_Group_Tobacco_Additives_FINAL_26-08-14pdf?MOD=AJPERES.

Wullner U., Gundisch D., Herzog H., Minnerop M., Joe A., Warnecke M., Jessen F., Schutz C., Reinhardt M., Eschner W., Klockgether T., Schmaljohann J. (2008). Smoking upregulates alpha4beta2* nicotinic acetylcholine receptors in the human brain. *Neurosci Lett* 430, 34-7.

Yang G.Y., Zhang C.L., Liu X.C., Qian G., Deng, D.Q. (2013). Effects of cigarette smoke extracts on the growth and senescence of skin fibroblasts in vitro. *International Journal of Biological Sciences* 9, 613-23.

Yao J., Gao Q., Mi Q., Li X., Miao M., Cheng P., Luo Y. (2013). In vitro micronucleus assay for the analysis of total particulate matter in cigarette smoke: comparison of flow cytometry and laser scanning cytometry with microscopy. *Mutat Res.* 755(2), 120-5.

Yararbas G., Keser A., Kanit L., Pogun S. (2010). Nicotine-induced conditioned place preference in rats: sex differences and the role of mGluR5 receptors. *Neuropharmacology* 58, 374-82.

Yauk C.L., Williams A., Buick J.K., Chen G., Maertens R.M., Halappanavar S., White P.A. (2012). Genetic toxicology and toxicogenomic analysis of three cigarette smoke condensates in vitro reveals few differences among full-flavor, blonde, and light products. *Environmental & Molecular Mutagenesis* 53, 281-96.

Zellmer S., Schmidt-Heck W., Godoy P., Weng H., Meyer C., Lehmann T., *et al.* (2010). Transcription factors ETF, E2F, and SP-1 are involved in cytokine-independent proliferation of murine hepatocytes. *Hepatology* 52, 2127-36.

Zenzen V., Diekmann J., Gerstenberg B., Weber S., Wittke S., Schorp, M.K. (2012). Reduced exposure evaluation of an electrically heated cigarette smoking system. Part 2: Smoke chemistry and in vitro toxicological evaluation using smoking regimens reflecting human puffing behavior. *Regul Toxicol Pharm* 64, S11-S34.

Zhang L., Tsai K.C., Du L., Fang H., Li M., Xu W. (2011). How to generate reliable and predictive CoMFA models. *Curr Med Chem* 18, 923-30.

Zhou S., Xu Y., Wang C., Tian Z. (2011). Pyrolysis behavior of pectin under the conditions that simulate cigarette smoking. *Journal of Analytical and Applied Pyrolysis* 91, 232-40.

Zubieta J.K., Bueller J.A., Jackson L.R., Scott D.J., Xu Y., Koeppe R.A., Nichols T.E., Stohler C.S. (2005). Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *J Neurosci* 25, 7754-62.

Zuo Y., Mukhin A.G., Garg S., Nazih R., Behm F.M., Garg P.K., Rose J.E. (2015). Sex-Specific Effects of Cigarette Mentholation on Brain Nicotine Accumulation and Smoking Behavior. *Neuropsychopharmacology* 40(4), 884-92.

9 Annex I

GUIDANCE AND TEMPLATE

A. GENERAL ISSUES

This brief guidance intends to support the understanding of “what was done”. Authors should take responsibility to be clear of the definitions and provide proper citations for any terms/data they use.

1. TITLE PAGE

The title page should contain the following information:

Identification of the additive

Abstract and keywords, if applicable

Name of sponsor (and bodies that fund or commission the analysis)

Name and affiliation of person or persons responsible for producing and signing off the report

Date and version of report.

2. SUMMARY

The summary is intended to provide a concise description of the key elements.

3. REPORTING SOURCES OF INFORMATION

This section should describe any data source or sources that were used (e.g. existing data and/or databases, *in silico* techniques/models used, experimental studies).

This section addresses the key features of the design.

The rationale for the overall study design should be documented

If needed also ethical approval (approval number – approved by ...- date) for *in vivo* experiments (animals or humans) should be given.

4. REPORTING DATA QUALITY / DATA COLLECTION QUALITY ASSURANCE

This section addresses the reporting of the elements of data collection and pre-processing that could influence data quality.

- How was the literature search conducted and which quality controls were in place and used
- In own experimental studies, were quality controls in place:

In silico

In vitro

In vivo

5. PUBLIC SUMMARY OF THE DOSSIER

The target group of a public summary is the non-professional public. The structure and content of the public summary shall be elaborated accordingly. The document should be less extensive compared to the *summary*. A scientific/professional terminology shall be avoided if possible.

6. CD/DVD

The applicant shall submit a dossier with the full information on standard electronic media such as CD ROMs or DVDs. Two or three CD ROMs or DVDs shall be submitted.

Common electronic formats should be used (e.g. MS Office, Adobe Acrobat Reader) allowing content copying and printing (no content copy protection). The text of the files should be searchable using the search facilities of standard software packages. The CD or DVD shall be structured in folders that reflect the structure of the submission.

Also a full paper copy of the dossier is requested, it has to be declared by the applicant on a separate sheet or in the accompanying letter that the electronic and the paper versions are identical.

7. LIST OF PARTS OF THE DOSSIER REQUESTED TO BE TREATED AS CONFIDENTIAL

Applicants have the right to request a confidential treatment of certain information. They shall indicate which sections and data they wish to be treated as confidential (and give verifiable justification for each part for which a confidential treatment is required).

Furthermore, the applicant shall provide the Commission with two electronic versions of the dossier, namely the complete dossier and a second version of the complete dossier without confidential information.

B. SPECIFIC ISSUES – GENERAL LAYOUT

1. CHEMICAL AND PHYSICAL SPECIFICATIONS OF ADDITIVE

A. Chemical identity

Primary name and/or INCI name

Chemical names

Trade names and abbreviations

CAS / EC number

Structural formula

Empirical formula

B. Physical form

Molecular weight

Purity, composition and substance codes

Impurities / accompanying contaminants

Solubility

Partition coefficient (Log Pow)

Additional physical and chemical specifications

Where relevant:

- organoleptic properties (colour, odour, taste if relevant)

- vapour pressure (include also temperature used for the assessment)

- pKa

- ...

C. Function and use of the additive

2. (Each identified) PYROLYSIS PRODUCTS

Chemical identity

Molecular weight

% formed (at specific temperature)

Solubility

Partition coefficient (Log Pow)

Additional physical and chemical specifications

Where relevant:

- organoleptic properties (colour, odour, taste if relevant)

- vapour pressure (include also temperature used for the assessment)

- density

-- pKa

- ...

3. TOXICOLOGICAL EVALUATION

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original (own) study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report a study summary should be submitted, including, if applicable, the following (the summary reports should usually only be 1 page long):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one toxicological endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the Toxicology section, a brief general conclusion should be formulated.

4. ADDICTIVENESS ASSESSMENT

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original (own) study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report a study summary should be submitted, including, if applicable, the following (the summary reports should not usually exceed 1 page):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one addictiveness endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the section addictiveness assessments, a brief general conclusion should be formulated.

5. CHARACTERISING FLAVOUR AND INHALATION FACILITATION PROPERTIES ASSESSMENT

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report, a study summary should be submitted, including the following (the summary reports should usually only be 1 page in length):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one attractiveness endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the section attractiveness assessments a brief general conclusion should be formulated.

6. ASSESSMENT OF INTERACTION OF ADDITIVES WITH OTHER ADDITIVES/INGREDIENTS

For each study, regardless of whether it is an own study or data were assessed from literature, a full study report should be given:

- If data is derived from an original study, all original (rough) data should be submitted
- If data is derived from literature, the full paper/report should be submitted.

Next to the full report, a study summary should be submitted, including the following (the summary reports should usually only be 1 page):

Guideline:

GLP/quality control measure:

Test system: (in silico/in vitro/in vivo/human):

N° independent assessments – group size:

Test substance:

Batch:

Purity:

Vehicle:

Dose level:

Route of exposure:

Exposure duration:

Exposure duration & observation period:

Study date/period:

Specific methodological issues:

Brief summary of the results: (summary):

Brief conclusion:

If more studies were reported for one endpoint, a final conclusion should be formulated, taking into account the data of the different related studies.

Summary: Finally, at the end of the section, a brief general conclusion should be formulated on the Interactions with other additives/ingredients.

SUMMARY / OVERALL CONCLUSIONS

In the final section, a summary and an overall conclusion shall be formulated covering all issues discussed above (chemical and physical specifications, use, toxicity, addictiveness, attractiveness and interactions with other ingredients).

Evaluation of certain food additives

Eighty-sixth report of the Joint
FAO/WHO Expert Committee on
Food Additives



Food and Agriculture
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United Nations



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Organization



World Health
Organization

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Evaluation of certain food additives

Eighty-sixth report of the Joint
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does not necessarily represent the decisions or the stated policy of the World Health Organization*



**Food and Agriculture
Organization of the
United Nations**



**World Health
Organization**

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Geneva, 12–21 June 2018

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¹ Unable to attend the meeting.

List of abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AMC	anionic methacrylate copolymer
BMC	basic methacrylate copolymer
bw	body weight
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CCFA49	Forty-ninth Session of the Codex Committee on Contaminants in Food Additives
CIFOCos	FAO/WHO Chronic Individual Food Consumption Database – Summary statistics
CITREM	citric and fatty acid esters of glycerol
CXG	Codex Guideline
CXS	Codex Standard
EFSA	European Food Safety Authority
EHC 240	<i>Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food</i>
EINECS	European Inventory of Existing Commercial Chemical Substances
F	filial generation (F_0 , F_1 , F_2 , etc.)
FAO	Food and Agriculture Organization of the United Nations
GEWR	Glycerol ester of wood rosin
GLP	good laboratory practice
GRAS	Generally Recognized as Safe
GSFA	General Standard for Food Additives
HPLC	high-performance liquid chromatography
i.p. or IP	intraperitoneal OR intraperitoneal injection
IACM	International Association of Color Manufacturers
IARC	International Agency for Research on Cancer
IC ₅₀	median inhibitory concentration
INS	International Numbering System for Food Additives
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K_m	Michaelis constant (affinity)
LD ₅₀	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
LOQ	limit of quantification
M	male
mADI	microbiological acceptable daily intake

MSDI	maximized survey-derived intake
NCI	National Cancer Institute
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NMC	neutral methacrylate copolymer
no. / No.	number
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NR	not reported
NR	not required
NR	not relevant
NTP	National Toxicology Program
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
OECD	Organisation for Economic Co-operation and Development
<i>P</i>	probability
P90	90th percentile
P95	95th percentile
ppm	parts per million
SCE	sister chromatid exchange
SPET	single-portion exposure technique
TDI	tolerable daily intake
TG	test guideline
TSH	thyroid-stimulating hormone
USA	United States of America
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UV	ultraviolet
UVA	ultraviolet (radiation from about 320–400 nm in wavelength)
v/v	volume per volume
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WHO	World Health Organization

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 12 to 21 June 2018. The meeting was opened by Dr Kazuaki Miyagishima, Director of the Department of Food Safety and Zoonoses of the World Health Organization (WHO), on behalf of WHO Director-General Dr Tedros Adhanom Ghebreyesus. Dr Miyagishima reported that the 2018 World Health Assembly adopted the general programme of work for 2019–2023, and that Codex continues to be a high priority.

Dr Miyagishima welcomed all the delegates. In his opening remarks, he noted the long-standing service of a number of experts, which demonstrates their dedication to JECFA's goals. He also noted the number of new experts, along with the efforts to increase the geographical and gender balance of the Committee, exemplifying JECFA's commitment to knowledge transfer. This was considered particularly welcome as the list of food additives and flavourings awaiting evaluation continues to grow, a sign of the recognition of the quality and importance of JECFA's work.

Dr Miyagishima welcomed JECFA's constant drive to improve its methodology, pointing out that the new methodology for evaluating flavouring agents, agreed on 2 years ago, would be put into practice for the first time by the current Committee. He also informed the Committee that an agreement had been reached between WHO and the International Agency for Research on Cancer (IARC) on new interim standard operating procedures that would streamline the evaluation of compounds and give due recognition of JECFA's primary role in food risk assessments.

Dr Markus Lipp, FAO Joint Secretary, and Dr Angelika Tritscher, WHO Joint Secretary, also welcomed the delegates and thanked them for their hard work before the meeting and their continuing efforts.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the eighty-sixth meeting had completed declaration of interest forms. No conflicts of interest were identified. There were no responses to the public posting of the list of participants for this meeting.

1.2 Modification of the agenda

The agenda (see [Annex 4](#)) was modified to include sorbitol syrup in the list of specific food additives based on request of the CCFA to clarify if sorbitol syrup can be covered by the same acceptable daily intake (ADI) as sorbitol.

The agenda (see [Annex 4](#)) was modified to include Corrigenda for specifications monographs.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives (JECFA), held in September 1955 (1), there have been 85 previous meetings of the Committee ([Annex 1](#)). The present meeting was convened on the basis of a recommendation made at the eighty-fourth meeting ([Annex 1](#), reference 234).

The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives including flavouring agents ([section 3](#));
- review and prepare specifications for certain food additives ([sections 3 and 4](#) and [Annex 2](#)); and
- undertake safety evaluations of certain food additives ([sections 3 and 4](#) and [Annex 2](#)).

2.1 Report from the Fiftieth Session of the Codex Committee on Food Additives (CCFA)

Dr Yongxiang Fan, Chair of the CCFA, provided the Committee with an update on the work of CCFA since the eighty-fourth meeting of JECFA in 2017 ([Annex 1](#), reference 234).

The Fiftieth Session of CCFA (CCFA50) noted the conclusions of the eighty-fourth meeting of JECFA on the safety of nine food additives. CCFA50 agreed to include gum ghatti (International Numbering System for Food Additives [INS] 419) and tamarind seed polysaccharide (INS 437) in the Codex *General Standard for Food Additives* (CXS 192-1995) (GSFA) Table 3 (“Additives Permitted for Use in Food in General, Unless Otherwise Specified, in Accordance with Good Manufacturing Practice”). CCFA50 noted the conclusions for β -carotene-rich extract from *Dunaliella salina*, Brilliant Blue FCF (INS 133) and Fast Green FCF (INS 143). CCFA50 called on members to provide more data to JECFA to complete the evaluation for Jagua (Genipin–Glycine) Blue, metatartaric acid (INS 353), tannins (oenological tannins) and yeast extracts containing mannoproteins.

CCFA50 finalized work on over 320 provisions for food additives for inclusion in the General Standard on Food Additives (GSFA) and forwarded specifications for the identity and purity of 10 food additives (two new and eight revised specifications) prepared by the eighty-fourth meeting of JECFA for adoption by the forty-first session of the Codex Alimentarius Commission (CAC).

CCFA50 agreed on a revised Priority List of Substances for Evaluation (or re-evaluation) by JECFA, which currently includes 46 food additives and eight flavourings. CCFA50 agreed to amend the circular letter on the Priority List in order to expedite the process of confirming requests and to provide a mechanism for members to confirm the requests without having to attend the in-session working group meetings. CCFA50 also agreed to change the Priority List table by introducing a summary of information about the request such as its basis and possible trade issues.

CCFA50 established an electronic working group that will develop an inventory of data available on nitrates and nitrites with a view to consulting with JECFA and CCFA regarding next steps on the use of these substances,

The Fifty-first Session of CCFA (CCFA51), to be held on 25–29 March 2019 in China, will continue its work on the food additives provisions for inclusion in the GSFA, including further alignments of food additive provisions in the Codex commodity standards with the corresponding provisions of the GSFA.

2.2 Update on activities relevant to JECFA

2.2.1 Update of Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food (EHC 240)

The Secretariat informed the Committee about ongoing activities on risk assessment methodology and update of certain chapters of EHC 240. In particular, more detailed guidance on the interpretation and evaluation of genotoxicity of compounds in food, including the interpretation of results of genotoxicity tests, is under development. In addition, Chapter 5 on dose-response assessment, including benchmark dose modelling, and the derivation of health-based guidance values is being updated, as is Chapter 6 on dietary exposure assessment. Guidance for the evaluation of enzyme preparations will also be updated. Final drafts are expected in 2019, following wide stakeholder input including public calls for comments.

2.3 Food additive specifications and analytical methods

2.3.1 Corrigenda for specifications monographs

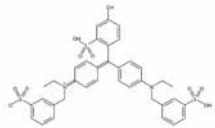
The following requests for corrections, reported to the JECFA Secretariat, were evaluated by the eighty-sixth Committee and found to be necessary (see [Table 1](#)). These corrections, however, will only be made in the online database for specifications.

Table 1

Corrections in JECFA reports and monographs on food additives^a

Food additive	Original text	New text	Additional information
Calcium disodium ethylenediaminetetraacetate (INS 385) Monograph 1 (2006)	CAS No. 662-33-9	CAS No. 62-33-9	Transcription error
Chlorophyllins, copper complexes sodium and potassium salts (INS 141(ii)) Monograph 5 (2008) Test for "Free ionisable copper"	Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil. ...	Accurately weigh about 1 g of the sample and mix in 20 mL of arachid oil. ...	Correction
Curcumin (INS 100(ii)) Monograph 1 (2006)	The criteria for several residual solvents are listed under the heading "Residual solvents" (see Fig. 1).	Acetone: Not more than 30 mg/kg Hexane: Not more than 25 mg/kg Methanol: Not more than 50 mg/kg Ethanol: Not more than 50 mg/kg Isopropanol: Not more than 50 mg/kg Ethyl acetate: Not more than 50 mg/kg	Improves readability It was unclear whether the criterion "Not more than 50 mg/kg" extended to methanol, ethanol, isopropanol and ethyl acetate.
Ethyl acetoacetate ethyleneglycol ketal JECFA No.: 1969 JECFA 73 (2010)	CAS No. 1648615	CAS No. 6413-10-1	Transcription error
Ethyl 2-methyl pentanoate JECFA No.: 214 JECFA 55 (2000)	CAS No. 28959-02-6	CAS No. 39255-32-8	Wrong CAS number
<i>cis</i> -3-Hexen-1-ol JECFA No.: 315 JECFA 51 (1998)	98.0% (sum of (Z) and (E) isomers, =<92.0% (Z))	98.0% (sum of (Z) and (E) isomers, =>92.0% (Z))	Transcription error
Monosodium <i>L</i> -glutamate (INS 621) Monograph 1 (2006)	CAS No. 142-47-2	CAS No. 6106-04-3	Wrong CAS number
Myrcene JECFA No.: 1327 JECFA 63 (2004)	Specific gravity: 0.789–1.793	Specific gravity: 0.789–0.793	Transcription error
Polyoxyethylene (20) sorbitan monostearat (Polysorbate 60) (INS 435) Monograph 16 (2014)	CAS No. 9005-07-6	CAS No. 9005-67-8	Wrong CAS number
Sodium aluminium silicate (INS 554) Monograph 20 (2017)	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on dried basis".	Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed "on ignited basis".	Transcription error
Silicon dioxide, amorphous (INS 551)	CAS No. 112696-00-8 (hydrated silica)	CAS No. 112926-00-8 (hydrated silica)	Transcription error

Table 1 (continued)

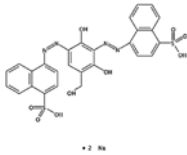
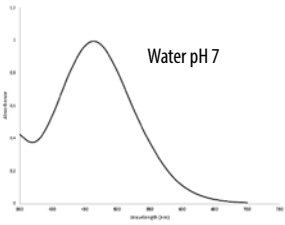
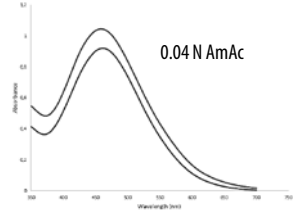
Food additive	Original text	New text	Additional information
Monograph 20 (2017)	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water.	Pyrogenic silica is produced in an essentially anhydrous state, whereas the wet process products are obtained as hydrates or contain surface adsorbed water.	Transcription error
Sodium thiosulfate (INS 539) Monograph 1 (2006)	CAS No. 7772-98-7	CAS No. 10102-17-7	CAS No. 7772-98-7 refers to the anhydrous form. The specifications in the monograph refer to the pentahydrate form.
Brown HT and its aluminium lake (FAO JECFA Monographs 19, 82nd meeting, 2016)	Text in Table 1: “Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry”	See Table 1, below	
Fast Green FCF (FAO JECFA Monographs 19, 82nd meeting, 2016)	Chemical structure in Table 1: “Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry”		

CAS: Chemical Abstracts Service; INS: International Numbering System for Food Additives; JECFA: Joint FAO/WHO Expert Committee on Food Additives; No.: number
* Bolding and underlining for clarity only. This formatting will not be shown in the online database.

Fig. 1
Residual solvent criteria for curcumin as displayed in Monograph 1, 2006

Residual solvents (Vol. 4)	Acetone:	Not more than 30 mg/kg
	Hexane:	Not more than 25 mg/kg
	Methanol:	}
	Ethanol:	Not more than 50 mg/kg
	Isopropanol:	
	Ethyl acetate:	

Table 2
Replacement of the text for the spectrophotometric data for Brown HT and its aluminium lake originally published in “Table 1. Values for synthetic colours for use in performing tests for Colouring Matters Content by Spectrophotometry” (FAO JECFA Monographs 19, 82nd meeting, 2016)

JECFA name	Sample weight	Structure	Spectral data	Visible absorption spectrum
Brown HT	245.6 mg		Water, pH 7 λ_{max} = 464 $A = 0.9957$ Spec abs = 403 $a = 40.3$ Water λ_{max} = 464 $A = 0.9804$ Spec abs = 397 $a = 39.7$ 0.04 N AmAc λ_{max} = 461 $A = 0.9206$ Spec abs = 373 $a = 37.3$	
Brown HT Aluminium Lake	53.3 mg	—	Straight colour (blue) 0.04 N AmAc λ_{max} = 461 $A = 0.9206$ Lake (red) 0.04 N AmAc λ_{max} = 458 $A = 1.0451$	

3. Specific food additives (other than flavouring agents)

3.1 Toxicological evaluation, exposure assessment and establishment of specifications

3.1.1 Anionic methacrylate copolymer

Explanation

Anionic methacrylate copolymer (AMC; E 1207; International Numbering System Number [INS No.] 1207; Chemical Abstracts Service [CAS] No. 26936-24-3; acrylates copolymers; methyl acrylate, methyl methacrylate, methacrylic acid polymer; methacrylic acid polymer with methyl acrylate and methyl methacrylate) is a copolymer manufactured from the monomers methacrylic acid, methyl methacrylate and methyl acrylate in the molar ratio of 7:3:1.

AMC has been evaluated by the European Food Safety Authority (EFSA) and is approved for use as a food additive in the European Union. Its use in the European Union is restricted to a maximum level of 100 000 mg/kg in solid food supplements, category 17.1 [1].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. AMC is soluble above pH 7 and is used for its taste- and odour-masking properties; as protection from heat, light, moisture and oxidation; and to prevent the fast release of active ingredients once they leave the stomach.

AMC has not previously been evaluated by the Committee. The Committee evaluated the use of AMC as a coating or glazing agent for solid food supplements and foods for special medical purposes when provided in the form of solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 10%, at the request of the Forty-ninth Session of the CCFA (CCFA49 [2]). AMC is also used in pharmaceuticals.

Toxicological data submitted for the evaluation included distribution studies, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the monomers but no additional studies on AMC. The Committee considered the data on the residual monomers, as well as that for AMC itself, because the monomers are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. Due to the low levels of residual monomers present in AMC, the Committee evaluated only absorption, distribution, metabolism and excretion (ADME) data and long-term toxicity and genotoxicity data on the monomers.

The Committee was also aware that AMC contains an oligomer fraction of up to 2%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 1000 Da, with around 75% of the oligomer fraction having a molecular weight between 5000 and 10 000 Da, it is unlikely that the oligomers would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the distribution and toxicity studies was prepared from an aqueous dispersion with 1.5% emulsifier and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of AMC.

The Committee evaluated toxicological and exposure data on sodium lauryl sulfate, polysorbate 80 and simethicone, residual components of AMC that can be present in the final product because they are used in the manufacture of the copolymer. The Committee concluded that these residual components did not pose a safety concern at the maximum estimated exposure levels.

Chemical and technical considerations

AMC is manufactured by emulsion polymerization of the monomers methacrylic acid, methyl methacrylate and methyl acrylate with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low molecular weight substances and coagulum.

AMC has a weight-average molecular weight of 280 000 Da and a number-average molecular weight of 77 000 Da.

Although organic solvents are not used in the manufacture of AMC, methanol may be present at a level not exceeding 1000 mg/kg as a result of hydrolysis of esterified carboxyl groups incorporated in the polymer. The copolymer is standardized as a 30% aqueous dispersion with sodium lauryl sulfate (0.3%) and polysorbate 80 (1.2%). Simethicone emulsion is used as an antifoaming agent during the manufacture and is present in the dispersion at not more than 20 mg/kg. The copolymer dispersion may contain residual monomers: methyl acrylate (not more than 1 mg/kg); methyl methacrylate (not more than 3 mg/kg); and methacrylic acid (not more than 1 mg/kg).

Biochemical aspects

AMC

Radiolabelled AMC administered to rats was excreted in the faeces with mean recovery amounting to 92.38% of the total dose after 72 hours and 94.07% ($\pm 3.42\%$) of the total dose 10 days after dosing. Radioactivity was detected at very low levels in the urine of four of the eight treated animals in this group; this may have been due to contamination with faecal matter [3].

Residual monomers

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration in rats. On the basis of available data, methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [4, 5, 6].

Methyl acrylate is rapidly absorbed. About half the dose is exhaled as carbon dioxide, while the rest is excreted in the urine in the form of cysteine conjugates and other thioethers [7, 8].

Methacrylic acid is rapidly absorbed after oral or inhalational exposure. After a single oral administration of the sodium salt of methacrylic acid to rats, the maximum concentration in blood was found after 10 minutes. After 60 minutes, no more methacrylic acid was detectable [9].

Toxicological studies

AMC

In a good laboratory practice (GLP)-compliant study, a single dose of 2000 mg/kg body weight (bw) of AMC was administered by gavage to rats. Following a 14-day recovery period, there were no abnormal clinical observations, no deaths and no statistically significant differences in body weights between the test animals and historical controls. No abnormalities were observed at necropsy [10].

When gavage doses of 0, 200, 500 or 1500 mg/kg bw per day AMC were administered to rats for 4 weeks, no treatment-related effects were observed. The no-observed-adverse-effect level (NOAEL) was 1500 mg/kg bw per day, the highest dose tested [11]. In a GLP-compliant 26-week oral toxicity study in rats using gavage doses of 0, 200, 500 or 1500 mg/kg bw per day, there were also no treatment-related effects. Some statistically significant findings were observed, but these did not exhibit a dose-response relationship. The NOAEL was 1500 mg/kg bw per day, the highest dose tested [12]. In a 4-week dog study, AMC was administered as copolymer-coated cellulose pellets at doses of the test substance at 0, 100, 200 or 400 mg/kg bw per day. There were no treatment-related effects. The NOAEL was 400 mg/kg bw per day, the highest dose tested [13].

Two reverse mutation assays in bacteria, a mouse lymphoma assay and a chromosomal aberration assay in vitro and an in vivo mouse micronucleus assay all gave negative results. The Committee concluded that AMC was not of concern for genotoxicity.

No long-term toxicity or carcinogenicity studies were available on AMC.

In a GLP-compliant developmental toxicity study, female rats administered AMC at 0 or 1000 mg/kg bw per day by gavage on gestation days 5–19 showed no treatment-related effects. The NOAEL was 1000 mg/kg bw per day, the only dose tested [14].

Studies on cytotoxicity, dermal toxicity, inhalation toxicity, ocular toxicity and phototoxicity produced no effects.

Residual monomers

Methyl methacrylate

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight increased in females at the highest dose but no treatment-related histopathological effects were observed in any organs or tissues [15]. Based on the results of this study, a tolerable daily intake (TDI) of 1.2 mg/kg bw per day was determined [16].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [15].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and sister chromatid exchange (SCE) assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days of exposure. These results were judged by the Committee to be inconclusive.

The Committee concluded that there was some evidence of mutagenicity and clastogenicity in vitro but that there was a lack of adequate in vivo tests following up the equivocal in vitro findings.

Methyl acrylate

In a 2-year inhalation study, groups of rats ($n = 86/\text{sex}$) were exposed to methyl acrylate by inhalation at 0, 15, 45 or 135 parts per million (ppm; 0, 53, 158 and 475 mg/m³) in air for 6 hours per day on 5 days per week for 2 years. No significant difference in mortality was observed between the groups. The incidence of soft-tissue sarcomas varied considerably between the groups but there was no dose-dependence. No increased frequency of any tumour type in any organ could be related to a carcinogenic effect of the test substance [17].

There are no epidemiological data in humans. The IARC [18] concluded that there was inadequate evidence in experimental animals for carcinogenicity and that methyl acrylate was not classifiable as to its carcinogenicity in humans.

The Committee evaluated the genotoxicity studies on methyl acrylate. Reverse mutation studies in bacteria were negative. A well-performed genotoxicity study on L5178Y mouse lymphoma cells, without exogenous metabolism, produced positive outcomes for gene mutation and chromosomal aberrations but only at cytotoxic concentrations. The in vivo study designs have methodological shortcomings. A mouse bone marrow micronucleus assay using intraperitoneal dosing showed an increase in micronuclei induction, but this was limited in magnitude and not dose related. A mouse bone marrow micronucleus assay using oral dosing was negative, but it is not clear if the target tissues were exposed to the test substance. Overall, the Committee concluded that the data were not sufficient to draw conclusions on the genotoxicity of methyl acrylate.

Methacrylic acid

There were no long-term chronic toxicity/carcinogenicity studies on methacrylic acid. The Committee noted that studies on methyl methacrylate, which is metabolized into methacrylic acid, found no carcinogenicity in mice, rats or hamsters [16].

There are several in vitro genotoxicity studies on methacrylic acid, but the most useful data were from negative studies on gene mutations in bacteria. Positive results were obtained in two comet assays and a γ -H2AX assay in human gingival fibroblasts. There were no studies on chromosomal aberrations or gene mutation in mammalian cells and no in vivo studies on methacrylic acid. The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid.

Observations in humans

No human data were available on AMC.

Assessment of dietary exposure

The Committee evaluated exposure to AMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes. As another major use of AMC is in pharmaceuticals, this use was also evaluated in the exposure assessment. The level of use of AMC is a maximum of 10%.

The Committee evaluated exposure to AMC and its residual monomers, methacrylic acid, methyl methacrylate and methyl acrylate. As the Committee evaluated the toxicology of methacrylic acid, a total exposure to this monomer was estimated from the sum of the methacrylic acid monomer exposure and the methacrylic acid product of methyl methacrylate metabolism.

The exposure assessment included estimates provided by the sponsor and an evaluation by EFSA [1] based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. The national consumption data were from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOcOss) and data from Australia and New Zealand submitted to the Committee. A comprehensive literature search was also conducted; no additional studies were identified.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals, given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are not relevant for the general healthy population. This use was not further evaluated by the Committee.

The total monomeric content of AMC is less than 0.01%. This level was used to calculate the exposure to individual monomers based on the ratio of each monomer in the copolymer. The total exposure to methacrylic acid is from the sum of the exposure to methacrylic acid monomers and methacrylic acid from methyl methacrylate with a conversion using molecular weights (Table 3).

The Committee noted that AMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA ranged between 8.0 and 13.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

Evaluation

There were no concerns for the toxicity of AMC itself. However, the presence in AMC of the residual monomer methyl acrylate, for which it is not possible to conclude on genotoxic potential, and the insufficient carcinogenicity data for methyl acrylate preclude establishing an ADI for AMC.

The available toxicology data for AMC itself do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for AMC range from 400 to 1500 mg/kg bw per day, the highest doses tested. Estimated exposures to AMC range from 2.9 to 43 mg/kg bw per day.

Toxicological data on the residual monomers, apart from the genotoxicity data, do not give rise to concerns when taking into account the low

Table 3
Summary of range of estimated exposures to AMC, its monomers and total methacrylic acid from uses in food supplements for average and high exposures

Population group	Range of estimated dietary exposures ^{a,b}				Total methacrylic acid exposure (µg/kg bw per day) ^c
	AMC (mg/kg bw per day)	Methacrylic acid (µg/kg bw per day)	Methyl methacrylate (µg/kg bw per day)	Methyl acrylate (µg/kg bw per day)	
Adults	3.5–37	0.04–0.4	0.1–0.9	0.2–2.4	0.1–1.2
Children	2.9–43	0.03–0.4	0.1–1.1	0.2–2.8	0.1–1.4

AMC: anionic methacrylate copolymer; bw; body weight
^a All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated average exposures, and the upper end of the range is the highest of the estimated high exposures.
^b Includes exposures estimates submitted by the sponsor and the European Food Safety Authority [1], and national estimates calculated by the Committee based on a concentration of 100 mg per 1 g dosage unit.
^c The total methacrylic acid exposure is the sum of the exposure to methacrylic acid monomers and methacrylic acid from methyl methacrylate, with a conversion using molecular weights.

exposures. Genotoxicity data for methyl methacrylate suggest a potential risk for mutagenicity and clastogenicity in vitro, and there is a lack of adequate data on genotoxicity in vivo. However, in carcinogenicity studies in mice, rats and hamsters with methyl methacrylate given by inhalation, there was no evidence of any carcinogenic effects. In a 2-year study in rats given methyl methacrylate in drinking-water, the NOAEL was 121 mg/kg bw per day; from this NOAEL, a TDI of 1.2 mg/kg bw was derived [16]. Estimated exposures to methyl methacrylate range from 0.1 to 1.1 µg/kg bw per day, which are below the TDI.

Data on methyl acrylate are limited. ADME studies suggest that methyl acrylate is rapidly absorbed and excreted. The genotoxicity data are insufficiently adequate to draw conclusions on the genotoxic potential of methyl acrylate. Although a rat carcinogenicity study on methyl acrylate by the inhalation route was negative, no suitable long-term oral toxicity studies were available to support the safety of methyl acrylate. The Committee was unable to conclude on the safety of methyl acrylate as a residual monomer in AMC. Estimated exposures to methyl acrylate range from 0.2 to 2.8 µg/kg bw per day.

There were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid, and no long-term carcinogenicity studies were available. The Committee noted that there was evidence that methyl methacrylate is metabolized to methacrylic acid, and therefore the four negative long-term toxicity oral (via drinking-water) and inhalation carcinogenicity studies on methyl methacrylate could be used to support the safety of methacrylic acid. The Committee concluded that the exposure to methacrylic acid from the sum of the levels present in AMC and as a metabolite of methyl methacrylate, ranging from 0.1 to 1.4 µg/kg bw per day, would be unlikely to be a health concern.

The Committee was unable to complete the evaluation of AMC. While the copolymer itself is not a health concern, genotoxicity concerns remain for the residual monomer methyl acrylate.

A toxicological and dietary exposure monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared. The specifications were made tentative pending the completion of the safety evaluation of AMC.

References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS): Scientific Opinion of the safety of anionic methacrylate copolymer for the proposed uses as a food additive. *EFSA J.* 2010;8(7):1656 [25 pp.] doi:10.2903/j.efsa.2010.1656.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Absorption, distribution and excretion study in the rat following a 13-day administration period. Huntingdon Life Sciences, Eye, Suffolk, England, United Kingdom; 1999. Unpublished study report no. 010/984481 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
4. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer.* 1977;36(1):114–9 [cited in reference 16].
5. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
6. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995 (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
7. Vodicka P, Gut I, Frantík E. Effects of inhaled acrylic acid derivatives in rats. *Toxicology.* 1990;65(1–2):209–21.
8. Sapota A. The disposition and metabolism of methyl acrylate in male Wistar albino rats. *Pol J Occup Med Environ Health.* 1993;6(2):185–93.
9. Bereznowski Z, Mariewski M, Somolenski RT. High performance liquid chromatographic determination of methacrylate in blood serum. *Biomed Chromatogr.* 1994;8(1):42–4 [cited in European Commission, 2002].
10. Acute oral toxicity study in the rat. Research Toxicology Centre, Rome, Italy; 1995. Unpublished study report no. 5090/T/211/95 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Four-week oral toxicity study in rats followed by a 2-week recovery period. Research Toxicology Centre, Rome, Italy; 1998. Unpublished study report no. 861/T/215/98 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
12. Twenty-six week oral toxicity study in rats followed by a 4-week recovery period. Unpublished study by Research Toxicology Centre, Rome, Italy; 2000. Report no. 7250/T/346/2000 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

13. Four-week oral toxicity study in dogs. Unpublished study by Research Toxicology Centre, Rome, Italy; 2004. Report no. 8101 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. Prenatal developmental toxicity study in rats. Unpublished study by Harlan Bioservice for Science, Walsrode, Germany; 2003. Study report no. 20-4-0144-02 submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. Borzelleca JF, Larson PS, Hennigar GR, Hluf EG, Crawford EM, Blackwell Smith R. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 16].
16. Methyl methacrylate. Geneva: World Health Organization; 1998 (International Programme on Chemical Safety II. Concise international chemical assessment document 4).
17. Reininghaus W, Koestner A, Klimisch HJ. Chronic toxicity and oncogenicity of inhaled methyl acrylate and *n*-butyl acrylate in Sprague-Dawley rats. *Food Chem Toxicol.* 1991;29(5):329–39.
18. Reevaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 71. Lyon: International Agency for Research on Cancer; 1999.

3.1.2 Basic methacrylate copolymer

Explanation

Basic methacrylate copolymer (BMC; E 1205; INS No. 1205; CAS No. 24938-16-7; basic butylated methacrylate copolymer; amino methacrylate copolymer; aminoalkyl methacrylate copolymer E; butyl methacrylate, dimethylaminoethyl methacrylate, methyl methacrylate polymer; butyl methacrylate, methyl methacrylate, dimethylaminoethyl methacrylate copolymer) is a cationic copolymer manufactured using the monomers dimethylaminoethyl methacrylate [2-(dimethylamino)ethyl methacrylate], butyl methacrylate [*n*-butyl methacrylate] and methyl methacrylate in the molar ratio of 1:2:1.

BMC has been evaluated by EFSA and is an approved food additive within the European Union where its use is restricted to a maximum level of 100 000 mg/kg in solid food supplements, category 17.1 [1].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. BMC is soluble below pH 5 and is used for its taste- and odour-masking properties; as protection from heat, light, moisture and oxidation; and to prevent the fast release of active ingredients in the stomach.

BMC has not previously been evaluated by the Committee. The Committee evaluated the use of BMC as a coating or glazing agent for solid food supplements and foods for special medical purposes in the form of solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 10%, at the request of CCFA49 [2]. Another proposed use of BMC is for microencapsulation, which enhances the stability of micronutrients in food

fortification, specifically for populations with nutrient deficiencies. BMC is also used in pharmaceuticals.

Toxicological data submitted for the evaluation included ADME, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the monomers but no additional studies on BMC. The Committee considered the data on the residual monomers because these are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. As exposure to monomers from BMC is higher than to AMC and neutral methacrylate copolymer (NMC), the Committee evaluated ADME, short- and long-term toxicity studies, and genotoxicity and reproductive and developmental toxicity data on the monomers.

The Committee was also aware that BMC contains an oligomer fraction of up to 10%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 1000 Da, and around two thirds of the oligomer fraction has a molecular weight of between 4000 and 5000 Da, it is unlikely that the oligomers would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the distribution and toxicity studies was prepared from an aqueous dispersion and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of BMC.

The Committee evaluated toxicological and exposure data on 2-propanol, butanol and methanol, the residual components of BMC that can be present in the final products because they are used in the manufacture of the copolymer. The Committee concluded that these residual components do not pose a safety concern at the maximum estimated exposure levels.

Chemical and technical considerations

BMC is manufactured by a controlled polymerization process using a free radical donor initiator system. After completion of polymerization, the viscous copolymer solution is fed into an extruder to remove solvents and volatile substances, by continuous degassing through vacuum and heating. The solid granules of BMC formed in the extruder can be milled to a powder in which not more than 10% of the particles have a diameter less than 3 µm.

BMC has a weight-average molecular weight of 47 000 Da and number-average molecular weight of 22 000 Da.

During the manufacture of BMC, 2-propanol is added as a production aid. Most of the solvent evaporates during the polymerization and extrusion steps in the manufacture of BMC. The methyl methacrylate integrated in the polymer chain may hydrolyse to methacrylic acid and methanol. The methacrylic acid remains in the polymer chain but methanol is released. Similarly, butyl

methacrylate integrated in the polymer chain may hydrolyse to methacrylate and butanol. BMC may contain 2-propanol (not exceeding 0.5%); butanol (not exceeding 0.5%); and methanol (not exceeding 0.1%). The copolymer may also contain residual monomers: dimethylaminoethyl methacrylate (not exceeding 500 mg/kg); butyl methacrylate (not exceeding 200 mg/kg); and methyl methacrylate (not exceeding 50 mg/kg).

Biochemical aspects

BMC

When radiolabelled BMC (purity >98%) was administered to adult rats in two separate studies, the majority was excreted in the faeces within 2–3 days of administration. Levels of radioactivity in the gut returned to normal within 7 days. From the levels of radioactivity in the urine, it can be concluded that less than 0.02% of the radioactivity was absorbed [3].

Residual monomers

There were no toxicokinetic studies on 2-(dimethylamino)ethyl methacrylate. Studies using simulated saliva or intestinal fluids show that this residual monomer is rapidly hydrolysed with 86–90% degradation [4].

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration to rats. Methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [5, 6, 7].

Like methyl methacrylate, *n*-butyl methacrylate, is rapidly metabolized by carboxylesterases. Hydrolysis of *n*-butyl methacrylate yields methacrylic acid and *n*-butanol, which are further metabolized, with methacryl CoA, a physiological substrate of the valine pathway [8].

Toxicological studies

BMC

In acute toxicity studies in mice, no effects were observed after oral, intraperitoneal and subcutaneous administration of BMC (purity >98%) at 3.0, 2.0 and 1.0 g/kg bw, respectively, although deaths occurred at higher doses via the intraperitoneal and oral routes. In rats, the oral median lethal dose (LD₅₀) was greater than 15 g/kg bw. Orally administered lactose granules (125 g) coated in test material (BMC at 5.9 g/kg bw) resulted in deaths in rats, but this mortality was attributed to the amount of the administered dose. When doses of BMC of up to 6 g/kg bw were administered to rats in feed, no adverse effects were observed [9, 10, 11].

Short-term toxicity studies were available in rats and dogs. A 6-month study in rats administered BMC (purity >98%) in feed at 500 or 2000 mg/kg bw per day showed no treatment-related effects [12]. A 28-day study in dogs administered BMC (>98% BMC) in gelatin capsules showed no treatment-related effects at doses of 100–750 mg/kg bw per day [13].

No long-term toxicity or carcinogenicity studies were available on the copolymer itself.

A reverse mutation assay in bacteria, a gene mutation assay in mammalian cells and an in vivo mouse micronucleus assay all produced negative results. The Committee concluded that BMC was not of concern for genotoxicity.

A developmental study in rats showed no treatment-related effects at the only dose tested, 1000 mg/kg bw per day, when administered in feed between gestation days 6 and 16 [14].

Studies of cytotoxicity, dermal toxicity, ocular toxicity and phototoxicity with BMC showed no effects.

Residual monomers

2-(Dimethylamino)ethyl methacrylate

A short-term toxicity study with 2-(dimethylamino)ethyl methacrylate in rats given gavage doses of 0, 100, 200 or 500 mg/kg bw per day for 13 weeks showed no treatment-related adverse effects. The NOAEL was 500 mg/kg bw per day, the highest dose tested [15].

No long-term toxicity studies were available on 2-(dimethylamino)ethyl methacrylate. In genotoxicity studies, 2-(dimethylamino)ethyl methacrylate was negative in two reverse mutation assays in bacteria, apart from one isolated positive response in *Salmonella typhimurium* TA1537 in the absence of metabolic activation (negative in the presence of metabolic activation), and negative in a gene mutation assay in mammalian cells in vitro. The monomer was positive for clastogenicity in two chromosomal aberration tests in mammalian cells in vitro, but negative in a GLP-compliant in vivo mouse erythrocyte micronucleus test conducted according to Organisation for Economic Co-operation and Development (OECD) guideline 474. The Committee concluded that 2-(dimethylamino)ethyl methacrylate does not raise concerns for genotoxicity in vivo.

In a reproductive toxicity study with 2-(dimethylamino)ethyl methacrylate, rats received gavage doses of 0, 40, 200 and 1000 mg/kg per day from 14 days before mating, for 43 days in males and until lactation day 3 in females. There were no effects on reproductive parameters. At the highest dose, maternal toxicity was observed and there were some deaths among dams, with total loss of litters in some dams during the lactation period, reduced pup body weight and reduced pup viability. The NOAEL was 200 mg/kg bw per day [16].

In a developmental toxicity study, 2-(dimethylamino)ethyl methacrylate was administered to female rats by gavage at dose levels of 0, 100, 300 or 600 mg/kg bw per day from gestation days 6 to 19. There were no treatment-related effects on maternal or embryo/fetal parameters. The NOAEL was 600 mg/kg bw per day, the highest dose tested [17]

Methyl methacrylate

In most of the numerous studies on the short-term toxicity of methyl methacrylate in mice, rats, hamsters and dogs, the monomer was administered by inhalation. Most commonly observed effects were decreases in body-weight gain and irritation of the skin, nasal cavity and eye at high concentrations (generally 500 ppm [2050 mg/m³]). At even higher concentrations, renal cortical necrosis and tubular degeneration (in rats and mice) and hepatic necrosis (in mice) were also reported [18].

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight in females increased at the highest dose, but no treatment-related histopathological effects were observed in any organ or tissue [19]. Based on the results of this study, a TDI of 1.2 mg/kg bw per day was determined [18].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [19].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and SCE assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days of exposure. These results were judged by the Committee to be inconclusive. The Committee concluded that there was some evidence of mutagenicity and clastogenicity in vitro. There was a lack of adequate in vivo tests following up the equivocal findings.

There were no conventional reproductive toxicity studies on methyl methacrylate. A dominant lethal study in mice given 100, 1000 or 9000 ppm (410, 4100 and 36 900 mg/m³, respectively) of methyl methacrylate by inhalation for 6 hours/day for 5 days showed no effects on fertility [20].

In older studies on mice and rats, developmental effects including decreases in fetal weight and increases in embryo/fetal death and skeletal abnormalities were observed following inhalation of methyl methacrylate at concentrations that were toxic to the dams [21, 22].

In another older developmental toxicity study, pregnant mice were exposed to 1330 ppm (5450 mg/m³) of methyl methacrylate for 2 hours, twice daily, during gestation days 6–15. There were no adverse developmental effects [23].

In a developmental toxicity study, rats were given methyl methacrylate by inhalation at concentrations from 9 to 2028 ppm (406–8315 mg/m³) for 2 hours daily from gestation days 6 to 15. There were no treatment-related adverse effects. The NOAEL was 8315 mg/m³ [24].

n-Butyl methacrylate

In an inhalation study, rats were exposed to *n*-butyl methacrylate at concentrations of 310, 952 or 1891 ppm for 6 hours/day, 5 days/week for 4 weeks. Microscopic examination of the nasal cavities of the male and female rats exposed to 1891 ppm showed slight and localized bilateral degeneration of the olfactory epithelium lining of the dorsal meati. One male and one female rat exposed to 952 ppm had similar changes in the olfactory epithelium. Rats exposed to 310 ppm had no exposure-related microscopic changes in the nasal cavity [25].

No long-term studies were available on *n*-butyl methacrylate.

n-Butyl methacrylate tested negative in a range of in vitro and in vivo mutagenicity studies covering all the relevant end-points.

In a developmental toxicity study in rats on four methacrylates, including *n*-butyl methacrylate, animals were exposed by inhalation for 6 hours/day on gestation days 6–20. The exposure concentrations for *n*-butyl methacrylate were 0, 100, 300, 600 or 1200 ppm. Fetal toxicity was evident as decreases in fetal body weight at 600 ppm or greater. These exposure levels of *n*-butyl methacrylate were also maternally toxic. No significant increases in embryo/fetal deaths or fetal malformations were observed [26].

Observations in humans

No human data were available on BMC.

Assessment of dietary exposure

The Committee evaluated exposure to BMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes as well as

for micronutrient encapsulation for food fortification. Because another major use of BMC is in pharmaceuticals, this use was also evaluated in the exposure assessment. The level of use of BMC in food supplements, pharmaceuticals and foods for special medical purposes is a maximum of 10%. The level of use of BMC for food fortification differs depending on the nutrient or group of nutrients being microencapsulated.

The Committee evaluated exposure to BMC for the copolymer and its monomers, *n*-butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate.

The exposure assessment included submitted estimates and an evaluation by EFSA based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. National consumption data were from the CIFOCCOSS. Data from Australia and New Zealand were submitted to the Committee. A second sponsor provided estimates of dietary exposure from use in micronutrient encapsulation for food fortification. These estimates included exposures for 12 nutrients at a level that met 100% of their respective recommended dietary allowance (RDA). The exposure was estimated for each nutrient individually and also for the sum of all 12 nutrients.

A comprehensive literature search was also conducted; no additional studies were identified.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals, given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are therefore not relevant for the general healthy population. This use was therefore not further considered by the Committee.

The total monomeric content of BMC is less than 0.3%. This level was therefore used to calculate the exposure to total monomers from the copolymer exposure. Estimates of exposure to the individual monomers were based on exposure to total monomers, taking into account the ratio of each individual monomer in the copolymer.

The estimated exposures to BMC and its monomers for adults and children from uses in food supplements and in micronutrient encapsulation for food fortification are shown in [Table 4](#). All estimates of exposure from uses in food supplements are presented as a range from the lowest of the average exposures to the highest of the high exposures. All estimates of dietary exposure from micronutrient encapsulation for food fortification are presented as a range

Table 4

Summary of the range of estimated exposures to BMC and its monomers for average and high exposures

Population group	Source of exposure	Range of estimated dietary exposures ^{a,b}			
		Copolymer exposure (mg/kg bw per day)	Monomer exposure (µg/kg bw per day)		
			<i>n</i> -Butyl methacrylate	2-(Dimethylamino) ethyl methacrylate	Methyl methacrylate
Adults	Food supplements	3.5–37	2.6–28	5.2–56	2.6–28
	Micronutrient encapsulation for food fortification ^c	0.03–45	0.02–33	0.04–67	0.02–33
	All sources	3.5–82	2.6–61	5.2–120	2.6–61
Children	Food supplements	2.9–43	2.2–32	4.4–64	2.2–32
	Micronutrient encapsulation for food fortification ^c	0.05–92	0.04–69	0.1–140	0.04–69
	All sources	3.0–135	2.2–100	4.4–200	2.2–100

BMC: butyl methacrylate; bw: body weight

^a All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated mean exposures, and the upper end of each range is the highest of the estimated high exposures.^b Includes exposure estimates submitted by the sponsor and the European Food Safety Authority [1] and national estimates calculated by the Committee. Based on a concentration of 100 mg per 1 g dosage unit.^c Based on meeting 100% of the United States/Canadian recommended dietary allowance. The lower end of each range is lowest exposure for a single nutrient and the upper end of each range is the sum of exposure from 12 nutrients.

from the lowest exposure for a single nutrient to the exposure for the sum of the 12 nutrients assessed.

The Committee noted that the exposures estimated by the sponsor were to provide a presumed worst case for the purpose of the safety evaluation. The Committee also noted that national estimates of exposure would be required for the evaluation of safety based on their own nutrient reference values, fortification needs and food consumption patterns.

Estimates of exposure to BMC and its monomers from all sources combined (food supplements and micronutrient encapsulation for food fortification) are also shown in Table 4. The Committee noted that the upper end of the range representing high exposures from all sources is a worst case estimate, and unlikely in terms of actual long-term exposure to BMC.

The Committee noted that BMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA ranged between 8.0 and 13.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

Evaluation

The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not a safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification.

An ADI “not specified” was established for BMC.

The available toxicology data for BMC do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for BMC range from 750 to 2000 mg/kg bw per day, the highest doses tested.

Toxicological data on the residual monomers do not give rise to concerns when taking into account the low exposures. 2-(Dimethylamino) ethyl methacrylate and *n*-butyl methacrylate do not give rise to concerns for genotoxicity. Long-term, reproductive and developmental toxicity studies do not suggest a risk to health at the estimated exposure levels. Genotoxicity data for methyl methacrylate suggest a potential for mutagenicity and clastogenicity in vitro, but there is a lack of adequate in vivo genotoxicity data. However, in carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, there was no evidence of any carcinogenic effects. In a 2-year study in rats given methyl methacrylate in drinking-water, the NOAEL was 121 mg/kg bw per day; from this NOAEL, a TDI of 1.2 mg/kg bw per day was derived [18]. Estimated exposures to methyl methacrylate range from 2.2 to 100 µg/kg bw per day, which are below the TDI.

A toxicological and dietary exposure monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared.

References

1. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the use of basic methacrylate copolymer as a food additive. EFSA J. 2010;8(2):1513 [23 pp.] doi:10.2903/j.efsa.2010.1513.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. [¹⁴C]-Eudragit-E-100: The excretion and tissue distribution of radioactivity after oral administration to rats. Report no. 79/RGC024/278. Life Science Research, Stock, Essex, England, United Kingdom; 1979. Unpublished study report. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

4. 2-Dimethylaminoethyl methacrylate: Basic toxicokinetics. EC no.: 220-688-8. CAS no.: 2867-47-2. Helsinki: European Chemicals Agency; 2015a (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/2/2>, accessed 6 November 2015).
5. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer*. 1977;36(1):114–9 [cited in reference 18].
6. Canadian Environmental Protection Act: Priority Substances List assessment report for methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993.(Cat. no.: En40-215/10E) [cited in reference 18].
7. ECETOC. Methyl methacrylate – CAS no. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995 (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 18].
8. IUCLID [database]. Butyl methacrylate (97-88-1) [2000 CD-ROM edition] [48 pp.] European Commission; 2010 (<http://esis.jrc.ec.europa.eu/>).
9. Determination of acute oral toxicity in rats. Unpublished study report by Battelle-Institute, Frankfurt am Main, Germany; 1966. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
10. The acute toxicity of preparation 2787 on oral administration to rats. Unpublished study report. Hamburg, Germany: Laboratory for Pharmacology and Toxicology; 1970. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Acute toxicity of Eudragit E. Unpublished study by Department of Pharmacology, Nagasaki University, Nagasaki, Japan; 1970. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
12. Six-month toxicity of 2787 B in Sprague-Dawley rats with administration in the feedstuff. Report no. 100692 by Laboratory for Pharmacology and Toxicology, Hamburg, Germany; 1973. Unpublished study report submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
13. Twenty-eight day oral toxicity study of Eudragit E PO in the dog. Unpublished study report no. 20-2-0145-02 by Harlan Bioservice for Science, Walsrode, Germany; 2003. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. Experiments to determine the effect of 2577G and 2697 on pregnant rats and their foetuses. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg, Germany; 1968. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. 2-Dimethylaminoethyl methacrylate. EC no.: 220-688-8. CAS no.: 2867-47-2. Repeated dose toxicity: oral. Helsinki: European Chemicals Agency; 2015b (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/6/2>, accessed 20 June 2018).
16. 2-Dimethylaminoethyl methacrylate: toxicity to reproduction. EC no.: 220-688-8. CAS no. 2867-47-2. Helsinki: European Chemicals Agency; 2015c (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/9/2>, accessed 20 June 2018).
17. 2-Dimethylaminoethyl methacrylate. EC no.: 220-688-8. CAS no.: 2867-47-2. Developmental toxicity / teratogenicity. Helsinki: European Chemicals Agency; 2015d (<https://echa.europa.eu/registration-dossier/-/registered-dossier/14994/7/9/3>, accessed 20 June 2018).
18. Methyl methacrylate. Concise international chemical assessment document 4. Geneva: World Health Organization; 1998 (International Programme on Chemical Safety, Concise International Chemical Assessment Document No. 4).

19. Borzelleca JF, Larson PS, Hennigar GR Jr, Huf EG, Crawford EM, Smith RB Jr. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 18].
20. Anderson D, Hodge MCE. Methyl methacrylate monomer: Dominant lethal study in the mouse. ICI, Macclesfield, Cheshire, England, United Kingdom; 1976. Report no. CTL/P/295 [cited in reference 18].
21. Hodge MCE, Palmer S. Methylmethacrylate monomer teratogenicity studies in the rat. Unpublished study by Rohm and Haas Company, Philadelphia, Pennsylvania, USA, to the US Environmental Protection Agency, Washington (DC), USA, under TSCA Section 8(d), July 1979; 1977 [cited in reference 18].
22. Nicholas CA, Lawrence WH, Autian J. Embryotoxicity and fetotoxicity from maternal inhalation of methyl methacrylate monomer in rats. *Toxicol Appl Pharmacol.* 1979;50(3):451–8 [cited in reference 18].
23. McLaughlin RE, Reger SI, Barkalow JA, Allen MS, Diffazio CA. Methyl methacrylate: A study of teratogenicity and fetal toxicity of the vapor in the mouse. *J Bone Joint Surg Am.* 1978;60(3):355–8 [cited in reference 18].
24. Solomon HM, McLaughlin JE, Swenson RE, Hagan JV, Wanner FJ, O'Hara GP et al. Methyl methacrylate: Inhalation developmental toxicity study in rats. *Teratology.* 1993;48(2):115–25.
25. ECHA. Butyl methacrylate. EC no.: 202-615-1. CAS no.: 97-88-1. Repeated dose toxicity: Inhalation. Helsinki: European Chemicals Agency; 2018 (<https://echa.europa.eu/registration-dossier/-/registered-dossier/15151/7/6/3>, accessed 20 June 2018).
26. Saillenfait AM, Bonnet P, Gallissot F, Peltier A, Fabriès JF. Developmental toxicities of methacrylic acid, ethyl methacrylate, *n*-butyl methacrylate, and allyl methacrylate in rats following inhalation exposure. *Toxicol Sci.* 1999;50(1):136–45 [cited in reference 25].

3.1.3 Erythrosine

Explanation

Erythrosine (INS No. 127; CAS No. 16423-68-0) is a xanthene dye permitted as a food colour in China, the European Union, the USA and other regions. It is used for colouring foods including baked goods, breakfast cereals, confectionery products, dairy products, decorations for baking, dressings and sauces, dried fruit, frostings and icings, frozen breakfast foods, frozen treats, hot beverages, juice drinks and processed foods (fish, meat and egg products).

The Committee previously evaluated the safety of erythrosine at its eighth, thirteenth, eighteenth, twenty-eighth, thirtieth, thirty-third and thirty-seventh meetings ([Annex 1](#), references 8, 19, 35, 66, 73, 83 and 94) and for dietary exposure at its fifty-third meeting ([Annex 1](#), reference 143). Toxicological monographs or monograph addenda were published after the thirteenth, eighteenth, twenty-eighth, thirtieth, thirty-third and thirty-seventh meetings ([Annex 1](#), references 20, 36, 67, 74, 84 and 95). At its eighteenth meeting the Committee allocated an ADI of 0–2.5 mg/kg bw. This ADI was reduced at the twenty-eighth meeting to 0–1.25 mg/kg bw and made temporary following observations that erythrosine

produced effects on thyroid function in short-term toxicity studies in rats and that, in long-term toxicity studies, male rats receiving 4% erythrosine in the diet developed benign thyroid tumours.

At the thirtieth meeting the Committee reduced the temporary ADI to 0–0.6 mg/kg bw, based on biochemical effects of erythrosine on thyroid hormone metabolism and regulation in rats. The Committee at the thirtieth meeting requested further data from pharmacokinetic studies relating the amount of erythrosine absorbed to the amount ingested to enable the establishment of a correlation between blood/tissue erythrosine levels and effects on the thyroid. At the thirty-third meeting the Committee further reduced the temporary ADI to 0–0.05 mg/kg bw. This ADI was based on a NOAEL from a study showing slightly increased thyroid-stimulating hormone (TSH) responsiveness in humans ingesting erythrosine at 60 mg per person per day (equivalent to 1 mg/kg bw per day) for 14 days, and applying an uncertainty factor² of 20. The Committee again requested the pharmacokinetic studies required by the previous Committee. At its thirty-seventh meeting, the Committee re-evaluated previously reviewed studies that had since been published and newer studies on thyroid physiology in rats. The Committee concluded that the thyroid tumours in male rats previously reported in long-term toxicity studies were secondary to thyroid hormone changes and species-specific sensitivity.

In view of the differences in thyroid physiology between humans and rats, the Committee based its evaluation on the human data and allocated an ADI of 0–0.1 mg/kg bw on the NOAEL of 60 mg per person per day from the 14-day study in human subjects (equivalent to 1 mg/kg bw per day), with application of an uncertainty factor of 10.

At the present meeting, the Committee re-evaluated erythrosine at the request of CCFA49 [1].

A toxicological dossier that included new studies on genotoxicity, reproductive and developmental toxicity, neurological effects and hypersensitivity was submitted. A comprehensive literature search conducted in PubMed retrieved three additional studies relevant to the present evaluation. The Committee also considered studies evaluated at previous meetings.

Chemical and technical considerations

Erythrosine consists of the disodium salt of 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Erythrosine is manufactured by iodination of fluorescein, the condensation product of resorcinol and phthalic anhydride. Impurities include unreacted starting materials ($\leq 0.4\%$), subsidiary colouring matters except fluorescein

² The previous Committee used the term “safety factor”.

($\leq 4\%$), fluorescein (≤ 20 mg/kg), inorganic iodides ($\leq 0.1\%$), lead (≤ 2 mg/kg) and zinc (≤ 50 mg/kg).

Biochemical aspects

Erythrosine is poorly absorbed and mainly excreted unchanged in rat faeces.

Following oral administration of radiolabelled erythrosine, less than 1% of the dose was excreted in the urine. Blood and plasma radioactivity reached maximum levels by 1 hour, while liver and kidney levels peaked after 4–12 hours; no radioactivity was detectable in the brain or pituitary. No erythrosine was accumulated in the thyroid or other tissues [2].

No additional metabolic or kinetic studies have become available since the previous evaluation by the Committee.

Toxicological studies

Erythrosine has low oral acute toxicity in mice, rats, gerbils and rabbits.

A short-term toxicity study with erythrosine showed pigment deposition in renal tubules in female rats at the 2% dietary level and in males at all but the lowest dietary levels, in a dose-related manner. The NOAEL was 0.25% in the diet (equal to 160 mg/kg bw per day) [3]. No compound-related effects were observed in other short-term toxicity studies in rats, gerbils, dogs and pigs. No additional short-term toxicity studies have become available since the previous evaluation by the Committee.

Several long-term oral toxicity studies showed no compound-related increases in tumour incidences in mice, rats and gerbils. In one long-term study of oral toxicity [4], mice showed decreased body weights at 3.0% erythrosine in the diet. The NOAEL was 1.0% erythrosine in the diet (equal to 1474 mg/kg bw per day). Body-weight decreases were also observed in a rat study [5]. The NOAEL was 1% erythrosine in the diet (equivalent to 500 mg/kg bw per day).

Two long-term feeding studies with erythrosine found an increase in the incidence of thyroid follicular cell adenomas in male rats [6, 7]. The previous Committee considered the occurrence of thyroid follicular tumours in rats secondary to hormonal effects based on results from studies on thyroid function and morphology. Another study indicated that erythrosine promoted the development of thyroid follicular tumours in partially thyroidectomized rats, but not in non-thyroidectomized rats [8]. The present Committee noted that the rat is not considered a suitable model for potential effects on the thyroid in humans [9].

A large number of in vitro and in vivo genotoxicity tests have been conducted on erythrosine. The Committee confirmed that the overall weight of evidence indicates that erythrosine is not genotoxic.

Two short-term oral toxicity studies indicated that erythrosine may affect testicular function in mice. Abdel-Aziz et al. [10] observed decreased epididymal sperm counts and sperm motility at dose levels of 68 and 136 mg/kg bw per day and a significant increase in the incidence of abnormal sperm head morphology at doses of 680 and 1360 mg/kg bw per day. Vivekanandhi et al. [11] observed decreases in sperm motility and sperm counts and increases in sperm abnormalities, with a lowest-observed-adverse-effect level (LOAEL) of 64 mg/kg bw per day. No effects on sperm or on fertility were observed in rats in long-term toxicity studies and a multigeneration study conducted with higher doses administered via the diet [6, 7, 12].

No developmental toxicity was reported in rats.

In a newly available reproductive toxicity study in mice, administration of erythrosine at dietary concentrations of 0, 0.005%, 0.015% or 0.045% (equivalent to 0, 7.5, 22.5 and 67.5 mg/kg bw per day, respectively) resulted in no adverse effects on reproductive parameters or functional developmental parameters in offspring. Significant changes in some measures of exploratory behaviour and motor activity were reported in high-dose dams and their offspring [13]. Motor activity and brain serotonin levels in rat single-dose and repeated-dose studies were inconsistent [14, 15]. In view of the lack of consistency in the data, the poor oral absorption of erythrosine, the evidence that erythrosine does not penetrate the blood–brain barrier and the lack of evidence of behavioural toxicity in a rat study with dose levels equivalent to up to 1000 mg/kg bw per day [16], the Committee concluded that the findings in the Tanaka [13] and Dalal & Poddar [14, 15] studies did not provide robust evidence of behavioural effects and could not be used in the risk assessment.

Observations in humans

Studies in human volunteers showed increased blood iodine levels but no changes in thyroid hormone levels in repeated-dose studies of up to 25 mg/day for 3 weeks or single-dose studies of up to 80 mg. A study with 30 healthy male volunteers found that erythrosine slightly increased thyroid-stimulating hormone (TSH) responsiveness to thyrotropin-releasing hormone (TRH), but there were no effects on other thyroid hormone parameters at the highest dose level of 200 mg per person per day for 14 days [17]. No effect was observed at 60 mg per person per day. At its thirty-third meeting, the Committee concluded that the NOAEL was 60 mg per person per day (equivalent to 1 mg/kg bw per day), and the present Committee concurred.

Assessment of dietary exposure

The Committee previously evaluated dietary exposure to erythrosine at its fifty-third meeting, but significant changes in methodologies for the exposure

Table 5

Summary of the range of estimates of dietary exposure for erythrosine

Source of estimates	Population	Range of estimated dietary exposures (mg/kg bw per day)	
		Means	High percentile (P90 and P95) ^a
Analytical levels ^b	Children	0.00–0.01	0.00–0.09
	Adolescents	0.00–0.02	0.00–0.04
	Adults	0.00–0.03	0.00–0.05
Maximum use levels ^c	Toddlers	0–0.2	0–0.4
	Children	0–0.1	0–0.1
	Adolescents	0–0.1	0–0.1
	Adults	0–0.1	0–0.2
	Elderly adults	0–0.1	0–0.1

bw: body weight; P90: 90th percentile; P95: 95th percentile

^a The upper bound of the range is the maximum of the 90th and 95th percentiles.^b Studies from the Republic of Korea [18] and the USA [19, 20] for consumers only.^c From published national studies [21, 22]) and the Committee's assessment based on consumption data from CIFOss for 36 countries.

assessments precluded comparisons between the previous and the current assessments.

A comprehensive literature search retrieved five relevant studies other than those submitted by the sponsor. The Committee reviewed published estimates of dietary exposure to erythrosine conducted in several countries. The Committee also conducted a conservative assessment using consumption data from 36 countries from the CIFOss database and Codex maximum use levels. A summary of the dietary exposure estimates is provided in [Table 5](#).

Dietary exposure estimates based on individual consumption data and maximum use levels range from 0 to 0.4 mg/kg bw. Because erythrosine has been in use for many years, national estimates based on analytical data were also available to the Committee. National exposure estimates based on analytical data range from 0.00 to 0.05 mg/kg bw per day for adults and from 0.00 to 0.09 mg/kg bw per day for children, considering consumers only.

The Committee also noted that exposure through pharmaceuticals was previously estimated to occur at up to approximately 0.1 mg/kg bw per day in specific populations, generally over a short period of time. However, the Committee considered that such exposure should not be taken into account in the assessment of exposure to erythrosine as a food additive when looking at long-term exposure in a healthy population.

The Committee considered the approach based on analytical data to be more realistic for preparing long-term dietary exposure estimates than the approach based on maximum use levels. Because the scenarios available from the national studies examined consumers only, the Committee concluded that the

highest estimate of 0.09 mg/kg bw per day for children should be considered in the safety assessment of erythrosine.

Evaluation

The evidence newly available at this meeting indicates that there are no concerns with respect to genotoxicity and reproductive and developmental toxicity of erythrosine. The previously established ADI of 0–0.1 mg/kg bw is based on a NOAEL of 60 mg per person per day (equivalent to 1 mg/kg bw per day for a 60 kg person) identified in a human study, with a default uncertainty factor of 10. In this study [17], minimal effects on thyroid function were observed at 200 mg per person per day (equivalent to 3.3 mg/kg bw per day). Effects in experimental animals were observed at doses at least 60-fold higher than the NOAEL in this human study; these effects supported the use of the human data as the basis for the ADI.

The Committee concluded that the new data that have become available since the previous evaluation do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw.

The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to erythrosine for all age groups do not present a safety concern.

A consolidated toxicological and dietary exposure monograph was prepared.

At the present meeting, the existing specifications for erythrosine were revised. High-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

A Chemical and Technical Assessment was prepared.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
2. Obrist J, LeVan L, Puhl RJ, Duan RJ. Final report: Metabolism of FD&C Red No. 3 in rats, study no. 6145–100. Unpublished report by Hazleton Laboratories America, Inc., 1986. Submitted to WHO by the Certified Color Manufacturers Association, Inc., Washington, DC, USA.

3. Butterworth KR, Gaunt IF, Grasso P, Gangolli SD. Acute and short-term toxicity studies on erythrosine BS in rodents. *Food Cosmet Toxicol.* 1976;14(6):525–31.
4. Borzelleca JF, Hallagen JB. Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in mice. *Food Chem Toxicol.* 1987;25(10):735–7.
5. Hansen WH, Davis KJ, Graham SL, Perry CH, Jacobson KH. Long-term toxicity studies of erythrosine. II. Effects on haematology and thyroxine and protein-bound iodine in rats. *Food Cosmet Toxicol.* 1973;11(4):535–45.
6. Brewer L, Jefferson ND, Blair M, Thorstenson JB, Nair KPC, Richter WR et al. Long-term dietary toxicity/carcinogenicity study in rats. FDC Red No. 3. Unpublished report no. 410–002 from International Research and Development Corporation, Mattawan, MI, USA; 1981. Submitted to WHO by the Certified Color Manufacturers Association Inc., Washington, DC, USA.
7. Borzelleca JF, Capen CC, Hallagen JB. Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in rats. *Food Chem Toxicol.* 1987;25(10):723–33.
8. Hiasa Y, Ohshima M, Kitahori Y, Konishi N, Shimoyama T, Sakaguchi Y et al. The promoting effects of food dyes, erythrosine (Red 3) and Rose Bengal B (Red 105), on thyroid tumors in partially thyroidectomized *N*-bis(2-hydroxypropyl)-nitrosamine-treated rats. *Jpn J Cancer Res.* 1988;79(3):314–9.
9. Part 1: IPCS framework for analysing the relevance of a cancer mode of action for humans and case-studies. Geneva: World Health Organization, International Programme on Chemical Safety; 2007; pp. 1–125. Harmonization Project Document No. 4.
10. Abdel-Aziz AH, Shouman SA, Attia AS, Saad SF. A study on the reproductive toxicity of erythrosine in male mice. *Pharmacol Res.* 1997;35(5):457–62.
11. Vivekanandhi J, Devi CPA, Jayaraman K, Raghavan L. Effect of erythrosine on testicular function of mice. *Toxicol Int.* 2006;13(2):119–25.
12. Aldridge D, Schardein JL, Blair M, Goldenthal EI, Benson BW. FD & C Red No. 3. Three generation reproduction study. Unpublished report no. 410–013 from International Research and Development Corporation, Mattawan, MI, USA; 1981. Submitted to WHO by the Certified Color Manufacturers Association, Inc., Washington, DC, USA.
13. Tanaka T. Reproductive and neurobehavioural toxicity study of erythrosine administered to mice in the diet. *Food Chem Toxicol.* 2001;39(5):447–54.
14. Dalal A, Poddar MK. Short-term erythrosine B-induced inhibition of the brain regional serotonergic activity suppresses motor activity (exploratory behavior) of young adult mammals. *Pharmacol Biochem Behav.* 2009;92(4):574–82.
15. Dalal A, Poddar MK. Involvement of high plasma corticosterone status and activation of brain regional serotonin metabolism in long-term erythrosine-induced rearing motor hyper activity in young adult male rats. *Toxicol Mech Methods.* 2010;20(6):287–97.
16. Vorhees CV, Butcher RE, Brunner RL, Wootten V, Sobotka TJ. A developmental toxicity and psychotoxicity evaluation of FD and C Red Dye No. 3 (erythrosine) in rats. *Arch Toxicol.* 1983;53(4):253–64.
17. Gardner DF, Utiger RD, Schwartz SL, Witorsch P, Meyers B, Braverman LE et al. Effects of oral erythrosine on thyroid function in normal men. *Toxicol Appl Pharmacol.* 1987;91(3):299–304.
18. Ha MS, Ha SD, Choi SH, Bae DH. Exposure assessment of synthetic colours approved in Korea. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2013;30(4):643–53.

19. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry SE. Exposure estimate for FD&C colour additives for the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2016;33(5):782–97.
20. Bastaki M, Farrell T, Bhusari S, Bi X, Scrafford C. Estimated daily intake and safety of FD&C food-colour additives in the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2017;34(6):891–904.
21. Gilsenan MB, Gibney MJ. Assessment of the influence of energy under-reporting on intake estimates of four food additives. *Food Addit Contam.* 2004;21(3):195–203.
22. Final assessment report. Application A603. Red 3 erythrosine in food colouring preparations. Canberra: Food Standards Australia New Zealand; 2010.

3.1.4 Indigotine

Explanation

Indigotine (INS No. 132) consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate (the principal component; CAS No. 860-22-0), disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (an isomer) and subsidiary colouring matters. Indigotine is used in China, the European Union, Japan, the USA and other regions. It is used for colouring foods including blueberry bagels, breakfast cereals, cakes and cupcakes, candies including chocolate, chewing gum, dairy products, decorations for baking, frozen treats, sauces and seasonings.

The Committee evaluated the safety of indigotine at its thirteenth and eighteenth meeting ([Annex 1](#), references 19 and 35). At its eighteenth meeting, the Committee established an ADI of 0–5 mg/kg bw based on a NOAEL of 500 mg/kg bw per day (1% in the diet) in a chronic dietary toxicity study in rats [1], and prepared specifications for indigotine ([Annex 1](#), reference 35). At its seventy-third meeting, the Committee revised the specifications ([Annex 1](#), references 202).

Indigotine was on the agenda for re-evaluation of safety and revision of specifications at the request of CCFA49 [2].

A toxicological dossier was submitted summarizing the available toxicity data, together with relevant study reports and publications. A comprehensive literature search retrieved eight other relevant studies.

Chemical and technical considerations

Indigotine is an indigoid dye. Indigotine consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate, disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (an isomer) and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Indigotine is manufactured by heating indigo in the presence of sulfuric acid. The indigo (or indigo paste) is manufactured by the fusion of

N-phenylglycine (prepared from aniline and formaldehyde) in a molten mixture of sodamide and sodium and potassium hydroxides under ammonia pressure. It is isolated and subjected to purification procedures prior to sulfonation. Impurities include unreacted starting materials ($\leq 0.5\%$), subsidiary colouring matters ($\leq 1\%$), unsulfonated primary aromatic amines ($\leq 0.01\%$ calculated as aniline) and lead (≤ 2 mg/kg).

Biochemical aspects

Although indigotine was poorly absorbed from the gastrointestinal tract in rats, one of its metabolites, 5-sulfoanthranilic acid, formed through microbial fermentation, was absorbed to a greater extent. After administration of a single oral dose of 50 mg/rat, unchanged indigotine, isatin-5-sulfonic acid and 5-sulfoanthranilic acid were identified in urine (0.53%, 0.63% and 0.28% of the dose, respectively). The metabolites isatin-5-sulfonic acid and 5-sulfoanthranilic acid were also identified in bile [3]. After in vitro incubation of indigotine with caecal microflora of rats, four unidentified metabolites were found [4].

In vitro, indigotine significantly inhibited the CYP2A6 monooxygenase activity in a noncompetitive manner, with a median inhibitory concentration (IC_{50}) of 0.05 mmol/L [5, 6].

Toxicological studies

Indigotine has a low acute toxicity. No adverse effects were seen in pigs fed indigotine (purity $>85\%$) at dose levels of 0, 150, 450 and 1350 mg/kg bw per day for 90 days [7].

When mice were fed diets containing 0.2%, 0.4%, 0.8% or 1.6% indigotine (purity $\geq 85\%$) for 80 weeks, the only effect seen was a slight anaemia in animals at 0.8% or 1.6% [8]. The previous Committee concluded that indigotine was not carcinogenic and that the “no-untoward-effect level was 0.4% of the diet equivalent to an intake of approximately 550 mg/kg/day” ([Annex 1](#), references 35 and 36).

In a 2-year combined toxicity and carcinogenicity study, mice were fed indigotine (purity 93%; 7% volatile matter) at dietary concentrations of 0, 0.5%, 1.5% or 5.0% (equal to 0, 825, 2477 and 8259 mg/kg bw per day, respectively). A moderate incidence of blue-green discoloration of the gastrointestinal tract, with occasional discoloration of the liver, gall-bladder and urine, was observed at all doses. There was no evidence of carcinogenicity. The NOAEL was 5% in the diet (equal to 8259 mg/kg bw per day), the highest dose tested [9].

In a long-term combined toxicity and carcinogenicity study, which included an in utero phase, indigotine (purity 93%, 7% volatile matter) was fed to rats at dietary levels of 0, 0.5%, 1.0% or 2.0% (equal to 0, 304, 632 and 1282 mg/kg bw per day, respectively). Treatment began approximately 2 months prior

to mating, and the long-term phase was initiated after random selection of the F₁ animals [10]. No consistent substance-related adverse effects were noted, with the exception of statistically significant increases in incidences of malignant mammary gland tumours and gliomas in males, but not females, at the highest dose. The incidence of mammary gland tumours (carcinomas/adenocarcinomas) in high-dose males was 5.9% (3/51 compared with 0/114 control animals). The incidence of gliomas in high-dose males was 9.9% (7/71) compared with 2.9% in controls (4/140 animals). No increase was seen in the low and mid dose groups (incidence 2.9%, 2/70 animals) or in female rats.

The previous Committee noted the statistically significant increase in incidence of malignant mammary gland tumours and gliomas in male rats at the highest dose level of 1282 mg/kg bw per day. In the absence of any indications for genotoxicity, the Committee concluded that the NOAEL in this study was 632 mg/kg bw per day.

Rats fed a diet containing 1% indigotine (equivalent to 500 mg/kg bw per day) for 2 years showed no treatment-related pathological signs, and fewer malignant and benign tumours than the controls; they also survived for longer than the controls [11]. When indigotine was fed to groups of rats at dietary levels of 0, 0.5%, 1.0%, 2.0% or 5.0% for 2 years, the only effect seen was a reduced growth in males at 2.0% and 5.0% [1]. The NOAEL was 1% in the diet, equivalent to 500 mg/kg bw per day.

Indigotine tested negative in a series of bacterial mutagenicity assays as well as in *in vitro* mammalian cell chromosomal aberration assays and a number of comet assays. Indigotine was also not genotoxic in *in vivo* micronucleus tests in mice and rats and a comet assay in mice. The Committee concluded that indigotine does not raise any concerns with respect to genotoxicity.

No reproductive or developmental toxicity was observed in one 3-generation rat study (doses up to 250 mg/kg bw); two 2-generation rat studies (doses up to 250 or 500 mg/kg bw); one rat teratogenicity study (doses up to 250 mg/kg bw); and two rabbit teratogenicity studies (doses up to 250 mg/kg bw).

Assessment of dietary exposure

Dietary exposure to indigotine has not been previously reviewed by the Committee.

A comprehensive literature search retrieved five relevant studies other than those submitted by the sponsor. The Committee reviewed published estimates of dietary exposure to indigotine conducted in several countries and regions. A summary of these estimates, showing the results from mean and high percentile calculations, is provided in [Table 6](#).

As indigotine has provisions in 51 food categories in the General Standard on Food Additives (GSFA), an exposure assessment based on maximum use

Table 6
Summary of the range of estimates of dietary exposure for indigotine

Source of estimates	Population	Range of estimated dietary exposures (mg/kg bw per day)	
		Mean	High percentile (P90 and P95) ^a
National ^b	Children	0.00–0.01	0.00–0.09
	Children	0.00–0.03	0.0–0.3
	Adolescents	0.00–0.01	0.00–0.03
	Adults	0.00–0.01	0.00–0.02
Europe ^c	Toddlers	0.0–0.2	0.3–0.8
	Children	0.0–0.1	0.2–0.8
	Adolescents	0.0–0.1	0.1–0.5
	Adults	0.0–0.4	0.1–0.3
	Elderly adults	0.0–0.03	0.1–0.2

bw: body weight; P90: 90th percentile; P95: 95th percentile

^a The upper bound of the range is the maximum of the 90th and 95th percentiles.

^b Data from Australia [12]; Kuwait [13]; the Netherlands [14]; the Republic of Korea [15]; and the USA [16, 17].

^c Based on consumption data from 17 European countries [18].

levels was considered by the Committee to be unrealistic. Moreover, analytical and reported use levels for the main contributing food categories were far below the maximum use levels, reinforcing the Committee's decision not to consider estimates based on maximum use levels for the exposure assessment.

Because indigotine has been authorized for use for many years, estimates based on analytical data were available and were considered by the Committee to be more appropriate for determining long-term dietary exposure estimates. Exposure estimates based on analytical data range from 0.0 to 0.8 mg/kg bw per day for adults, adolescents and children at the 95th percentile. Because of the conservative assumptions in high percentile exposure estimates, the Committee concluded that an estimate of 0.8 mg/kg bw per day for children and toddlers should be considered in the safety assessment for indigotine.

Evaluation

The Committee noted that indigotine is poorly absorbed from the gastrointestinal tract, has a low acute toxicity, is not genotoxic and does not show any potential for reproductive or developmental toxicity. The previous Committee identified a NOAEL of 500 mg/kg bw per day from a 2-year rat study of 1% indigotine in the diet and established an ADI of 0–5 mg/kg bw. The current Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies. In one long-term toxicity study, slight anaemia was observed in mice fed diets with 0.8% or 1.6% indigotine. In another long-term toxicity study, body-weight gain was reduced in male rats at 2.0% and 5.0%

indigotine in the diet. In a third long-term toxicity study, increased incidences of malignant mammary gland tumours and gliomas were observed in male rats at 1282 mg/kg bw per day but not at 304 and 632 mg/kg bw per day.

The Committee concluded that there are no reasons to revise the ADI and confirmed the ADI of 0–5 mg/kg bw.

The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper limit of the ADI of 0–5 mg/kg bw established for indigotine. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.

A consolidated toxicological and dietary exposure monograph was prepared.

The existing specifications for indigotine were revised. HPLC methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

The specifications monograph was revised, and a Chemical and Technical Assessment was prepared.

References

1. Hansen WH, Fitzhugh OG, Nelson AA, Davis KJ. Chronic toxicity of two food colors, brilliant blue FCF and indigotine. *Toxicol Appl Pharmacol*. 1966;8(1):29–36.
2. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
3. Lethco EJ, Webb JM. The fate of FD&C Blue No. 2 in rats. *J Pharmacol Exp Ther*. 1966;154(2):384–9.
4. Singh S, Das M, Khanna SK. Bio-metabolism of green S and indigo carmine through caecal microflora of rats. *Biochem Biophys Res Commun*. 1993;195(1):490–96.
5. Kuno N, Mizutani T. Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. *J Toxicol Environ Health A*. 2005;68(16):1431–44.
6. Mizutani T. Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner. *J Environ Public Health*. 2009;953952:1–9. doi:10.1155/2009/953952.
7. Gaunt IF, Kiss IS, Grasso P, Gangolli SD. Short-term toxicity study on indigo carmine in the pig. *Food Cosmet Toxicol*. 1969;7(1):17–24.
8. Hooson J, Gaunt IF, Kiss IS, Grasso P, Butterworth KR. Long-term toxicity of indigo carmine in mice. *Food Cosmet Toxicol*. 1975;13(2):167–76.
9. Borzelleca JF, Hogan GK. Chronic toxicity/carcinogenicity study of FD & C Blue No. 2 in mice. *Food Chem Toxicol*. 1985;23(8):719–22.

10. Borzelleca JF, Hogan GK, Koestner A. Chronic toxicity/carcinogenicity study of FD & C Blue No. 2 in rats. *Food Chem Toxicol.* 1985;23(6):551–8.
11. Oettel H, Froberg H, Nothdurft H, Wilhelm G. [Testing of some synthetic dyes for their suitability as food coloring agents]. *Arch Toxikol.* 1965;21(1):9–29 (in German).
12. Survey of added colours in foods available in Australia. Study of concentration in foods including dietary exposure assessment and risk characterisation. Canberra: Food Standards Australia New Zealand; 2008.
13. Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N et al. Estimates of dietary exposure of children to artificial food colours in Kuwait. *Food Addit Contam.* 2006;23(3):245–51.
14. Kist-van Holthe JK, Altenburg TM, Bolakhrif S, El Hamdi L, Man MW, Tu J et al. Consumption of artificial food colourings by school children in the Netherlands. *Adv Pediatr Res.* 2015;2(5). doi:10.12715/apr.2015.2.5.
15. Ha MS, Ha SD, Choi SH, Bae DH. Exposure assessment of synthetic colours approved in Korea. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2013;30(4):643–53.
16. Doell DL, Folmer DE, Lee HS, Butts KM, Carberry KM. Exposure estimate for FD&C colour additives for the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2016;33(5):782–97.
17. Bastaki M, Farrell T, Bhusari S, Bi X, Scrafford C. Estimated daily intake and safety of FD&C food-colour additives in the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2017;34(6):891–904.
18. Scientific Opinion on the re-evaluation of Indigo Carmine (E 132) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *EFSA J.* 2014;12(7):3768. doi:10.2903/j.efsa.2014.3768.

3.1.5 Lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic)

Explanation

Lutein esters from *Tagetes erecta* (INS No. 161b(iii)) and lutein from *Tagetes erecta* (INS No. 161b(i)) are used as food colouring agents and nutrient supplements in a wide range of baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogues, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 2 to 330 mg/kg.

Zeaxanthin (INS No. 161h(i)) is used as a nutritional supplement and colour in a wide range of foods such as baked goods, beverages, chewing gum, egg products, fats and oils, gravies and sauces, hard and soft candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 0.5 to 70 mg/kg.

Lutein esters contain lutein (all-*E*,3*R*,3'*R*,6'*R*)- β , ϵ -carotene-3,3'-diol, which is a naturally occurring oxygenated xanthophyll pigment and a macular carotenoid. Lutein occurs with its isomeric xanthophyll, zeaxanthin, in many

foods but particularly in vegetables and fruit. Lutein occurs either esterified to fatty acids or in a non-esterified “free” form. Studies show that *meso*-zeaxanthin, a structurally related xanthophyll of lutein, and zeaxanthin may originate from foodstuffs that are sources of these xanthophylls, rather than being derived from the bioconversion of retinal lutein [1]. Extracts containing xanthophylls (free and/or esterified) are used as colours and as nutritional supplements in a wide range of applications.

At the thirty-first meeting, the Committee prepared tentative specifications for xanthophylls obtained from *Tagetes erecta* petals, but no toxicological evaluation was performed ([Annex 1](#), reference 77). Xanthophyll preparations (*Tagetes* extract) containing lutein esters at low concentrations were evaluated at the fifty-fifth and fifty-seventh meetings ([Annex 1](#), references 149 and 154). The tentative specifications ([Annex 1](#), reference 151) were subsequently superseded by full specifications ([Annex 1](#), reference 156). At the sixty-third meeting, the Committee evaluated biochemical data and the results of toxicological and human studies on *Tagetes* preparations with a high content of non-esterified lutein (>80%) and established a group ADI of 0–2 mg/kg bw per day for lutein from *Tagetes erecta* and synthetic zeaxanthin ([Annex 1](#), reference 173). At the seventy-ninth meeting, the Committee evaluated the results of new toxicity studies on preparations with a higher content of xanthophyll esters (>60%), identified as “lutein esters from *Tagetes erecta*” ([Annex 1](#), reference 220). A temporary ADI “not specified” for lutein esters from *Tagetes erecta* was established; the ADI was made temporary because the specifications for lutein esters from *Tagetes erecta* were tentative.

At the eighty-second meeting, the Committee evaluated additional information on manufacturing and composition of lutein esters from *Tagetes erecta* that permitted the adoption of final specifications and the removal of the temporary designation ([Annex 1](#), reference 230). An ADI “not specified” for lutein esters from *Tagetes erecta* was established. The Committee was unable to consider establishing a group ADI “not specified” for lutein esters from *Tagetes erecta*, lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls, and recommended that this be taken up at a future meeting.

At the present meeting, newly available data for lutein, zeaxanthin and *meso*-zeaxanthin were submitted by the sponsor. A comprehensive literature search retrieved two other studies. The Committee assessed available toxicity studies for re-evaluation of safety, dietary exposure and specifications to consider establishing a group ADI “not specified” for lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic).

Chemical and technical considerations

Lutein, lutein esters (including lutein dipalmitate) and zeaxanthin are members of a group of pigments known as xanthophylls; they have no provitamin A activity.

Tagetes extract (INS No. 161b(ii)) is obtained by hexane extraction of dried petals of marigold (*Tagetes erecta* L.), with subsequent removal of the solvent. The major colouring principles are lutein (all-*E*,3*R*,3'*R*,6'*R*- β , ϵ -carotene-3,3'-diol) and lutein dipalmitate (helenien; β , ϵ -carotene-3,3'-diol dipalmitate). Hydroxy derivatives of carotenes together with other oxy derivatives, such as epoxides, may also be present in *Tagetes* extract. The product may contain fats, oils and waxes that occur naturally in the plant material. The articles of commerce are usually further formulated to standardize the colour content or to obtain water-soluble or dispersible products.

Lutein esters from *Tagetes erecta* (INS No. 161b(iii)) is a purified extract obtained from marigold (*Tagetes erecta* L.) oleoresin, which is extracted using organic solvents. The preparation contains lutein esters, of which lutein dipalmitate accounts for the major part; a smaller proportion of zeaxanthin esters is also present. The balance of the extract is made up of naturally occurring waxes.

Lutein from *Tagetes erecta* (INS No. 161b(i)) is a purified extract obtained from marigold (*Tagetes erecta* L.) oleoresin, which is extracted using organic solvents. The final product, after saponification and crystallization, contains lutein as the major component and a smaller proportion of zeaxanthin.

Zeaxanthin (synthetic) (INS No. 161h(i)) is the synthetic all-*trans* isomer of zeaxanthin (3*R*,3'*R*- β , β -carotene-3,3'-diol) produced by the Wittig reaction from raw materials that are commonly used in the production of other carotenoids with application in foods. Minor quantities of *cis*-zeaxanthins and by-products 12-apozeaxanthinal, parasiloxanthin, diatoxanthin and triphenyl phosphine oxide may be present in the final product.

Biochemical aspects

The ADME of lutein and lutein esters from *Tagetes erecta* was extensively described in the monographs at the sixty-third and seventy-ninth meetings ([Annex 1](#), references 174 and 221). The absorption of lutein involves emulsification by bile, followed by lipolysis by pancreatic lipases into the micellar fraction for absorption by intestinal cells. Absorption of xanthophylls from esters requires hydrolysis. Hydrolysis is an efficient process as esterified lutein is not normally found in human serum. These processes have been shown to be influenced by food matrices, for example, dietary fibres reduce absorption whereas dietary fats promote absorption due to the hydrophobic nature of these xanthophylls. The bioavailability of lutein following administration of lutein esters has been shown to be equivalent to the administration of free lutein.

Sheshappa et al. [2] investigated the influence of food matrices in lutein-deficient rats. Lutein levels in plasma, liver and eyes were higher than in the controls when animals were administered lutein-mixed micelles containing either 3% fat, phosphatidyl choline (PC) or lyso-phosphatidyl choline (lysoPC). In contrast, the administration of lutein-mixed micelles containing pectin and mixed xanthophylls resulted in lutein levels that were lower than in the controls. Evans et al. [3] found that in humans given a single dose of 20 mg of lutein in a starch-based matrix or cross-linked to an alginate matrix, lutein from the starch-based product was better absorbed than lutein from the alginate one.

In humans, dietary supplements of lutein (containing 5% zeaxanthin) at 10 mg/day for 6 months increased the mean serum level of lutein from a baseline value of 210 to 1000 nmol/L and of zeaxanthin from 56 to 95 nmol/L [4].

Albert et al. [5] observed that monkeys fed a xanthophyll-free diet supplemented with either pure lutein or pure zeaxanthin for 12–92 weeks formed 3'-dehydrolutein in plasma. In addition, two 3'-dehydrolutein diastereomers, (3*R*,6'*S*)- and (3*R*,6'*R*)-3'-dehydrolutein, were present in nearly equimolar concentrations. The authors considered these findings to be comparable to those in human plasma after dietary supplementation with either lutein or zeaxanthin at doses from 1 to 20.5 mg/day for 42 days [6, 7].

Toxicological studies

The Committee previously concluded that the NOAEL for lutein was 200 mg/kg bw per day, the highest dose tested in a 13-week rat toxicity study, and 1000 mg/kg bw per day, the highest dose tested in a rat developmental toxicity study. For lutein esters from *Tagetes erecta*, the NOAEL was 1000 mg/kg bw per day (equivalent to 540 mg/kg bw per day of lutein), the highest dose tested in both a 13-week and a developmental toxicity study in rats.

The studies reviewed by the Committee confirm that lutein, zeaxanthin and *meso*-zeaxanthin, tested in their free form, are of very low toxicity. The only substance-related finding was discoloration of faeces and fur or skin in animals at higher doses. The NOAELs in short-term and long-term toxicity studies were approximately 210–400 mg/kg bw per day for lutein, 87.5–260 mg/kg bw per day for zeaxanthin and 200–300 mg/kg bw per day for *meso*-zeaxanthin in rats and 10 mg/kg bw per day for lutein and zeaxanthin in monkeys. These NOAELs were generally the highest dose levels tested [8, 9, 10].

A 2-generation reproductive toxicity study in rats found that administration of zeaxanthin at up to 500 mg/kg bw per day, the highest dose tested, did not cause any adverse effects [11].

Based on previously and newly available data, the Committee considered that lutein, zeaxanthin and *meso*-zeaxanthin did not raise concerns with respect to genotoxicity.

Observations in humans

The Committee previously reviewed the results of clinical studies in which lutein and lutein esters from *Tagetes erecta* were given as nutritional supplements or therapeutic agents for age-related macular degeneration ([Annex 1](#), references 173 and 220). Although these studies were not designed as safety assessments, lutein and lutein esters were found to be well tolerated. This was confirmed based on evaluations of newly available clinical studies. These clinical studies included healthy and preterm infants given lutein in infant formula at concentrations equal to 0.5 mg/kg bw per day for 6 weeks. Doses for adults were up to 10 mg/person per day [4, 12].

Assessment of dietary exposure

At the sixty-third meeting, the Committee estimated mean and 90th percentile dietary exposure to lutein from *Tagetes erecta* as approximately 7 and 13 mg/day, respectively (equivalent to 0.12 and 0.22 mg/kg bw per day, assuming a 60 kg body weight) ([Annex 1](#), reference 173).

At the seventy-ninth meeting, the use of lutein esters from *Tagetes erecta* was considered to be substitutional for the use of lutein from *Tagetes erecta*.

The present Committee estimated exposure to lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic). When used as a food colour, these substances are substitutional on a molar basis, and exposures are expressed as lutein throughout the report. The estimates included exposure from the use in food supplements. Exposure to *meso*-zeaxanthin was not explicitly assessed by the Committee although it is present in some commercial food supplements.

A comprehensive literature search retrieved 20 additional references relevant to the dietary exposure assessment.

Dietary exposure from the use of lutein, zeaxanthin and their esters and zeaxanthin (synthetic) as a food colour was estimated as 0.3 mg/kg bw per day for adults and 1 mg/kg bw per day for children by EFSA [13]. These estimates, based on maximum reported use levels and national consumption data from 11 European countries, were higher than those estimated by the Committee at the sixty-third meeting.

Dietary exposure from the use of lutein, zeaxanthin and their esters and zeaxanthin (synthetic) in food supplements was estimated by the Committee from dosage information on product labels and from intervention studies to be 0.67 mg/kg bw per day for both adults and children.

Dietary exposure from natural occurrence in food was estimated as 0.13 mg/kg bw per day for adults and 0.69 mg/kg bw per day for children. These estimates were based on national food consumption data from China for adults

Table 7

Estimates of high dietary exposure to lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic)

Source of exposure	Estimated dietary exposure, mg/kg bw per day (%) ^a	
	Adults	Children
Food colour	0.4 (33)	1 (42)
Food supplements	0.67 (56)	0.67 (28)
Natural occurrence	0.13 (11)	0.69 (29)
Aggregated exposure	1.2	2.4

bw: body weight

^a Estimated dietary exposure in mg/kg bw per day and, in parentheses, the estimated exposure as a percentage of the total (aggregated) exposure.

[14] and from the Republic of Korea for children [15]. These estimates were also higher than the previous Committee's estimates.

The present Committee estimated a conservative aggregated high dietary exposure from the use of lutein, zeaxanthin and their esters from *Tagetes erecta* and zeaxanthin (synthetic) as food colour and from food supplements in combination with the natural occurrence of these xanthophylls to be 1.2 mg/kg bw per day for adults and 2.4 mg/kg bw per day for children (Table 7).

Evaluation

Free lutein, lutein esters and free zeaxanthin including *meso*-zeaxanthin are biochemically and toxicologically equivalent. The esters of lutein and zeaxanthin are hydrolysed in the gastrointestinal tract, and systemic exposure is to free lutein and zeaxanthin, compounds that differ only in the position of a single double bond. Lutein and zeaxanthin are naturally present in food. In addition, both xanthophylls are essential constituents of the primate retina; biologically controlled mechanisms and pathways exist for absorption of both xanthophylls from the diet and their distribution within the body and deposition in the retina.

The Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from *Tagetes erecta*, synthetic zeaxanthin and *meso*-zeaxanthin. The Committee considered the available toxicological data together with the dietary exposure of the general population.

No adverse effects were observed in a broad range of toxicological studies of free lutein, lutein esters and free zeaxanthin and *meso*-zeaxanthin in laboratory animals and in clinical studies in humans. Results from a new 2-generation reproductive toxicity study of zeaxanthin in rats indicated no adverse effects at up to 500 mg/kg bw per day, the highest dose tested [11].

The estimated dietary exposure from the use of free lutein, lutein esters and free zeaxanthin as colours or food supplements are in the same order of magnitude as the intakes from foods where these xanthophylls are naturally present.

Based on the absence of toxicity in a wide range of studies with NOAELs of approximately 210–400 mg/kg bw per day for lutein, 87.5–500 mg/kg bw per day for zeaxanthin and 200–300 mg/kg bw per day for *meso*-zeaxanthin and estimated dietary exposure of up to 2.4 mg/kg bw per day, the Committee established a group ADI “not specified” for lutein from *Tagetes erecta*, lutein esters from *Tagetes erecta* and zeaxanthin (synthetic).

Meso-zeaxanthin was not included in this group ADI as specifications are not currently available.

The group ADI of 0–2 mg/kg bw for lutein from *Tagetes erecta* and zeaxanthin (synthetic) was withdrawn.

A toxicological and dietary exposure monograph addendum was prepared.

The specifications for lutein from *Tagetes erecta* were revised, and the Chemical and Technical Assessment was updated. The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

References

1. Nolan JM, Meagher K, Kashani S, Beatty S. What is *meso*-zeaxanthin, and where does it come from? *Eye* (Lond). 2013;27(8):899–905.
2. Sheshappa MB, Ranganathan A, Bhatiwada N, Talahalli RR, Vallikannan B. Dietary components affect the plasma and tissue levels of lutein in aged rats with lutein deficiency—a repeated gavage and dietary study. *J Food Sci*. 2015;80(10):2322–30.
3. Evans M, Beck M, Elliott J, Etheve S, Roberts R, Schalch W. Effects of formulation on the bioavailability of lutein and zeaxanthin. A randomized, double-blind, cross-over, comparative, single-dose study in healthy subjects. *Eur J Nutr*. 2013;52(4):1381–91.
4. Khachik F, de Moura FF, Chew EY, Douglass LW, Ferris FL, Kim J et al. The effect of lutein and zeaxanthin supplementation on metabolites of these carotenoids in the serum of persons aged 60 or older. *Invest Ophthalmol Vis Sci*. 2006;47(12):5234–42.
5. Albert GL, Hoeller U, Schierle J, Neuringer M, Johnson EJ, Schalch W. Metabolism of lutein and zeaxanthin in rhesus monkeys. Identification of (3R,6'R)- and (3R,6'S)-3'-dehydro-lutein as common metabolites and comparison to humans. *Comp Biochem Physiol B Biochem Mol Biol*. 2008;151(1):70–8.
6. Hartmann D, Thürmann PA, Spitzer V, Schalch W, Manner B, Cohn W. Plasma kinetics of zeaxanthin and 3'-dehydro-lutein after multiple oral doses of synthetic zeaxanthin. *Am J Clin Nutr*. 2004;79(3):410–7.
7. Thürmann PA, Schalch W, Aebischer JC, Tenter U, Cohn W. Plasma kinetics of lutein, zeaxanthin and 3'-dehydro-lutein after multiple oral doses of a lutein supplement. *Am J Clin Nutr*. 2005;82(1):88–97.
8. Ravikrishnan R, Rusia S, Ilamurugan G, Salunkhe U, Deshpande J, Shankaranarayanan ML et al. Safety assessment of lutein and zeaxanthin (Lutemax 2020): subchronic toxicity and mutagenicity studies. *Food Chem Toxicol*. 2011;49(11):2841–8.

9. Thurnham DI, Howard AN. Studies on *meso*-zeaxanthin for potential toxicity and mutagenicity. *Food Chem Toxicol.* 2013;59:455–63.
10. Ravi KB, Raghunatha Reddy KR, Shankaranarayanan J, Deshpande JV, Juturu V, Soni MG. Safety evaluation of zeaxanthin concentrate (OmniXan™): acute, subchronic toxicity and mutagenicity studies. *Food Chem Toxicol.* 2014;72:30–9.
11. Clode S. Zeaxanthin 10% WS beadlets (Ro 01-9509): Two generation oral (dietary administration) reproduction toxicity study in the rat. Amendment to final report study no.: 2416/001. Unpublished study by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom; 2018. Submitted to WHO by DSM Nutritional Products Ltd, Kaiseraugst, Switzerland.
12. Costa S, Giannantonio C, Romagnoli C, Vento G, Gervasoni J, Persichilli S et al. Effects of lutein supplementation on biological antioxidant status in preterm infants: a randomized clinical trial. *J Matern Fetal Neonatal Med.* 2013;26(13):1311–5.
13. Statement on the safety assessment of the exposure to lutein preparations based on new data on the use levels of lutein. *EFSA J.* 2012;10(3):2589. doi:10.2903/j.efsa.2012.2589.
14. Wang L, Li B, Pan MX, Mo XF, Chen YM, Zhang CX. Specific carotenoid intake is inversely associated with the risk of breast cancer among Chinese women. *Br J Nutr.* 2014;111(9):1686–95.
15. Lee HS, Cho YH, Park J, Shin HR, Sung MK. Dietary intake of phytonutrients in relation to fruit and vegetable consumption in Korea. *J Acad Nutr Diet.* 2013;113(9):1194–99.

3.1.6 Neutral methacrylate copolymer

Explanation

Neutral methacrylate copolymer (NMC; E 1206; INS No. 1206; CAS No. 9010-88-2; ethyl acrylate methyl methacrylate polymer; ethyl acrylate methyl methacrylate polymer; ethyl acrylate polymer with methyl methacrylate; methyl methacrylate ethyl acrylate polymer; methyl methacrylate polymer with ethyl acrylate) is a copolymer manufactured from the monomers ethyl acrylate and methyl methacrylate in the molar ratio of 2:1. NMC is described in the European Pharmacopeia [1] and United States Pharmacopeia and National Formulary [2].

The Committee considered three different copolymers: basic, anionic and neutral methacrylate copolymers. Each copolymer releases the active ingredients from within their coatings under different physiological conditions in different parts of the digestive tract. NMC is insoluble in aqueous media and its release is not pH dependent. NMC is used in sustained release formulations that enable continuous dissolution of an active ingredient over a defined time. Release periods can be controlled by changing the amount of copolymer used.

NMC has not previously been considered by the Committee. The Committee evaluated the use of NMC as a coating or glazing agent for solid food supplements such as capsules, pastilles, tablets, pills, pellets and powders, at levels not exceeding 20%, at the request of CCFA49 [3]. NMC is also used in pharmaceuticals.

Toxicological data submitted for evaluation included absorption, distribution and excretion studies, acute and short-term toxicity and genotoxicity studies as well as a developmental toxicity study. Limited data were also submitted on the residual monomers. A comprehensive literature search retrieved data on the residual monomers but no other studies on NMC. The Committee also considered the data on the residual monomers because the monomers are of low molecular weight and therefore likely to be absorbed from the gastrointestinal tract. Due to the low levels of residual monomers present in NMC, the Committee considered only the ADME, long-term toxicity and genotoxicity data on the monomers.

The Committee was also aware that NMC contains an oligomer fraction of between 0.06% and 0.13%. As the lower end of the molecular weight range for all the constituent oligomers is greater than 5000 Da, it is unlikely that they would be absorbed from the gastrointestinal tract. Therefore, the Committee did not consider the toxicological aspects of the oligomers.

Unless otherwise stated, the test substance used in the absorption, distribution and excretion and toxicity studies was prepared from an aqueous dispersion with 0.7% emulsifier and then freeze-dried to remove water. In all cases, doses have been expressed as dry weight of NMC.

The Committee evaluated toxicological and exposure data on polyethylene glycol monostearyl ether, methanol and ethanol, residual components of NMC that can be present in the final product because they are used in the manufacture of the copolymer. The Committee concluded that these residual components do not pose a safety concern at the maximum estimated exposure levels.

Chemical and technical considerations

NMC is manufactured by emulsion polymerization of the monomers ethyl acrylate and methyl methacrylate with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low molecular weight substances and coagulum.

NMC has a weight-average molecular weight of 600 000 Da and a number-average molecular weight of 220 000 Da.

Although organic solvents are not used in the manufacture of NMC, methanol may be present at a level not exceeding 100 mg/kg and ethanol may be present at a level not exceeding 1000 mg/kg. The copolymer is standardized as a 30% aqueous dispersion with polyethylene glycol monostearyl ether (0.7%). The copolymer dispersion may contain the residual monomers methyl methacrylate (not more than 50 mg/kg) and ethyl acrylate (not more than 20 mg/kg).

Biochemical aspects

NMC

In a pre-GLP study, NMC was found to be poorly absorbed and quickly eliminated from the body when single doses of ^{14}C -labelled NMC at 600 mg/kg bw per day were administered to rats by gavage. An average of 97.6% of the radioactivity was excreted in the faeces within 48 hours. Seven days after dosing, levels of radioactivity in tissues of treated animals did not differ significantly from that of control animals [4].

Residual monomers

Methyl methacrylate is rapidly absorbed and distributed following inhalation or oral administration to rats. Methyl methacrylate is metabolized to methacrylic acid and methanol, which is subsequently converted to carbon dioxide via the tricarboxylic acid cycle in both experimental animals and humans [5, 6, 7].

Ethyl acrylate is rapidly absorbed and metabolized following inhalation or oral administration in rats. Metabolism occurs via hydrolysis of the ester linkage by carboxylesterases, forming ethanol and acrylic acid, both of which are ultimately metabolized to carbon dioxide, or via conjugation of ethyl acrylate with glutathione. Following conjugation with glutathione, ethyl acrylate is rapidly eliminated via urinary excretion [8].

Toxicological studies

NMC

Two acute toxicity studies were available, in rats and in dogs. Rats received doses of 25.2–28.2 g/kg bw in their feed; dogs received doses of 7950 or 9100 mg/kg bw in their feed. No treatment-related effects were seen during the observation period or at macroscopic examination of the organs. Urine analysis also found no treatment-related effects [9, 10].

Two short-term toxicity studies were available in rats. In the first study, doses of 0, 500, 1000 or 2000 mg/kg bw per day were given by gavage for 35 days. It is not clear if the doses were expressed in dry weight of polymer or of the preparation. No treatment-related effects were observed [11]. In the second study, doses of 0, 500 or 2000 mg/kg bw per day were given in the feed for 6 months. No treatment-related effects were observed [12]. In both studies, the NOAELs were 2000 mg/kg bw per day, the highest doses tested. In a 26-week study, dogs were given NMC at doses of 0, 50, 125 or 250 mg/kg bw per day administered as NMC-coated cellulose pellets. Apart from decreases in body-weight gain and feed consumption at the highest dose, and the presence of white granular material in the gut, attributed to the physical characteristics of the test material, no treatment-related effects were observed. The NOAEL was 250 mg/kg

bw per day, the highest doses tested [13]. A 28-day study in mini pigs, in which NMC was given by gavage as NMC-coated cellulose pellets, the calculated doses were 0, 113, 227 and 454 mg/kg bw per day. Other than the presence of white granular material in the intestines, attributed to the physical characteristics of the test substance, no treatment-related effects were found. The NOAEL was 454 mg/kg bw per day, the highest dose tested [14].

Two reverse mutation studies in bacteria, one in vitro mouse lymphoma assay and an in vivo mouse micronucleus assay gave negative results. The Committee concluded that the NMC does not give rise to concern for genotoxicity.

No long-term toxicity or carcinogenicity studies with NMC were available.

Developmental toxicity studies were available in rats and rabbits, both using doses of 0, 500 or 2000 mg/kg bw per day in the feed during the period of organogenesis. No treatment-related effects were observed. The NOAEL for both studies was 2000 mg/kg bw per day, the highest doses tested.

Studies on cytotoxicity and dermal, inhalation and ocular toxicity found no effects.

Residual monomers

Methyl methacrylate

In a long-term toxicity study in rats given methyl methacrylate in drinking-water at 0, 6, 60 or 2000 mg/L (equal to 0, 0.4, 4 and 121 mg/kg bw per day for males and 0, 0.5, 5 and 146 mg/kg bw per day for females, respectively) for 2 years, relative kidney weight increased in females at the highest dose but no treatment-related histopathological effects were observed in any organs or tissues [15]. Based on the results of this study, a TDI of 1.2 mg/kg bw per day was determined [16].

In long-term toxicity and carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, the observed effects were, in general, similar to those reported in the short-term toxicity studies but also included inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium. There was no evidence of any carcinogenic effects [15].

Bacterial reverse mutation assays with methyl methacrylate gave mostly negative results. Mixed results (i.e. positive, weakly positive or negative) were obtained in in vitro chromosomal aberration and SCE assays. One in vitro micronucleus assay was unequivocally negative, whereas a second assay was negative at low concentrations but weakly positive at higher concentrations. Three mouse lymphoma assays for gene mutations were positive. A mouse bone marrow micronucleus assay was negative, but it is not clear if the target tissue was exposed to the test substance. A rat micronucleus assay with exposure by inhalation was positive following 1 day of exposure but negative following 5 days

of exposure. These results were judged by the Committee to be inconclusive. The Committee concluded that there was some evidence of mutagenicity and clastogenicity *in vitro*. There was a lack of adequate *in vivo* tests following up the equivocal findings.

Ethyl acrylate

Studies in mice and rats administered ethyl acrylate at 0, 100 or 200 mg/kg bw per day for 103 weeks by gavage showed an increased incidence of squamous cell hyperplasia and papillomas in the forestomach of both species, and, in male rats, squamous cell carcinomas in the forestomach. In contrast, studies using other routes of administration including via inhalation (with concentrations up to 310 mg/m³) and in the drinking-water (with doses up to 280 mg/kg bw per day) showed no such effects. An IARC working group concluded that the mechanism of formation of the forestomach tumours in rodents is not relevant to humans; rather it can be attributed to the irritating effect of high bolus doses of ethyl acrylate delivered to the contact site (forestomach) by gavage [17]. This conclusion was recently confirmed by Health Canada [8]. The Committee concluded that ethyl acrylate is not a carcinogenic risk to humans.

Genotoxicity results for ethyl acrylate are mixed, with some positive results *in vitro*, some negative results in reverse mutation assays in bacteria and positive results in a mouse lymphoma assay. There were both positive and negative results in *in vitro* SCE assays and in chromosomal aberration assays with metabolic activation, but no evidence of clastogenicity in the absence of metabolic activation.

In *in vivo* studies on ethyl acrylate, two early mouse micronucleus studies using intraperitoneal dosing gave positive results, although one of these gave positive results in only one of the five experiments carried out [18, 19]. However, three other micronucleus studies in mice (one dermal exposure, two by intraperitoneal injection) all gave negative results ([Annex 1](#), reference 174). A chromosomal aberration assay and an *in vivo* SCE assay and a recent point mutation assay in mice were also negative. The Committee concluded that the genotoxic potential observed in some *in vitro* studies was not expressed *in vivo*. The Committee noted that Health Canada [8] reached a similar conclusion.

Observations in humans

No human data were available on NMC.

Assessment of dietary exposure

The Committee evaluated exposure to NMC from its use as a glazing or coating agent in food supplements and foods for special medical purposes. As another

major use of NMC is in pharmaceuticals, this use was also considered in the exposure assessment. The level of use of NMC is a maximum of 20%.

The Committee evaluated exposure to NMC for the copolymer and its monomers, methyl methacrylate and ethyl acrylate. The exposure assessment included estimates submitted by the sponsor and an evaluation by EFSA [20] based on consumption of food supplements and pharmaceuticals. The Committee also estimated exposure based on national food consumption data for food supplements using the concentration proposed by the sponsor. The national consumption data were from CIFOCoSS and data submitted to the Committee from Australia and New Zealand. A comprehensive literature search was also conducted; no additional studies relevant to the exposure assessment were found.

No quantitative estimates of exposure could be determined for foods for special medical purposes. The sponsor indicated that it is not anticipated that foods for special medical purposes would increase exposures above that of food supplements and pharmaceuticals given the conservative nature of those calculations. In addition, the consumers of foods for special medical purposes will generally be under medical supervision, and exposures for these consumers are therefore not relevant for the general healthy population. This use was therefore not further considered by the Committee.

The total monomeric content of NMC is less than 0.01%. This level was used to calculate the exposure to total monomers from the copolymer exposure. Estimates of exposure to the individual monomers were based on the exposure to total monomers, taking into account the ratio of each individual monomer in the copolymer.

All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures.

The estimated exposures to NMC and its monomers from uses in food supplements are shown in [Table 8](#).

The Committee noted that NMC is used in pharmaceuticals. Estimated exposures from this use from the sponsor and EFSA [20] ranged between 10.0 and 23.3 mg/kg bw per day for adults and children. These estimates were within the range of exposures from food supplements. However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure for a healthy population.

Evaluation

New specifications for NMC were prepared and made tentative, requiring a suitable validated method for its assay. A Chemical and Technical Assessment was prepared.

The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the

Table 8

Summary of range of estimated exposures to NMC and its monomers from uses in food supplements for average and high exposures

Population group	Range of estimated dietary exposures ^{a,b}		
	Copolymer exposure (mg/kg bw per day)	Monomer exposure (µg/kg bw per day)	
		Methyl methacrylate	Ethyl acrylate
Adults	6.9–74	0.2–2.2	0.5–5.2
Children	5.8–86	0.2–2.5	0.4–6.0

bw: body weight; NMC: neutral methacrylate copolymer

^a All estimates of exposure are presented as a range from the lowest of the average exposures to the highest of the high exposures. The lower end of each range is the lowest of the estimated mean exposures, and the upper end of each range is the highest of the estimated high exposures.

^b Includes exposure estimates submitted by the sponsor and EFSA (2010) and national estimates calculated by the Committee, based on a concentration of 200 mg per 1 g dosage unit.

food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes.

The Committee therefore established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative.

The available toxicology data for NMC do not give rise to concerns for toxicity. The substance is poorly absorbed and is excreted in the faeces. In short-term and developmental toxicity studies, the NOAELs for NMC range from 454 to 2000 mg/kg bw per day, and these were the highest doses tested. Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day.

Toxicological data on the residual monomers do not give rise to concerns when taking into account the low exposures. Genotoxicity data for methyl methacrylate suggest a potential risk for mutagenicity and clastogenicity in vitro, and there is a lack of adequate data on genotoxicity in vivo. However, in carcinogenicity studies in mice, rats and hamsters given methyl methacrylate by inhalation, there was no evidence of any carcinogenic effects. In a 2-year drinking-water study on methyl methacrylate in rats, the NOAEL was 121 mg/kg bw per day, from which a TDI of 1.2 mg/kg bw per day was derived [16]. Estimated exposures to methyl methacrylate range from 0.2 to 2.5 µg/kg bw per day, which are below the TDI.

Although there were some positive genotoxicity findings for ethyl acrylate, the Committee concluded that the genotoxic potential observed in some in vitro studies was not expressed in vivo. Long-term toxicity studies on ethyl acrylate in mice and rats produced forestomach tumours, but the Committee concurred with the conclusions of IARC [17] and, more recently, Health Canada [8], that the mechanism of forestomach tumour formation in rats and mice is not relevant to humans. The Committee was also reassured by the long-term carcinogenicity studies on ethyl acrylate that did not use gavage as the route of

administration; these suggest that ethyl acrylate is not carcinogenic at the doses tested. In the 2-year drinking-water study on ethyl acrylate, the NOAEL was 280 mg/kg bw per day. Estimated dietary exposures to ethyl acrylate range from 0.4 to 6 µg/kg bw per day. The margin of exposure (MOE) based on the highest estimated exposure was calculated to be 46 000.

Assessments of dietary exposure to methyl methacrylate and ethyl acrylate due to their residual occurrence in NMC suggest that exposure to these monomers from the uses of NMC is not a safety concern.

A toxicological and dietary exposure monograph was prepared.

References

1. Polyacrylate Dispersion 30 Per Cent. 9 01/2017:0733. European Pharmacopeia; 2017.
2. United States Pharmacopeia and National Formulary (USP 41-NF 36); Ethyl acrylate and methyl methacrylate copolymer dispersion. Vol. 1. Rockville, Maryland: United States Pharmacopeial Convention; 2017.
3. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).
4. The excretion and tissue distribution of radioactivity after oral administration to rats. Huntingdon Life Sciences, Suffolk, England, United Kingdom; 1986. Report no. RTS 010/984481(2nd amendment). Unpublished study report submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
5. Bratt H, Hathway DE. Fate of methyl methacrylate in rats. *Br J Cancer*. 1977;36(1):114–9 [cited in reference 16].
6. Canadian Environmental Protection Act: Priority Substances List assessment report: Methyl methacrylate. Prepared by Health Canada and Environment Canada. Ottawa: Government of Canada; 1993 (Cat. No.: En40-215/10E) [cited in reference 16].
7. Methyl methacrylate – CAS No. 80-62-6. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals; 1995. (Joint Assessment of Commodity Chemicals No. 30) [cited in reference 16].
8. Screening assessment for the challenge 2-propenoic acid, ethyl ester (ethyl acrylate). Chemical Abstracts Service Registry Number 140-88-5. Ottawa (ON): Environment Canada, Health Canada; 2011.
9. The acute toxicity of preparations 2807B and 2837B on oral administration in rats. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1971. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
10. The acute toxicity of preparations 2807B and 2837B on oral administration in dogs when administered in the feedstuff. Unpublished study by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1972a. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
11. Subacute toxicity study with rats by oral administration for one month and the recovery study. Unpublished study by BoZo Research Centre, Co Ltd, Japan; 1981. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.

12. Six-month toxicity of 2837D in Sprague Dawley rats by administration in the diet. Unpublished study report by Laboratory for Pharmacology and Toxicology, Hamburg-Hausbruch, Germany; 1972b. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
13. 26-Week oral toxicity study in dogs followed by a 3-week recovery period. Unpublished study report by Research Toxicology Centre, Rome, Italy; 2007a. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
14. 4-Week oral toxicity study in minipigs. Unpublished study report no. 31030 by Research Toxicology Centre, Rome, Italy; 2006. Submitted to WHO by Evonik Nutrition and Care GmbH, Darmstadt, Germany.
15. Borzelleca JF, Larson PS, Hennigar GR Jr, Huf EG, Crawford EM et al. Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. *Toxicol Appl Pharmacol.* 1964;6:29–36 [cited in reference 16].
16. Methyl methacrylate. Geneva: World Health Organization; 1998. (International Programme on Chemical Safety II. Concise International Chemical Assessment Document No. 4).
17. Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluation carcinogenic risks to humans. Views and Expert Opinions of an IARC Working Group. Lyon: International Agency for Research on Cancer, World Health Organization; 2003 (IARC Technical Publication No. 39) [cited in [Annex 1](#), reference 174].
18. Przybojewska B, Dziubałtowska E, Kowalski Z. Genotoxic effects of ethyl acrylate and methyl acrylate in the mouse evaluated by the micronucleus test. *Mutat Res.* 1984;135(3):189–91.
19. Ashby J, Richardson CR, Tinwell H. Inactivity of ethyl acrylate in the mouse bone marrow micronucleus assay. *Mutagenesis.* 1989;4(4):283–5.
20. Scientific Opinion on the safety of anionic methacrylate copolymer for the proposed uses as a food additive. *EFSA J.* 2010;8(7):1655. doi: 10.2903/j.efsa.2010.1656.

3.1.7 Sorbitol syrup

Explanation

Sorbitol syrup (INS No. 420(ii)) is currently included in the Codex GSFA ([Annex 3](#)) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. At its Fiftieth Session, the Codex Committee on Contaminants in Food Additives [1] requested the Committee to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances and advise on the need for a separate evaluation of sorbitol syrup or if the ADI “not specified” for sorbitol is also applicable for sorbitol syrup.

Evaluation

The Committee acknowledges that sorbitol and sorbitol syrup are chemically similar but distinct substances that should be assigned separate ADIs.

Sorbitol syrup consists of not less than 99.0% of hydrogenated saccharides and not less than 50.0% of D-sorbitol on an anhydrous basis. The D-sorbitol portion of sorbitol syrup is not of toxicological concern, and the Committee

established an ADI “not specified” for sorbitol at its twenty-sixth meeting ([Annex 1](#), reference 61).

The remaining portion of sorbitol syrup consists of other hydrogenated saccharides. These hydrogenated saccharides were evaluated previously by the Committee. Evaluations of maltitol syrup ([Annex 1](#), reference 83) and polyglycitol syrup ([Annex 1](#), reference 137) concluded that the presence of maltitol and higher-order polyols in these syrups was not of toxicological concern, and each of the syrups was assigned an ADI “not specified”. The evaluations of these syrups were based on toxicological findings and biochemical aspects.

The hydrogenated saccharides are poorly absorbed. They are partially hydrolysed by enzymes in the gastrointestinal tract and also metabolized by colonic microflora. The previous Committee concluded that these substances were fully metabolized into natural body constituents ([Annex 1](#), reference 70) and not of toxicological concern.

Based on the similarity of the chemical constituents of sorbitol syrup to sorbitol, maltitol syrup and polyglycitol syrup, the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI “not specified” for sorbitol syrup.

No toxicological monograph was prepared.

Reference

1. Report of the Fiftieth Session of the Codex Committee on Food Additives, Xiamen, China, 26–30 March 2018. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2018 (REP18/FA).

3.1.8 *Spirulina* extract

Explanation

Spirulina extract (INS No. 134) is used as a food additive, as a blue colouring agent. *Spirulina* extract is used in food in China, the European Union, Japan, Mexico and the USA, among others. It is used as a colour in a wide range of foods and beverages including flavoured dairy products, cheese, dairy-based desserts, processed fruits and vegetables, baked goods and baking mixes, alcoholic and non-alcoholic beverages and beverage bases, breakfast cereals, cocoa products, confectionery products (including soft and hard candy and chewing gum), egg products, gravies and sauces, herbs and spices, condiments and soup and soup mixes. It is also used as a colouring agent in nutritional supplements and in pharmaceuticals. Intended use levels range from 400 to 40 000 mg/kg, depending on the food item or category and the colour strength of the formulated *spirulina* extract used.

Spirulina extract has not been evaluated previously by the Committee. It was placed on the agenda for its use as a food colour at the request of CCFA49 [1].

A toxicological dossier submitted by the sponsor summarized the available toxicity data on spirulina, together with relevant study reports and publications. A few of the studies were conducted using spirulina extract, but most toxicity studies used dried spirulina as the test material. A comprehensive literature search retrieved an additional 17 relevant toxicological study reports.

Chemical and technical considerations

Spirulina extract is produced from *Arthrospira platensis* (commonly called *Spirulina platensis*), an edible cyanobacterium cultivated in open or covered ponds or in bioreactors. Commercial cultivation occurs in alkaline aqueous medium containing sodium bicarbonate, nitrates, phosphates, sulfates and other nutrients including trace minerals. No herbicides or additional solvents are used during the cultivation of *A. platensis* or in the manufacture of spirulina extract. Cultivation conditions are optimized to control for contaminating organisms.

Following cultivation, the culture is harvested, concentrated, washed and prepared as a paste or dried to a powder. The dried or fresh biomass undergoes aqueous extraction and pH adjustment, as needed. Following extraction, the mixture undergoes centrifugation and filtration to remove cell debris and water-insoluble components. The resulting aqueous phase contains proteins, carbohydrates, minerals and two phycobiliproteins (also referred to as phycocyanins) that impart the blue colour. The mixture is concentrated to the desired pigment concentration and then pasteurized and/or sterilized before packaging. Spirulina extract products may undergo additional standardization and/or drying to achieve the desired formulation and pigment concentration. Commercially available products occur in either liquid (aqueous) or powder form with a wide range of pigment concentrations.

The primary colouring principles in spirulina extract are C-phycocyanin (CAS No. 11016-15-2; EINECS No. 234-248-8; ~30 kDa) and allophycocyanin (no CAS number assigned; ~105 kDa), in various ratios, with C-phycocyanin occurring in higher proportions. Phycocyanins are complexes of proteins with the pigment molecule phycocyanobilin [2]. The total content of C-phycocyanin and allophycocyanin in spirulina extract varies depending on the desired colour effect and degree of dilution of the extract. Phycocyanin concentrations in spirulina extract range from 1.5–15% in liquid products to 1.5–65% in powder products, as the sum of C-phycocyanin and allophycocyanin.

Commercial spirulina extracts typically contain peptides and proteins (10–90%, dry weight, including the proteins complexed with phycocyanobilin), carbohydrates and polysaccharides (≤65%, dry weight), fat (<1%, dry weight), fibre (<6%, dry weight), minerals/ash (<6%, dry weight) and water (<6% for

powder products and $\leq 95\%$ for liquid formulations). Spirulina extract may contain trace amounts ($<1\%$) of carotenoids and chlorophylls, which are largely removed during production.

Biochemical aspects

ADME data on spirulina extract are not available.

Spirulina extract is described as consisting of various proportions of proteins and carbohydrates as well as much smaller amounts of fibre and fats. These extract components are digested through normal biochemical pathways like other common dietary constituents.

An in vitro simulated gastric fluid digestion assay demonstrated that the protein portion of the C-phycocyanin is rapidly digested by pepsin into small chromopeptides, consisting of two to 13 amino acids [3]. Because the chemical structure of the chromophore phycocyanobilin is very similar to biliverdin, a non-reduced form of bilirubin [4], phycocyanobilin is expected to be metabolized and excreted like bilirubin, through the bile and into the faeces [5, 6].

No evidence of bioaccumulation of coloured matter was observed in repeated-dose toxicity studies in laboratory animals fed spirulina extract or dried spirulina.

Toxicological studies

As noted, most of the tests conducted used dried spirulina and not spirulina extract. However, based on the similarity of the constituents and the high concentrations of the dried spirulina test materials used in the toxicity studies, the Committee considered this acceptable in the evaluation of spirulina extract. In some studies conducted with spirulina extract, the phycocyanin content was reported; no study conducted with dried spirulina reported its phycocyanin content. The Committee also noted that the source of the test material in some studies was *Spirulina maxima*, as opposed to *S. platensis*. Given the chemical, genetic and nutritional similarity of these two species of edible cyanobacteria [7, 8, 9], this was considered acceptable in the evaluation of spirulina extract. Most reports did not state if the studies were GLP or guideline compliant. However, the Committee concluded that the studies were of acceptable quality and the findings valid.

In acute gavage toxicity studies, no clinical signs of toxicity were observed in mice administered spirulina extract (phycocyanin content not reported) at a dose of up to 3000 mg/kg bw [10]; in rats administered spirulina extract (24–26% phycocyanin content) at a dose of up to 5000 mg/kg bw [11, 12]; or in rats administered dried spirulina at a dose of up to 10 000 mg/kg bw [13].

No toxicity was seen in rats administered spirulina extract (24% phycocyanin content) by gavage at a dose of 3000 mg/kg bw per day for 14 days

[12]; in rats administered spirulina extract (phycocyanin content not reported) by gavage at doses of up to 4000 mg/kg bw per day for 12 weeks [14]; in rats fed diets with spirulina extract (26% phycocyanin content) at a concentration of 0.4% (equivalent to 400 mg/kg bw per day) for 14 weeks [11]; or in rats in a long-term toxicity study where the dietary concentration of spirulina extract (8–9% phycocyanin content) was 1.0% (equivalent to 500 mg/kg bw per day) for 12 months [15].

In a short-term mouse toxicity study conducted with dietary concentrations of dried spirulina of up to 5% (equivalent to 7500 mg/kg bw per day) for 6 months, the NOAEL was the highest concentration tested [16]. In a set of well-conducted toxicity studies conducted with dietary concentrations of dried spirulina of up to 30% for 13 weeks, the NOAELs were the highest concentrations tested (equivalent to 45 000 and 30 000 mg/kg bw per day in mice and rats, respectively) [17, 18]. Several additional short-term feeding studies conducted with dried spirulina in mice [19] and rats [13, 20, 21] showed no toxicity under the conditions of the studies. Similarly, no evidence of systemic toxicity or carcinogenicity was observed in long-term feeding studies in rats where the dietary concentration of dried spirulina was 30% (equivalent to 15 000 mg/kg bw per day) for 84 weeks [22].

With dried spirulina, a dose-related increase in relative seminal vesicle weight was the only treatment-related effect observed in 13-week-long toxicity studies in mice [18] and rats [17]. This finding was not reproduced in other short-term or long-term toxicity studies where seminal vesicle weight was investigated. As there were no histopathological changes in the seminal vesicles and no effects on reproduction in the test animals, the Committee considered this finding of no toxicological relevance.

A bacterial reverse mutation assay and in vitro and in vivo chromosomal aberration assays with spirulina extract (26% phycocyanin content) showed no evidence of genotoxicity [12]. Dominant lethal assays conducted in mice and rats with dried spirulina also showed no evidence of genotoxicity under the conditions of the assays [23, 24].

There was no evidence of reproductive toxicity when mice and rats were fed dietary concentrations of dried spirulina of up to 30% (equivalent to 45 000 and 18 000 mg/kg bw per day, respectively) prior to mating (male mice and rats for 9 weeks, female mice for 8 weeks, female rats for 2 weeks) and during mating and gestation. No toxicity was observed in dams or pups when mice, rats and hamsters were fed dietary concentrations of dried spirulina up to 30% (equivalent to 45 000, 30 000 and 27 000 mg/kg bw per day, respectively) over the entire gestation period [25, 26, 27].

In a 2-generation toxicity study in which dosing was limited to F₀ parental females from gestation day 15 through to lactation day 21, mice were fed

diets containing dried spirulina at 0, 10%, 20% or 30% (equivalent to 0, 15 000, 30 000 and 45 000 mg/kg bw per day, respectively). F_1 and F_2 offspring were not directly exposed to the test material. At 30% dried spirulina in the diet, reduced F_1 pup weight at birth and reduced F_1 survival rate on postnatal days 0–4 were reported; no effects were observed in the F_2 animals [26]. The Committee noted that there was no effect on pup survival rate in a 2-generation toxicity study in which F_0 female rats were exposed from gestation day 17 to lactation day 21, in a dosing schedule similar to that used in the Chamorro et al. mouse study [26, 28]. There were also no effects on fetal weight or pup weight at birth in several other reproductive and developmental toxicity studies in the mouse, rat and hamster.

Nutritional studies of dried spirulina fed to rats, rabbits, pigs, sheep and cows showed that the animals maintained good health [29] even when the dietary concentrations were very high (e.g. up to 40% of the diets in rats, equivalent to 40 000 mg/kg bw per day). From a toxicological perspective, these nutritional studies were limited in terms of their observations.

Observations in humans

Observations in humans included case reports, clinical studies and nutritional studies.

The case reports of adverse effects were relatively few considering the long history and widespread use of dried spirulina as a food ingredient and dietary supplement [30]. A few reports cited adverse immunological reactions, such as allergy, associated with the ingestion of dried spirulina dietary supplements. The Committee noted that dried spirulina was well tolerated in clinical and nutritional studies conducted with gram quantities consumed daily for months.

Assessment of dietary exposure

Dietary exposure to spirulina extract from its use as a food colour was assessed by the present Committee.

A comprehensive literature search retrieved nine studies relevant for the assessment of dietary exposure. Consumption of spirulina or components of spirulina occurs from uses other than food colour, that is, dried spirulina in dietary supplements, dried spirulina and spirulina extract as food ingredients, and spirulina extract in coatings for dietary supplements and pharmaceuticals. In order to assess the aggregated dietary exposure from these uses, exposures have been normalized based on phycocyanin content. This was considered appropriate since the colour component of spirulina extract is due to its content of phycocyanins (C-phycocyanin and allophycocyanin). This approach allowed for a comparison of the two test substances used in the toxicological assessments, dried spirulina and spirulina extract.

Dietary exposure expressed as phycocyanins from the use of spirulina extract as a food colour was estimated using the budget method [31]. The theoretical maximum daily exposure was estimated to be 123 and 385 mg/kg bw per day for adults and children respectively. The conversion to phycocyanin content was based on the content of phycocyanins (28%) in the spirulina extracts proposed for use by the sponsor.

Dietary exposure expressed as phycocyanins from the use of dried spirulina in dietary supplements was estimated to be 33 and 133 mg/kg bw per day for adults and children, respectively. This estimate was based on dosage information on product labels and from intervention studies. The conversion to phycocyanin content was based on phycocyanin content (20%) in products from the high end of the range reported in Generally Recognized as Safe (GRAS) notices for commercial dried spirulina.

Dietary exposure expressed as phycocyanins from the use of dried spirulina or spirulina extract as food ingredients was estimated for high consumers to be 33 and 133 mg/kg bw per day for adults and children, respectively. This estimate was based on proposed uses in four GRAS notices, with the conversion to phycocyanin content based on information in the GRAS documentation.

Dietary exposure expressed as phycocyanins from use of spirulina extract in coatings of dietary supplements was estimated for high consumers to be 0.1 and 0.2 mg/kg per day for adults and children, respectively. The conversion to phycocyanin content was based on the assumption that the concentration was the same as for food colour, that is, 28% in spirulina extract.

The Committee noted that spirulina extract could be used in coatings of pharmaceuticals comparable to the use in coatings for dietary supplements, resulting in a similar dietary exposure (0.1 mg/kg bw per day for adults and 0.2 mg/kg bw per day for children). However, the Committee considered that such use should not be taken into account in the assessment of long-term dietary exposure in a healthy population.

The Committee estimated a conservative aggregated exposure to dried spirulina and spirulina extract from all the assessed uses to be 190 and 650 mg/kg bw per day for adults and children, expressed as phycocyanins (Table 9). Based on this assessment, the estimated exposure to phycocyanins from the use of dried spirulina and spirulina extract as a food colour contributes approximately 60% to this total exposure and as dietary supplements and food ingredients contributes approximately 20% each, while the contribution from the use in coatings of dietary supplements is negligible.

Evaluation

The Committee established a temporary ADI “not specified” for spirulina extract. The ADI was based on the absence of toxicity in repeated-dose animal studies

Table 9
Estimates of dietary exposure to phycocyanins from the use of spirulina products

Use (source of phycocyanins)	Exposure to phycocyanins, mg/kg bw per day (%) ^a	
	Adults	Children
Food colour (spirulina extract)	123 (65)	385 (59)
Dietary supplements (dried spirulina)	33 (17)	133 (20)
Food ingredients (dried spirulina and spirulina extract)	33 (17)	133 (20)
Dietary supplement coating (spirulina extract)	0.1 (0.06)	0.2 (0.03)
Aggregated exposure	190	650

bw: body weight

^a Estimated dietary exposure in mg/kg bw per day and, in parentheses, the estimated exposure as a percentage of the total (aggregated) exposure.

conducted with spirulina extract and dried spirulina. These included well-conducted short-term toxicity studies in mice and rats fed dried spirulina at doses of up to 45 000 and 30 000 mg/kg bw per day, respectively. Assuming a phycocyanin content of 10% based on commercial dried spirulina, the doses of phycocyanin were estimated to be 4500 and 3000 mg/kg bw per day, respectively. No evidence of carcinogenicity or systemic toxicity was observed in long-term toxicity studies in rats fed spirulina extract or dried spirulina. There were no concerns regarding genotoxicity. Reproductive and developmental toxicity were not of concern based on the absence of toxicity in feeding studies conducted with dried spirulina in mice, rats and hamsters.

Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour, based on the budget method, and exposure to spirulina extract and dried spirulina from other dietary sources including food ingredients, dietary supplements and coatings of dietary supplements was 190 mg/kg bw for a 60 kg adult and 650 mg/kg bw for a 15 kg child. The Committee concluded that this dietary exposure does not present a health concern.

The ADI “not specified” was made temporary due to the tentative nature of the specifications.

A toxicological and dietary exposure monograph was prepared.

A new tentative specifications monograph and a Chemical and Technical Assessment were prepared.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

2. Glazer AN, Fang S. Chromophore content of blue-green algal phycobiliproteins. *J Biol Chem.* 1973;248(2):659–62.
3. Minic SL, Stanic-Vucinic D, Mihailovic J, Krstic M, Nikolic MR, Cirkovic Velickovic T. Digestion by pepsin releases biologically active chromopeptides from C-phycocyanin, a blue-colored biliprotein of microalga *Spirulina*. *J Proteomics.* 2016;147:132–9.
4. Schram BL, Kroes HH. Structure of phycocyanobilin. *Eur J Biochem.* 1971;19(4):581–94.
5. McCarty MF. Clinical potential of spirulina as a source of phycocyanobilin. *J Med Food.* 2007;10(4):566–70.
6. Eriksen NT. Production of phycocyanin – a pigment with applications in biology, biotechnology, foods and medicine. *Appl Microbiol Biotechnol.* 2008;80(1):1–14.
7. Ciferri O. *Spirulina*, the edible microorganism. *Microbiol Rev.* 1983;47(4):551–78.
8. Scheldeman P, Baurain D, Bouhy R, Scott M, Mühling M, Whitton BA et al. *Arthrospira* ('*Spirulina*') strains from four continents are resolved into only two clusters, based on amplified ribosomal DNA restriction analysis of the internally transcribed spacer. *FEMS Microbiol Lett.* 1999;172(2):213–22.
9. Kwei CK, Lewis D, King K, Donohue W, Nielan BA. Molecular classification of commercial *Spirulina* strains and identification of their sulfolipid biosynthesis genes. *J Microbiol Biotechnol.* 2011;21(4):359–65.
10. Romay C, Ledón N, González R. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm Res.* 1998;47(8):334–8.
11. Naidu AK, Sarada R, Manoj G, Khan MY, Swamy MM, Viswanatha S et al. Toxicity assessment of phycocyanin – a blue colorant from blue green alga *Spirulina platensis*. *Food Biotechnol.* 1999;13(1):51–66.
12. Chen YH, Chang GK, Kuo SM, Huang SY, Hu IC, Lo YL et al. Well-tolerated *Spirulina* extract inhibits influenza virus replication and reduces virus-induced mortality. *Sci Rep.* 2016;6(24253):1–11.
13. Hutadilok-Towatana N, Reanmongkol W, Satitit S, Panichayupakaranant P, Ritthisunthorn P. A subchronic toxicity study of *Spirulina platensis*. *Food Sci Technol Res.* 2008;14(4):351–58.
14. Song L, Liu B, Zhao Y, Tian J, Qin S. Chronic toxicity study of phycocyanin on Sprague Dawley rats. *Chin Med Herald.* 2012;9(33):15–21.
15. Takemoto K. Chronic toxicity study of phycocyanin (Linablu A). Unpublished study by Saitama Medical School, Moroyama, Saitama Prefecture, Japan; 1980. Submitted to WHO by the International Association of Color Manufacturers (IACM), Washington, DC, USA.
16. Yang Y, Park Y, Cassada DA, Snow DD, Rogers DG, Lee J. In vitro and in vivo safety assessment of edible blue-green algae, *Nostoc commune* var. *sphaeroides* Kützting and *Spirulina plantensis*. *Food Chem Toxicol.* 2011;49(7):1560–4.
17. Chamorro GA, Herrera G, Salazar M, Salazar S, Ulloa V. Subchronic toxicity study in rats fed spirulina. *J Pharm Belg.* 1988;43(1):29–36.
18. Salazar M, Martinez E, Madrigal E, Ruiz LE, Chamorro GA. Subchronic toxicity study in mice fed *Spirulina maxima*. *J Ethnopharmacol.* 1998;62(3):235–41.
19. Yang Y, Kim B, Park YK, Lee JY. Effects of long-term supplementation of blue-green algae on lipid metabolism in C57BL/6J mice. *J Nutrit Health Food Sci.* 2014;1(1):1–6.

20. Vidé J, Romain C, Feillet-Coudray C, Bonafos B, Cristol JP, Fouret G et al. Assessment of potential toxicological aspects of dietary exposure to silicon-rich spirulina in rats. *Food Chem Toxicol.* 2015;80:108–13.
21. Bigagli E, Cinic L, Niccolai A, Tredici MR, Biondi N, Rodolfi L et al. Safety evaluations and lipid-lowering activity of an *Arthrospira platensis* enriched diet: a 1-month study in rats. *Food Res Int.* 2017;102:380–6.
22. Chamorro-Cevallos G. Toxicologic study of the spirulina algae pilot plant for the production of proteins (Spirulina de Soso, Texcoco LLP). Vienna: United Nations Industrial Development Organization (UNIDO); 1980 (UNIDO/IO 387).
23. Chamorro G, Salazar M. Dominant lethal assay of *Spirulina maxima* in male CD-1 mice after short-term and prolonged-term feeding. *J Food Prot.* 1989;52(2):125–7.
24. Chamorro G, Salazar M, Pages N. Dominant lethal study of *Spirulina maxima* in male and female rats after short-term feeding. *Phytother Res.* 1996;10:28–32.
25. Chamorro G, Salazar S, Salazar M, Pages N. [Evaluation of the teratological effect of Spirulina in hamster.] *Belg J Food Chem Biotech.* 1987;42:188–91 (in French).
26. Chamorro G, Salazar S, Favila-Castilo L, Steele C, Salazar M. Reproductive and peri- and postnatal evaluation of *Spirulina maxima* in mice. *J Appl Phycol.* 1997;9(2):107–12.
27. Chamorro G, Salazar M. Teratogenic study of Spirulina in mice. *Arch Latinoam Nutr.* 1990;40(1):86–94.
28. Salazar M, Chamorro GA, Salazar S, Steele CE. Effect of *Spirulina maxima* consumption on reproduction and peri- and postnatal development in rats. *Food Chem Toxicol.* 1996;34(4):353–9.
29. Madeira M, Cardoso C, Lopes P, Coelho D, Afonso C, Bandrra N et al. Microalgae as feed ingredients for livestock production and meat quality: A review. *Livest Sci.* 2017;205:111–21.
30. Marles RJ, Barrett ML, Barnes J, Chavez ML, Gardiner P, Ko R et al. United States pharmacopeia safety evaluation of spirulina. *Crit Rev Food Sci Nutr.* 2011;51(7):593–604.
31. Hansen SC. Conditions for use of food additives based on a budget for an acceptable daily intake. *J Food Prot.* 1979;42:429–32.

3.2 Revision of specifications and analytical methods

3.2.1 Cassia gum

Cassia gum was on the agenda at the request of the Committee at its eighty-second meeting. The specifications for cassia gum were made tentative by the eighty-second JECFA ([Annex 1](#), reference 230) pending submission of a validated method for the determination of anthraquinones. The Committee, at the current meeting, considered the analytical methods provided and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with *n*-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified.

The tentative specifications were revised and the tentative status was removed. The Chemical and Technical Assessment was revised.

3.2.2 Citric and fatty acid esters of glycerol

Citric and fatty acid esters of glycerol (CITREM) was on the agenda at the request of the Committee at its seventy-ninth and eighty-second meetings ([Annex 1](#), references 174 and 230), requesting a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in the specifications monographs. The Committee did not receive any suitable replacement method. The specifications for CITREM were made tentative, pending a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.

The Committee noted that the method for total glycerol still uses chloroform. The Committee encourages the submission of a method for total glycerol that eliminates the use of chloroform.

The Committee noted that neutralizing agents, other than sodium and potassium hydroxides, are in use for the partial or full neutralization of CITREM products intended for some food applications. The Committee noted that a request was made to extend the definition of CITREM to include the use of alternative neutralizing agents. However, the Committee emphasizes that such requests shall be made through the corresponding Codex committees.

Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by December 2019.

A Chemical and Technical Assessment was prepared.

3.2.3 Glycerol ester of wood rosin

Glycerol ester of wood rosin (GEWR) was on the agenda of the current meeting at the request of Codex Committee on Food Additives [1] to allow the use of additional species of pine as source materials in the manufacture of GEWR. The current specifications refer to two pine species: *Pinus palustris* and *Pinus elliottii*. The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species, namely *Pinus halepensis* and *Pinus brutia*, as source materials. The total esterified abietic acids in GEWR (abietic acid, dehydroabietic acid and neoabietic acid) prepared from the four pine species range from approximately 72% to 85%. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. The Committee further noted that this harmonizes the JECFA specifications with those of other regulatory authorities.

Identification of GEWR is based on the presence of characteristic acids. Since the specifications monograph for GEWR does not contain an assay, the Committee recommends that the JECFA Secretariat be notified upon the development and validation of an appropriate assay.

The existing specifications were revised. The Chemical and Technical Assessment was revised.

References

1. Report of the Forty-ninth Session of the Codex Committee on Food Additives, Macao SAR, China, 20–24 March 2017. Rome: Food and Agriculture Organization of the United Nations and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2017 (REP17/FA).

3.2.4 Modified starches

General considerations

The Committee at its seventy-ninth meeting ([Annex 1](#), reference 220) recommended the separation of the combined specifications for the modified starches into 16 individual monographs (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451). The recommendation was based on the difficulty of revising individual specifications for any given modified starch within the existing combined specifications monograph.

The Committee at its eighty-second meeting ([Annex 1](#), reference 230) reviewed individual specifications monographs for each of the 16 modified starches and, based on the limited information received, prepared full specifications for three (INS Nos 1404, 1420, 1451) and tentative specifications for the remaining 13 (INS Nos 1400, 1401, 1402, 1403, 1405, 1410, 1412, 1413, 1414, 1422, 1440, 1442, 1450). The Committee noted that all the modified starches may also be subjected to bleaching and therefore included the appropriate purity tests in all the revised specifications. Data and information necessary to complete and revise the 13 individual tentative specifications monographs, as well as information on the method of manufacture for each of the 16 modified starches, were requested through a further call for data.

At the current meeting, the Committee reviewed data on the method of manufacture, identity and purity of all 16 modified starches.

The Committee noted the following:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered a read-across approach to be appropriate for the toxicological evaluation of these substances.

- All 16 modified starches had been assigned an ADI “not specified”.
- All modified starches can be additionally bleached or fragmented; therefore, revision of the specifications of bleached or fragmented starches would require the revision of all 16 monographs.
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals content and microbiological considerations). Revision of those common specifications would affect all 16 monographs.
- Because of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, unavailable and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.

Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture and the corresponding diversity of impurities.

The Committee recommended that all modified starches be included in a modular monograph titled “Modified Starches” that contains common requirements (“General specifications for modified starches”) consisting of specifications that apply to all 16 modified starches (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451) and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The annexes are as follows:

- Annex 1 – Fragmentation
- Annex 2 – Bleaching
- Annex 3 – Esterification and/or crosslinking with phosphorus-containing compounds
- Annex 4 – Acetylation
- Annex 5 – Oxidation
- Annex 6 – Esterification with octenyl succinic anhydride
- Annex 7 – Etherification with propylene epoxide
- Annex 8 – Esterification and crosslinking with adipic anhydride

Table 10

List of modified starches considered and applicable annexes

Modified starch	INS No.	Annex
Dextrin roasted starch	1400	1
Acid treated starch	1401	1
Alkaline treated starch	1402	1
Bleached starch	1403	2
Oxidized starch	1404	5
Enzyme-treated starch	1405	1
Monostarch phosphate	1410	3
Distarch phosphate	1412	3
Phosphated distarch phosphate	1413	3
Acetylated distarch phosphate	1414	3, 4
Starch acetate	1420	4
Acetylated distarch adipate	1422	4, 8
Hydroxypropyl starch	1440	7
Hydroxypropyl distarch phosphate	1442	3, 7
Starch sodium octenyl succinate	1450	6
Acetylated oxidized starch	1451	4, 5

INS: International Numbering System for Food Additives

Each modified starch should fulfil the specification requirements of the General specifications as well as the specification requirements of all the annexes applicable to it.

The Committee determined that, using this approach, revisions made to the section “General specifications for modified starches” would affect all the modified starches. However, annexes can be revised individually and the revisions would only affect the modified starches mentioned in that annex. A tentative status prompted by information missing from the General specifications would affect all 16 modified starches. A tentative status of an annex would only affect the status of modified starches covered by that annex.

In response to the considerations noted above, the Committee drafted a new modular specifications monograph titled “Modified starches” consisting of an explanatory introduction, “General specifications for modified starches”, and eight annexes. The new modular specifications monograph for modified starches is to be included in FAO Monographs 22, and will replace the 16 existing individual specifications for modified starches (INS Nos 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

Technical considerations

In line with previous decisions, the Committee noted that the processes of starch modification should not result in any additional contamination from heavy metals and therefore recommended that the limit for lead included in the General specifications be decreased from 2 to 0.2 mg/kg. This change is in line with the limit for lead in grains and cereals currently contained in the Codex General Standard for Contaminants in Food [1].

Starch sodium octenyl succinate (INS No. 1450) was on the agenda of the eighty-second JECFA meeting at the request of CCFA at its Forty-seventh Session [2] to assess the data on the levels of lead when the additive is used in infant formula and formula for special medical purposes for infants and to consider a specific limit in the specifications.

At the current meeting, the Committee received data for the lead content from 12 batches of starch sodium octenyl succinate for use in infant formula and formula for special medical purposes intended for infants. The limit of lead for starch sodium octenyl succinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications. This limit is in line with the recommendation from the seventy-ninth JECFA that the introduction of lead limits of 0.1 mg/kg for starch sodium octenyl succinate would result in this additive not exceeding the maximum limit for lead in the final infant formula (i.e. 0.01 mg/kg) if the additive were included in infant formula at the maximum use level reviewed by JECFA.

The Committee further noted that during the manufacture of hydroxypropyl starch (INS No. 1440) and hydroxypropyl distarch phosphate (INS No. 1442), propylene chlorohydrins can occur as impurities. The current specification for propylene chlorohydrins is "Not more than 1 mg/kg". Based on information available in the literature [3] and the limited data received, the Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in these modified starches in order to consider lowering this limit.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added. Should more suitable methods become available, the Committee recommends that these methods be communicated to the JECFA secretariat.

The Committee requests suitable microbiological acceptance criteria and supporting data for all modified starches. Information required in the annexes is summarized in [Table 11](#).

Table 11
Information required in the annexes

Annex	Modification	Starches	Information required
1	Minor fragmentation	INS No. 1400: Dextrin roasted starch INS No. 1401: Acid treated starch INS No. 1402: Alkaline treated starch INS No. 1405: Enzyme-treated starch All modified starches that are additionally fragmented.	A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes
2	Bleaching	INS No. 1403: Bleached starch All modified starches if additionally bleached.	Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s)
3	Esterification and/or crosslinking with phosphorus-containing compounds	INS No. 1410: Monostarch phosphate INS No. 1412: Distarch phosphate INS No. 1413: Phosphated distarch phosphate INS No. 1414: Acetylated distarch phosphate INS No. 1442: Hydroxypropyl distarch phosphate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches
4	Acetylation	INS No. 1420: Starch acetate INS No. 1414: Acetylated distarch phosphate INS No. 1422: Acetylated distarch adipate INS No. 1451: Acetylated oxidized starch	Currently no additional information required
5	Oxidation	INS No. 1404: Oxidized starch INS No. 1451: Acetylated oxidized starch	A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method
6	Esterification with octenyl succinic anhydride	INS No. 1450: Starch sodium octenyl succinate	Currently no additional information required
7	Etherification with propylene epoxide	INS No. 1440: Hydroxypropyl starch INS No. 1442: Hydroxypropyl distarch phosphate	A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of hydroxypropyl starch using the method
8	Crosslinking with adipic anhydride	INS No. 1422: Acetylated distarch adipate	A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches Levels of free adipic acid in at least 5 representative batches

INS: International Numbering System for Food Additives; No.: number

References

1. General standard for contaminants and toxins in food and feed. Rome: Food and Agriculture Organization of the United Nations, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 1995 (Codex Stan 193-1995).
2. Report of the Forty-seventh Session of the Codex Committee on Food Additives, Xi'an, China, 23–27 March 2015. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2015 (REP15/FA).
3. Scientific Opinion on the re-evaluation of oxidised starch (E 1404), monostarch phosphate (E 1410), distarch phosphate (E 1412), phosphated distarch phosphate (E 1413), acetylated distarch phosphate

(E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422), hydroxypropyl starch (E 1440), hydroxypropyl distarch phosphate (E 1442), starch sodium octenyl succinate (E 1450), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452) as food additives. EFSA J. 2017;15(10):4911 [96 pp.] doi:10.2903/j.efsa.2017.4911.

4. Flavouring agents

4.1 Toxicological evaluation and exposure assessment

4.1.1 Alicyclic primary alcohols, aldehydes, acids and related esters

Introduction

The Committee evaluated an additional three flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters. These three flavouring agents have not previously been evaluated by the Committee. The Committee also re-evaluated six previously evaluated flavouring agents in this group.

The Committee previously evaluated 26 members of this group of flavouring agents at its fifty-ninth meeting ([Annex 1](#), reference 160) and 11 members of this group at its seventy-third meeting ([Annex 1](#), reference 202). The Committee concluded that all 37 flavouring agents were of no safety concern at estimated dietary exposures.

The three additional flavouring agents in this group are the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253); (1-methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl) methanol (No. 2254); and ($\pm$)-bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester (No. 2255). These three flavouring agents have not been reported to occur as natural components of foods.

The six previously evaluated flavouring agents in this group that were re-evaluated at the present meeting are *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), *p*-mentha-1,8-dien-7-ol (No. 974), *p*-mentha-1,8-dien-7-yl acetate (No. 975), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), myrtenol (No. 981) and myrtenyl acetate (No. 982). These six flavouring agents are all reported to occur as natural components of foods, including bergamot oil, blackberry, black tea, cumin, ginger, grapefruit oil, kabosu oil, kumquat oil, lamb's lettuce, lemon peel oil, lime oil, mandarin oil, orange juice and oil, pepper, peppermint, pistachio, spearmint, thyme, yuzu oil and other foods [1].

Two of the six previously evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), contain an α,β -unsaturated aldehyde group that is considered to be a structural alert for genotoxicity [2]. The remaining four re-evaluated flavouring agents in this group are not α,β -unsaturated aldehydes, but are structurally related to Nos 973 and 980. Additional in vitro and in vivo genotoxicity data were available for all six of these previously evaluated flavouring agents. Also, one of the two major components of an additional flavouring agent evaluated by the Committee at the present meeting (No. 2253, which is a mixture of 1-vinyl-3-

cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde) contains an α,β -unsaturated aldehyde group. In vitro and in vivo genotoxicity data on No. 2253 were also available for evaluation.

The evaluations of the three additional members of this group and the re-evaluation of six previously considered flavouring agents in this group were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

Assessment of dietary exposure

The total annual volumes of production of the nine flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters are 36 kg in Europe, 34 kg in the USA, 3030 kg in Japan and 28 kg in Latin America [3, 4]. More than 97% of the annual production volume in Japan is accounted for by *p*-mentha-1,8-dien-7-al (No. 973). More than 97% of the annual production volume in Europe and the USA are accounted for by three flavouring agents: *p*-mentha-1,8-dien-7-al (No. 973); *p*-mentha-1,8-dien-7-yl acetate (No. 975); and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980). More than 96% of the annual production volume in Latin America is accounted for by three flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980) and myrtenol (No. 981).

Annual volumes of production in the USA for the three additional flavouring agents belonging to the group of alicyclic primary alcohols, aldehydes, acids and related esters are 0.4 kg each for Nos 2253 and 2254 and 0.1 kg for No. 2255. Annual production volume for these three flavouring agents in Europe, Japan or Latin America is reported as 0 kg [3, 4].

Dietary exposures were estimated using both the single-portion exposure technique (SPET) and the maximized survey-derived intake (MSDI) method, and the higher of the two values for each flavouring agent is reported in [Table 12](#). The SPET and MSDI method values are in the range of 30–1500 and 0.01–780 $\mu\text{g}/\text{day}$, respectively, with the SPET yielding the highest estimate for each flavouring agent. The estimated daily dietary exposure was highest for the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253) (1500 $\mu\text{g}/\text{day}$, the SPET value obtained for non-alcoholic beverages).

Absorption, distribution, metabolism and excretion

Information on the ADME of this group of flavouring agents was described in the reports of the fifty-ninth and seventy-third meetings ([Annex 1](#), references 160 and 202). No additional information was available for this meeting.

Consideration of genotoxicity data

The Committee considered new genotoxicity data on six members of this group. The six members were evaluated at the fifty-ninth meeting ([Annex 1](#), reference 160) and re-evaluated at the present meeting: *p*-mentha-1,8-dien-7-al (No. 973), *p*-mentha-1,8-dien-7-ol (No. 974), *p*-mentha-1,8-dien-7-yl acetate (No. 975), formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), myrtenol (No. 981) and myrtenyl acetate (No. 982). Two of these six flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) and formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980), contain an α,β -unsaturated aldehyde group considered to be a structural alert for genotoxicity [2].

The Committee considered the new genotoxicity data for No. 973 and noted that there were reproducible positive results in a reverse mutation assay in the TA98 strain of *Salmonella typhimurium* [5]. In addition, a study in rats investigated micronucleus induction in bone marrow and DNA damage in liver and duodenum (comet assay) [6, 7]. In this GLP-compliant study, the micronucleus assay was conducted according to OECD guideline 474 while the comet assay was conducted in accordance with published guidelines developed by an expert working group [8]. The results of the micronucleus assay indicated that *p*-mentha-1,8-dien-7-al (No. 973) did not induce an increase in micronucleated polychromatic erythrocytes. Although the comet assay did not indicate DNA damage in duodenum compared to the negative control, a 3-fold increase in DNA strand breaks (statistically significant, $P < 0.001$) was observed in liver at the highest dose tested (700 mg/kg bw per day), and there was a dose-dependent trend in the response ($P < 0.001$). Based on these new data, the Committee concluded that there are concerns for potential genotoxicity for *p*-mentha-1,8-dien-7-al (No. 973).

For No. 980, new in vitro reverse mutation [9, 10] and micronucleus induction [11, 12] studies were negative. Also available were new in vivo assays on micronucleus induction in bone marrow and DNA damage in liver and duodenum [13] conducted in the same laboratory and using the same protocols as used for the study on No. 973. The results of the micronucleus assay indicated no increase in micronucleated polychromatic erythrocytes and the results of the comet assay indicated no DNA damage in duodenum or liver. The Committee concluded that there are no concerns for potential genotoxicity for formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (No. 980).

The remaining four re-evaluated flavouring agents in this group are not α,β -unsaturated aldehydes but are structurally related to Nos 973 and 980. Additional in vitro and in vivo genotoxicity data were available on these flavouring agents for evaluation at the present meeting. None of these studies indicated potential for genotoxicity.

One of the two major components of an additional flavouring agent evaluated by the Committee at the present meeting (No. 2253, which is a mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde) also contains an α,β -unsaturated aldehyde group, and in vitro and in vivo genotoxicity data on No. 2253 were available for evaluation. In a bacterial reverse mutation assay, No. 2253 was negative in all five strains tested in both the presence and absence of metabolic activation [14]. In an in vitro mammalian chromosomal aberration assay, No. 2253 induced an increase in the percentage of aberrant cells [15]. However, a GLP-compliant in vivo micronucleus induction assay in mice, conducted in accordance with OECD guideline 474, was negative [16]. The Committee concluded that there are no concerns for potential genotoxicity for the mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde (No. 2253).

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Genotoxicity data on the three additional flavouring agents in this group (Nos 2253–2255) and five of the six re-evaluated flavouring agents in this group do not indicate that these flavouring agents have the potential for genotoxicity. For one of the re-evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (No. 973) the Committee concluded that there were concerns for genotoxicity. Therefore, No. 973 was not further considered using the Procedure for the Safety Evaluation of Flavouring Agents.

Step 2. In applying the Procedure, the Committee assigned six flavouring agents (Nos 974, 975, 980–982 and 2253) to structural class I, one flavouring agent (No. 2254) to structural class II and one flavouring agent (No. 2255) to structural class III [17].

Step 3. Dietary exposures were estimated using both the MSDI method and the SPET.

Step 4. The highest estimated dietary exposures for all eight flavouring agents were below the threshold of toxicological concern applicable to each flavouring agent. The Committee therefore concluded that these eight flavouring agents would not pose a safety concern at current estimated dietary exposures.

Table 12 summarizes the evaluations of the eight flavouring agents belonging to this group of alicyclic primary alcohols, aldehydes, acids and related esters that were considered at the present meeting (Nos 974, 975, 980–982 and 2253–2255).

Consideration of combined intakes from use as flavouring agents

The Committee previously considered the potential combined intakes for this group of alicyclic primary alcohols, aldehydes, acids and related esters and did not identify any safety concerns. The three additional flavouring agents in this

Table 12

Summary of the results of the safety evaluations of alicyclic primary alcohols, aldehydes, acids and related esters used as flavouring agents^{a,b,c,d}

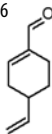
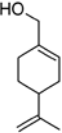
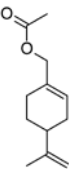
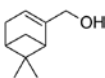
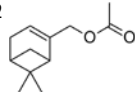
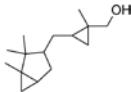
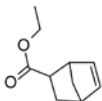
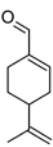
Flavouring agent	No.	CAS no. and structure	Step 4 ^e Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Structural class I				
Mixture of 1-vinyl-3-cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde	2253	1049017-63-1 	1049017-68-6 	No, SPET: 1 500 No safety concern
<i>p</i> -Mentha-1,8-dien-7-ol	974	536-59-4 	No, SPET: 1 000	No safety concern
<i>p</i> -Mentha-1,8-dien-7-yl acetate	975	15111-96-3 	No, SPET: 1 000	No safety concern
Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	564-94-3 	No, SPET: 1 000	No safety concern
Myrtenol	981	515-00-4 	No, SPET: 1 000	No safety concern
Myrtenyl acetate	982	1079-01-2 	No, SPET: 1 000	No safety concern
Structural class II				
(1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	198404-98-7 	No, SPET: 30	No safety concern
Structural class III				
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	10138-32-6 	No, SPET: 60	No safety concern

Table 12 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 ^e Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Flavouring agent excluded at Step 1 of the Procedure				
<i>p</i> -Mentha-1,8-dien-7-al (perillaldehyde)	973	2111-75-3 	NA	Genotoxicity data for <i>p</i> -mentha-1,8-dien-7-al raise concerns for potential genotoxicity

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; NA: not applicable; No.: number; NOAEL: no-observed-adverse-effect level; SPET: single-portion exposure technique

^a Thirty-seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 160 and 202). Six of those flavouring agents (Nos 973–975 and 980–982) and three additional flavouring agents in this group (Nos 2253–2255) were also evaluated at the present meeting.

^b Step 1: Genotoxicity data for one of the nine flavouring agents in this group, *p*-mentha-1,8-dien-7-al (No. 973), raise concerns for potential genotoxicity. Genotoxicity data for the remaining eight flavouring agents in this group do not indicate potential genotoxicity.

^c Step 2: The additional flavouring agents in this group are in structural class I (No. 2253), class II (No. 2254) and class III (No. 2255). The five re-evaluated flavouring agents in this group are in structural class I.

^d Step 3: Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in Table 12. The SPET gave the highest estimate for each flavouring agent. Dietary exposure values are expressed in µg/day.

^e Step 4: The thresholds for human dietary exposure for structural classes I, II and III are 1800, 540 and 90 µg/day, respectively.

group (Nos 2253–2255) have low MSDI values (0.01–0.04 µg/day) and therefore would make a negligible contribution to the combined intake of this group.

Consideration of additional data on previously evaluated flavouring agents

In addition to new genotoxicity data on the six flavouring agents that were re-evaluated at the present meeting, the Committee considered additional data on several other previously evaluated flavouring agents in this group. Acute toxicity data were evaluated for Nos 961, 967, 977 and 981, and studies of genotoxicity were evaluated for Nos 967, 977–979 and 984. These new toxicological data support the conclusions of previous Committee evaluations that these flavouring agents are not safety concerns.

Conclusions

In previous evaluations of flavouring agents in this group of alicyclic primary alcohols, aldehydes, acids and related esters, studies of hydrolysis; ADME; acute, short-term and long-term toxicity; and genotoxicity were available. None of the 37 previously evaluated flavouring agents raised safety concerns.

At the present meeting, the Committee concluded that the three flavouring agents (Nos 2253–2255) that are additions to the group of alicyclic primary alcohols, aldehydes, acids and related esters, would not give rise to safety concerns at current estimated dietary exposures.

The Committee also concluded that five of the six previously evaluated flavouring agents in this group (Nos 974, 975 and 980–982) that were re-evaluated at the present meeting do not give rise to safety concerns. For one of the re-evaluated flavouring agents, *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), the Committee concluded that there were concerns for potential genotoxicity. Therefore No. 973 was not further considered using the Procedure.

An addendum to the monograph was prepared.

References

1. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 5 January 2018).
2. Eder E, Scheckenbach S, Deininger C, Hoffman C. The possible role of alpha, beta-unsaturated carbonyl compounds in mutagenesis and carcinogenesis. *Toxicol Lett.* 1993;67(1–3):87–103.
3. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Bowen R. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2011. Study no. 8221207. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Beevers C. *p*-Mentha-1,8-dien-7-al: Combined bone marrow micronucleus test and comet assay in the liver of treated rats. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2014a. Study no. 8288455. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Beevers C. *p*-Mentha-1,8-dien-7-al: Analysis of comet slides from Covance study 8288455. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2014b. Study no. 8307194. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
8. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR et al. Fourth International Workgroup on Genotoxicity testing: results of the in vivo comet assay workgroup. *Mutat Res.* 2007;627(1):31–5.
9. McGarry S. Myrtenal: Bacterial reverse mutation assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2016b. Study no. 8332788. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

10. Dakoulas E. 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS No. 564-94-3): Bacterial reverse mutation assay. BioReliance Corporation, Maryland, USA; 2017. Study no. AE67ZC.503.BTL. Unpublished report to the Research Institute for Fragrance. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
11. McGarry S. Myrtenal: In vitro human lymphocyte micronucleus assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2016a. Study no. 8332792. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
12. Lloyd M. Myrtenal: In vitro human lymphocyte micronucleus assay. Covance Laboratories Ltd, North Yorkshire, England, United Kingdom; 2017. Study no. 8351223. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
13. Pant K. In vivo mammalian erythrocyte micronucleus assay and mammalian alkaline comet assay in rats. BioReliance Corporation, Maryland, USA; 2018. Study no. AF08WZ.433M.BTL. Unpublished report to the International Organization of the Flavor Industry. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
14. Sokolowski A. *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay with GR-50-0091. Harlan Laboratories Ltd, Rossdorf, Germany; 2011. Study no. 1406304. Unpublished report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
15. Hall C. In vitro chromosome aberration test in Chinese hamster V79 cells with G-50-0091. Harlan Laboratories Ltd, Rossdorf, Germany; 2011a. Study no. 1406305. Unpublished report to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
16. Hall C. Micronucleus assay in bone marrow cells of the mouse with [redacted]. Harlan Laboratories Ltd, Rossdorf, Germany; 2011b. Study no. 1439400. Unpublished report to the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
17. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 1978;16(3):255–76.

4.1.2 Carvone and structurally related substances

Introduction

The Committee evaluated five flavouring agents belonging to the previously evaluated group of carvone and structurally related substances. The Committee re-evaluated two flavouring agents, (+)-carvone (No. 380.1; *d*-carvone) and (–)-carvone (No. 380.2; *l*-carvone), and evaluated three additional flavouring agents. These three additional flavouring agents included two esters, pinocarvyl isobutyrate (No. 2242) and carvyl palmitate (No. 2243), and one alicyclic secondary alcohol, 6-hydroxycarvone (No. 2244). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference

230). (+)-Carvone (No. 380.1) and (–)-carvone (No. 380.2) were re-evaluated because of new data had become available.

The Committee previously evaluated nine members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), reference 137). The Committee concluded that all nine flavouring agents were of no safety concern at estimated dietary exposures.

Carvone (Nos 380.1 and 380.2) was evaluated at the eleventh meeting ([Annex 1](#), reference 14) at which a conditional³ ADI of 0–1.25 mg/kg bw for the (+)- and (–)-enantiomers was established. At the twenty-third meeting ([Annex 1](#), reference 50), a temporary ADI of 0–1 mg/kg bw was established for (+)- and (–)-carvone. This temporary ADI was extended at the twenty-fifth, twenty-seventh, thirtieth and thirty-third meetings ([Annex 1](#), references 56, 62, 73 and 83). At its thirty-seventh meeting, the Committee determined that the (+)- and (–)-enantiomers of carvone should be evaluated separately ([Annex 1](#), reference 94). The Committee established an ADI for (+)-carvone of 0–1 mg/kg bw per day based on a no-observed-effect level (NOEL) of 93 mg/kg bw per day from a 3-month toxicity study in rats. The temporary ADI for (–)-carvone was not extended because insufficient data were available for the toxicological evaluation of this enantiomer. The Committee at its fifty-first meeting maintained the ADI of 0–1 mg/kg bw for (+)-carvone (No. 380.1) ([Annex 1](#), reference 137).

Two of the five flavouring agents, (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), have been reported to occur naturally in foods, mainly botanicals. (+)-Carvone (No. 380.1) has been reported in *Carum* (caraway) and *Anethum* (dill). (–)-Carvone (No. 380.2) has been reported in the oils of *Mentha* (spearmint) [2].

A comprehensive literature search for toxicological data was performed in Scopus; no additional relevant references were identified.

Assessment of dietary exposure

The total annual volume of production of the three new flavouring agents belonging to the group of carvone and structurally related substances is 2 kg in Japan [3, 4]. The total production volume for the flavouring agents presented for re-evaluation, (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), is 48 300 kg in Europe, 53 700 kg in the USA, 1780 kg in Japan and 6340 kg in Latin America [3, 4]. Separate production volumes for the two enantiomers are not available.

Dietary exposure was estimated using both the SPET and the MSDI method, with the highest values reported in [Table 13](#). The estimated daily dietary exposure is highest for carvone (Nos 380.1 and 380.2) at 37 800 µg/day, the SPET

³ "Conditional ADI is a term no longer used by JECFA to signify a range above the 'unconditional ADI', which may signify an acceptable intake when special problems, different patterns of dietary intake, and special groups of the population that may require consideration are taken into account." [1]

value obtained from beer and malt beverages. The MSDI values for carvone range from 350 to 5573 µg/day for the four regions. For the other flavouring agents, the estimated daily dietary exposures range from 0.05 to 0.3 µg/day (MSDI values) and from 3 to 450 µg/day (SPET values).

Absorption, distribution, metabolism and excretion

Relevant information apart from that described in the monographs of the eleventh, twenty-third, thirty-seventh and fifty-first meetings ([Annex 1](#), references 15, 51, 95, 138) was available on the ADME of the flavouring agents belonging to the group of carvone and structurally related substances.

In general, carboxylesterases or esterases catalyse the hydrolysis of esters to their corresponding alcohol and carboxylic acid. The carvyl esters in this group (Nos 2242 and 2243) can be expected to readily hydrolyse to pinocarveol (No. 1403) and carveol (No. 974), respectively, and their respective carboxylic acids. Carvone (Nos 380.1 and 380.2) is metabolized to carveol, dihydrocarveol, carvonic acid, dihydrocarvonic acid and uroterponolone in humans [5]. Oxidation of (+)- or (–)-carvone by human or rat liver microsomes is stereospecific; (+)-carvone is oxidized exclusively to (+)-carveol and (–)-carvone to (–)-carveol, with (–)-carvone having a significantly higher affinity for microsomal enzymes (lower apparent K_m). Only (–)-carveol is converted to a glucuronide conjugate by human or rat liver microsomes [6]. This is expected to impact the toxicity profiles of the enantiomers and their metabolites. 6-Hydroxycarvone is expected to undergo conjugation with glucuronic acid or glutathione followed by excretion.

Flavouring agents not evaluated according to the Procedure for the Safety Evaluation of Flavouring Agents

Exposure to (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) occurs from different food and non-food sources as well as from their use as flavouring agents. Both enantiomers of carvone occur naturally in food and are used in one or more of the following applications: pesticides, feed additives, veterinary products, personal care products, natural insect repellents, food supplements and herbal medicinal products [2, 7].

For the current re-evaluation of carvone (Nos 380.1 and 380.2), additional biochemical data and studies of acute toxicity and genotoxicity were submitted by the flavour industry. The Scientific Committee of the EFSA published an evaluation of carvone based on data from the flavour and pesticide industries. An ADI of 0.6 mg/kg bw per day for (+)-carvone was established, but an ADI for (–)-carvone could not be established due to lack of data. The highest level of aggregated exposure to (+)-carvone was at the level of the ADI for (+)-carvone. The highest level of aggregated exposure to (–)-carvone was 3-fold higher than that of (+)-carvone [7].

The Committee previously established an ADI for (+)-carvone of 0–1 mg/kg bw per day based on a NOEL of 93 mg/kg bw per day from a 3-month toxicity study in rats [8]. At the current meeting, the Committee noted that when identifying the NOEL, no correction was made for the 5-day (rather than 7-day) dosing scheme. Shortly before the meeting, data from the pesticide industry (studies of acute toxicity, short-term toxicity and a 2-generation study) were made available to the Committee by the sponsor. The Committee considered these data and concluded that a review of the ADI for (+)-carvone is recommended based on the evaluation of all biochemical and toxicological data. Also, additional data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources to complete the re-evaluation of (+)-carvone (No. 380.1).

The Committee previously concluded that the ADI for (+)-carvone could not be extended to (–)-carvone because insufficient data were available for the toxicological evaluation of this enantiomer ([Annex 1](#), reference 94). Data are also needed in an oral exposure assessment to (–)-carvone from all sources to complete the re-evaluation of (–)-carvone (No. 380.2).

The Committee therefore did not re-evaluate (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) according to the Procedure for the Safety Evaluation of Flavouring Agents at the current meeting.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for the additional flavouring agents (Nos 2242–2244) in this group. Chemical-specific genotoxicity data on flavouring agents previously evaluated within this group do not indicate that the flavouring agents in this group have the potential to be genotoxic.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents to Nos 2242–2244, the Committee assigned Nos 2242 and 2243 to structural class I and No. 2244 to structural class III [9].

Steps 3 and 4. The highest estimated dietary exposures of Nos 2242–2244 are below their respective thresholds of toxicological concern (i.e. 1800 µg/day for structural class I and 90 µg/day for structural class III). The Committee therefore concluded that these three flavouring agents would not pose a safety concern at current estimated dietary exposures.

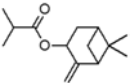
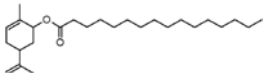
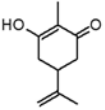
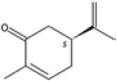
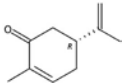
[Table 13](#) summarizes the evaluations of the three flavouring agents (Nos 2242–2244) in the group of carvone and structurally related substances.

Consideration of combined intakes from use as flavouring agents

The three additional flavouring agents in the group of carvone and structurally related substances have low MSDIs (0.05–0.3 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional

Table 13

Summary of the results of the safety evaluations of carvone and structurally related substances used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Conclusion based on current estimated dietary exposure
Structural class I				
Pinocaryl isobutyrate	2242	929116-08-5 	No, SPET: 450	No safety concern
Carvyl palmitate	2243	929222-96-8 	No, SPET: 3	No safety concern
Structural class III				
6-Hydroxycarvone	2244	51200-86-3 	No, SPET: 4	No safety concern
Flavouring agents not evaluated according to the Procedure				
(+)-Carvone	380.1	2244-16-8 	A review of the ADI is recommended based on an evaluation of all biochemical and toxicological data. Also, data are needed for an assessment of oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.	
(-)-Carvone	380.2	6485-40-1 	Additional toxicological data on (-)-carvone are necessary. Also, data are needed for an assessment of oral exposure to (-)-carvone from all sources to complete the evaluation for (-)-carvone.	

ADI: acceptable daily intake; CAS: Chemical Abstracts Service; no.: number; SPET: single-portion exposure technique

^a Nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 137).

^b Step 2: Two additional flavouring agents in this group are in structural class I and one additional flavouring agent in this group is in structural class III.

^c The threshold of toxicological concern for human dietary exposure for structural class I is 1800 µg/day and for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest estimated daily dietary exposures calculated using the SPET.

flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of additional data on previously evaluated flavouring agents

In the previous evaluations of substances in this group of carvone and structurally related substances, studies of biochemistry, acute toxicity, short-term and long-term toxicity and genotoxicity were available ([Annex 1](#), references 15, 51, 95 and 138). None of the nine flavouring agents of this group raised safety concerns.

Besides data on carvone (see the section “Flavouring agents not evaluated according to the Procedure for the Safety Evaluation of Flavouring Agents”), additional biochemical data on carveol (No. 381), a study of acute toxicity with *p*-menthan-2-one (No. 375) and studies of genotoxicity with dihydrocarveol (No. 378) and carveol (No. 381) were available. These data support the conclusions of the previous evaluation.

Conclusions

The Committee concluded that the three flavouring agents pinocarvyl isobutyrate (No. 2242), carvyl palmitate (No. 2243) and 6-hydroxycarvone (No. 2244), which are additions to the group of carvone and structurally related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures.

The Committee did not re-evaluate (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) according to the Procedure for the Safety Evaluation of Flavouring Agents given the lack of information on the oral exposure from all sources, the need to review the ADI of (+)-carvone and the lack of toxicological data on (–)-carvone.

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. In addition, data are needed for an assessment of oral exposure to (+)-carvone from all sources to complete the re-evaluation. This could not be completed during the current meeting.

For (–)-carvone (No. 380.2), the Committee concluded that additional toxicological data are necessary. Also, data are needed for an assessment of oral exposure to (–)-carvone from all sources to complete the re-evaluation.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within 3 years.

An addendum to the monograph was prepared.

References

1. FAO/WHO. Principles and methods for the risk assessment of chemicals in food. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. Geneva: World Health Organization, International Programme on Chemical Safety; 2009 (Environmental Health Criteria 240).

2. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
3. 2015 IOFI Global Poundage Survey. Brussels: International Organization of the Flavor Industry; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Engel W. Detection of a "nonaromatic" NIH shift during in vivo metabolism of the monoterpene carvone in humans. *J Agric Food Chem*. 2002;50(6):1686–94.
6. Jäger W, Mayer M, Platzer P, Reznicek G, Dietrich H, Buchbauer G. Stereoselective metabolism of the monoterpene carvone by rat and human liver microsomes. *J Pharm Pharmacol*. 2000;52(2):191–7.
7. EFSA. Scientific Opinion on the safety assessment of carvone, considering all sources of exposure. *EFSA J*. 2014;12(7):3806 [74 pp]. doi:10.2903/j.efsa.2014.3806.
8. National Toxicology Program. Toxicology and carcinogenesis studies of *d*-carvone (CAS No. 2244-16-8) in B6C3F1 mice and toxicology studies in F344/N rats (gavage studies). (NTP/TR No. 381, draft; NIH No. 90-2836). Washington, DC, USA: US Department of Health and Human Services; 1989. Draft report submitted to WHO by Flavor and Extract Manufacturer's Association of the United States, Washington, DC, USA [cited in [Annex 1](#), reference 94].
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol*. 1978;16(3):255–76.

4.1.3 Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

Introduction

The Committee re-evaluated 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (Nos 1491–1526 and 2103–2105). At three previous meetings, the Committee noted that there were positive in vitro genotoxicity data for several members of this group and that there was a paucity of in vivo genotoxicity data to allay concerns ([Annex 1](#), references 178, 190 and 211). Based on these considerations, the Committee concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group.

The concerns with this group arose primarily from the carcinogenicity of furan itself, which is believed to involve a reactive genotoxic metabolite formed by epoxidation and opening of the furan ring. Furan is not a member of this group of flavouring agents, but all the members of the group contain a furan ring with substituents of varying complexity. Four members of this group, namely 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), 2-ethylfuran (No. 1489) and 2-butylfuran (No. 1490), have short-chain alkyl substituents on the

furan ring; although considered at previous meetings, these four members are no longer supported by industry and were not considered in this re-evaluation.

At the present meeting, the Committee considered additional studies of in vitro genotoxicity (for seven flavouring agents: Nos 1495, 1497, 1503, 1504, 1511, 1514 and 1520) and in vivo genotoxicity (for four flavouring agents: Nos 1491, 1497, 1503 and 1511). Additional short-term studies of toxicity for two flavouring agents in this group (Nos 1491 and 1500) were also available.

Twenty of these 39 flavouring agents (Nos 1491–1494, 1497, 1499, 1503–1505, 1508–1513, 1520–1522, 2104 and 2105) have been reported to occur as natural components in foods including cheese, chicken, cocoa, coffee, honey, rye bread, spirituous beverages, tomatoes, wheaten bread, wine and other foods [1].

The re-evaluations of the 39 flavouring agents in this group were conducted using the Procedure for the Safety Evaluation of Flavouring Agents. The first step in the Procedure is consideration of genotoxicity ([Annex 1](#), reference 230).

Assessment of dietary exposure

The total annual volumes of production of the 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers is 585 kg in Europe, 798 kg in the USA, 246 kg in Japan and 490 kg in Latin America [2, 3]. More than 83%, 70%, 93% and 85% of the annual production volume in Europe, the USA, Japan and Latin America, respectively, is accounted for by 2-furyl methyl ketone (No. 1503).

Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported in [Table 14](#). Estimated daily dietary exposures range from 0.3 to 1200 µg/person (SPET values) and from 0.01 to 61 µg/person (MSDI method values). The estimated daily dietary exposure is highest for 2,5-dimethyl-3-oxo-(2*H*)-furan-4-yl butyrate (No. 1519) at 1200 µg/person, the SPET value for non-alcoholic beverages. The SPET yielded the highest estimated daily dietary exposure in each case with the exception of phenethyl 2-furoate (No. 1517; MSDI = 2 µg/person).

Absorption, distribution, metabolism and excretion

Detailed information on the ADME of this group of flavouring agents was described in the monograph of the sixty-ninth meeting of the Committee ([Annex 1](#), reference 191). At that meeting, the Committee noted that the biotransformation processes applicable to members of this group of furan-substituted flavouring agents are, in large part, dependent on the presence or absence of specific functional groups attached to the furan ring. The Committee also noted that at higher dose levels, low relative molecular mass alkyl furans (e.g. 2-methylfuran)

can undergo ring oxidation to yield reactive 2-ene-1,4-dicarbonyl intermediates that can react with protein and DNA. For example, furan has limited metabolic options and is biotransformed via ring oxidation to an enedialdehyde species that is a potent hepatotoxin. The Committee further noted that the presence of an extended side-chain attached to the furan ring would reduce the potential for epoxidation of the double bond and provide a site for detoxication via metabolism and elimination.

No new data on the ADME of specific members of this group of flavouring agents were available for the present meeting.

Consideration of genotoxicity data

Additional studies of genotoxicity on furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers were evaluated for the present meeting. A total of 16 in vitro genotoxicity studies were available for seven flavouring agents (Nos 1495, 1497, 1503, 1504, 1511, 1514 and 1520), and a total of eight in vivo genotoxicity studies were available for four flavouring agents (Nos 1491, 1497, 1503 and 1511).

A positive result was observed for 2-furyl methyl ketone (No. 1503) in an in vitro SCE assay. However, an in vitro chromosomal aberration assay was negative and in vivo studies of DNA damage (comet assays) and micronucleus induction were also negative. All other in vitro and in vivo genotoxicity assays considered at the present meeting were negative.

The Committee concluded that the newly available in vitro and in vivo genotoxicity data evaluated at the present meeting allay the previous concerns of the Committee. Those concerns arose primarily from the carcinogenicity of furan itself and from some positive genotoxicity findings for four flavouring agents with short-chain alkyl substituents on the furan ring. Those four flavouring agents, namely 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), 2-ethylfuran (No. 1489) and 2-butylfuran (No. 1490), are no longer supported by industry, and were not considered in this re-evaluation.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Genotoxicity data on members of this group of 39 flavouring agents do not raise concerns for genotoxicity.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned all 39 flavouring agents to structural class III [4].

Step 3. Dietary exposures were estimated using both the MSDI method and the SPET.

Step 4. The highest estimated dietary exposures for 14 flavouring agents (Nos 1492, 1500, 1502, 1512, 1517, 1518, 1522–1526, 2103–2105) are below the class III threshold of toxicological concern (i.e. 90 µg/day). The Committee

therefore concluded that these 14 flavouring agents would not pose a safety concern at current estimated dietary exposures.

The highest estimated dietary exposures for 25 flavouring agents (Nos 1491, 1493–1499, 1501, 1503–1511, 1513–1516, 1519–1521) are above the class III threshold of toxicological concern. Therefore, these 25 flavouring agents proceeded to Step 5 of the Procedure.

Step 5. For 2-pentylfuran (No. 1491) the NOAEL of 30 mg/kg bw per day from a 90-day study in rats [5] provides an adequate MOE of 10 000 in relation to the estimated dietary exposure to No. 1491 when used as a flavouring agent. This NOAEL is also appropriate for assessment of the structurally related flavouring agents 2-decylfuran (No. 1493) and 2,4-difurfurylfuran (No. 1496). This NOAEL provides adequate MOEs of 6000 and 4500 in relation to the estimated dietary exposure to No. 1493 and No. 1496, respectively, when used as flavouring agents.

For 3-(2-furyl)acrolein (No. 1497), the NOAEL of 45 mg/kg bw per day from a 90-day study in rats [6] provides an adequate MOE of 5400 in relation to the estimated dietary exposure to No. 1497 when used as a flavouring agent. This NOAEL is also appropriate for assessment of the structurally related flavouring agents 3-methyl-2-(3-methylbut-2-enyl)-furan (No. 1494), 2-methyl-3(2-furyl)acrolein (No. 1498), 3-(5-methyl-2-furyl)prop-2-enal (No. 1499) and 2-furfurylidene-butyraldehyde (No. 1501) and provides adequate MOEs of 9000, 10 800, 2700 and 10 800 in relation to the estimated dietary exposures to Nos 1494, 1498, 1499 and 1501, respectively, when used as flavouring agents.

For 2,3-dimethylbenzofuran (No. 1495), the NOAEL of 0.6 mg/kg bw per day from a 90-day study in rats [7] provides an adequate MOE of 360 in relation to the estimated dietary exposure to No. 1495 when used as a flavouring agent.

For 3-acetyl-2,5-dimethylfuran (No. 1506), the NOAEL of 10 mg/kg bw per day from a 14-day study in rats [8] provides an adequate MOE of 3000 in relation to the estimated dietary exposure to No. 1506 when used as a flavouring agent. The NOAEL of 10 mg/kg bw per day for No. 1506 is also appropriate for assessment of the structurally related flavouring agents 2-acetyl-5-methylfuran (No. 1504) and 2-acetyl-3,5-dimethylfuran (No. 1505) and provides adequate MOEs of 6000 and 600 in relation to the estimated dietary exposures to 1504 and 1505, respectively, when used as flavouring agents.

For 2-furyl methyl ketone (No. 1503), the NOAEL of 25 mg/kg bw per day obtained from a 90-day study in rats [6] provides an adequate MOE of 4300 in relation to the estimated dietary exposure to No. 1503 when used as a flavouring agent. The NOAEL of 25 mg/kg bw per day for No. 1503 is also appropriate for assessment of the structurally related flavouring agents 2-butyrylfuran (No. 1507), (2-furyl)-2-propanone (No. 1508), 2-pentanoylfuran (No. 1509), furfuryl methyl ether (No. 1520) and ethyl furfuryl ether (No. 1521) and provides adequate MOEs of 2400, 10 000, 1500, 7500 and 12 000, respectively, in relation to the estimated

dietary exposures to Nos 1507, 1508, 1509, 1520 and 1521, respectively, when used as flavouring agents.

For 4-(2-furyl)-3-buten-2-one (No. 1511), the NOAEL of 30 mg/kg bw per day from a 14-day study in rats [9] provides an adequate MOE of 2600 in relation to the estimated dietary exposure to No. 1511 when used as a flavouring agent. The NOAEL of 30 mg/kg bw per day for No. 1511 is also appropriate for assessment of the structurally related flavouring agents 1-(2-furyl)butan-3-one (No. 1510), ethyl 3-(2-furyl)propanoate (No. 1513), isobutyl 3-(2-furan)propionate (No. 1514), isoamyl 3-(2-furan)propionate (No. 1515), isoamyl 3-(2-furan)butyrate (No. 1516) provides adequate MOEs of 4500, 3900, 4500, 4500 and 12 000 in relation to the estimated dietary exposures to Nos 1510, 1513, 1514, 1515 and 1516, respectively, when used as flavouring agents.

For 2,5-dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate (No. 1519), the NOAEL of 200 mg/kg bw per day for the structurally related substance 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (No. 1446) obtained from a 2-year study in rats [10] provides an adequate MOE of 10 000 in relation to the estimated dietary exposure to No. 1519 when used as a flavouring agent.

Based on the adequate margins of exposure for each of the 25 flavouring agents considered at Step 5 of the Procedure, the Committee concluded that these 25 flavouring agents (Nos 1491, 1493–1499, 1501, 1503–1511, 1513–1516, 1519–1521) would not pose a safety concern at current estimated dietary exposures.

Consideration of combined intakes from use as flavouring agents

Twenty-five of the flavouring agents in this group have MSDI values of less than 0.1 µg/day, and the four highest MSDI values are 3, 6, 13 and 61 µg/day. The Committee considered that combined intakes of members of this group of flavouring agents do not raise safety concerns.

Consideration of secondary components

Two flavouring agents in this group (Nos 1519 and 1524) have a minimum assay value of less than 95% (see [Annex 3](#)). For No. 1519, the major secondary components are 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (present at 1–3%) and butyric acid (present at 1–3%). The SPET value for No. 1519 is 1200 µg/day and 3% of this value is 36 µg/day, which is below the class III threshold of toxicological concern (90 µg/day). Butyric acid (JECFA flavour No. 87) was evaluated at the forty-ninth meeting, and it was concluded that there were no safety concerns from its use as a flavouring agent ([Annex 1](#), reference 131). The major secondary components of No. 1519 are therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1519 as a flavouring agent.

For No. 1524, the major secondary component is di-(2-methyl-3-furyl) disulfide (present at 6–7%). The SPET value for No. 1524 is 10 µg/day and 7%

of this value is 0.7 µg/day, which is below the class III threshold of toxicological concern (90 µg/day). The major secondary component of No. 1524 is therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1524 as a flavouring agent.

Conclusions

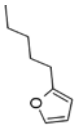
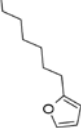
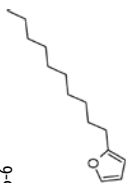
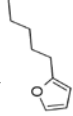
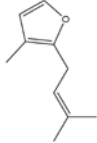
The Committee concluded that the 39 previously evaluated flavouring agents in this group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers that were re-evaluated at the present meeting (Nos 1491–1529 and 2103–2105) do not give rise to safety concerns. The Committee concluded that the additional genotoxicity data on members of this group allay the concerns that were raised at previous meetings.

An addendum to the monograph was prepared.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 10 February 2018).
2. 2015 IOFI Global Poundage Survey. Brussels: International Organization of the Flavor Industry; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
5. Bauter M. 2-Pentylfuran: A 90-day oral gavage study in rats. Product Safety Labs, Dayton NJ. Study no. 42348. Unpublished report to the International Organization of the Flavor Industry, 2017. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Lough R. A combined 28-day and 90-day toxicity study of four test articles [2-furyl methyl ketone, benzophenone, 3-(2-furyl)acrolein and isobutyl 3-(2-furyl) propionate] administered orally (in the diet) to the albino rat. Study no. 81238. Unpublished report from Bio-Research Laboratories, Ltd, Montréal, Quebec, Canada; 1985. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Long DW. TT 175: Acute oral toxicity and 3-month oral toxicity study in the rat. Study no. IFREB-R 770261. Unpublished report from Institut Français de Recherches et Essais Biologiques (IFREB), Les Oncins, France; 1977. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
8. Van Miller JP, Weaver EV. Fourteen-day dietary minimum toxicity screen (MTS) of 2-methyl-1-butanol blend, methyl-*o*-methoxy benzoate, 4,5,6,7-tetrahydro-3,6-dimethylbenzofuran, 3-actyl-2,5-dimethylfuran and furfuryl methyl ether in albino rats. Study no. 50-526. Unpublished report from

Table 14
Summary of the results of the safety evaluations of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers used as flavouring agents^{a,b,c,d}

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? ^e	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Structural class III						
2-Pentylfuran	1491	3777-69-3 	Yes, SPET: 180	Yes. The NOAEL of 30 mg/kg bw per day in a 90-day study in rats [5] is 10 000 times greater than the estimated dietary exposure to No. 1491 when used as a flavouring agent.	NR	No safety concern
2-Heptylfuran	1492	3777-71-7 	No, SPET: 6	NR	NR	No safety concern
2-Decylfuran	1493	83469-85-6 	Yes, SPET: 300	Yes. The NOAEL of 30 mg/kg bw per day in a 90-day study in rats [5] for the related substance 2-pentylfuran (No. 1491) is 6 000 times greater than the estimated dietary exposure to No. 1493 when used as a flavouring agent.	2-Pentylfuran (No. 1491) 	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	15186-51-3 	Yes, SPET: 300	Yes. The NOAEL of 45 mg/kg bw per day for the related substance 3-(2-furyl) acrolein (No. 1497) [6] is 9 000 times greater than the dietary exposure to No. 1494 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
2,3-Dimethylbenzofuran	1495	3782-00-1 	Yes, SPET: 100	Yes. The NOAEL of 0.6 mg/kg bw per day [7] is 360 times greater than the estimated dietary exposure to No. 1495 when used as a flavouring agent.	NR	No safety concern

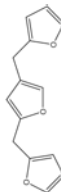
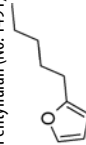
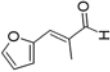
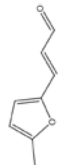
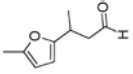
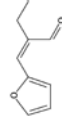
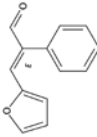
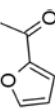
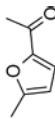
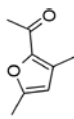
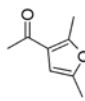
Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*				Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?		Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure		
Flavouring agent	No.	CAS no. and structure	Yes, SPET: 400	Yes, SPET: 500	Yes, SPET: 250	Yes, SPET: 1 000			
2,4-Difurfurylfuran	1496	64280-32-6 	Yes, SPET: 400				Yes. The NOAEL of 30 mg/kg bw per day [5] for the related substance 2-pentylfuran (No. 1491) is 4 500 times greater than the estimated dietary exposure to No. 1496 when used as a flavouring agent.	2-Pentylfuran (No. 1491) 	No safety concern
3-(2-Furyl)acrolein	1497	623-30-3 	Yes, SPET: 500				Yes. The NOAEL of 45 mg/kg bw per day [6] is 5 400 times greater than the dietary exposure to No. 1497 when used as a flavouring agent.	NR	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	874-66-8 	Yes, SPET: 250				Yes. The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 10 800 times greater than the dietary exposure to No. 1498 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	5555-90-8 	Yes, SPET: 1 000				Yes. The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 2 700 times greater than the estimated dietary exposure to No. 1499 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	31704-80-0 	No, SPET: 50				NR	NR	No safety concern
2-Furfurylidene-butyraldehyde	1501	770-27-4 	Yes, SPET: 250				Yes. The NOAEL of 45 mg/kg bw per day [6] for the related substance 3-(2-furyl)acrolein (No. 1497) is 10 800 times greater than the estimated dietary exposure to No. 1501 when used as a flavouring agent.	3-(2-Furyl)acrolein (No. 1497) 	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
2-Phenyl-3-(2-furyl)prop-2-enal	1502	65545-81-5 	No, SPET: 40	NR	NR	No safety concern
2-Furyl methyl ketone	1503	1192-62-7 	Yes, SPET: 350	Yes. The NOAEL of 25 mg/kg bw per day [6] is 4 300 times greater than the estimated dietary exposure to No. 1503 when used as a flavouring agent.	NR	No safety concern
2-Acetyl-5-methylfuran	1504	1193-79-9 	Yes, SPET: 100	Yes. The NOAEL of 10 mg/kg bw per day [8] for the related substance 3-acetyl-2,5-dimethylfuran (No. 1506) is 6 000 times greater than the estimated dietary exposure to No. 1504 when used as a flavouring agent.	3-Acetyl-2,5-dimethylfuran (No. 1506) 	No safety concern
2-Acetyl-3,5-dimethylfuran	1505	22940-86-9 	Yes, SPET: 1 000	Yes. The NOAEL of 10 mg/kg bw per day [8] for the related substance 3-acetyl-2,5-dimethylfuran (No. 1506) is 600 times greater than the estimated dietary exposure to No. 1505 when used as a flavouring agent.	3-Acetyl-2,5-dimethylfuran (No. 1506) 	No safety concern
3-Acetyl-2,5-dimethylfuran	1506	10599-70-9 	Yes, SPET: 200	Yes. The NOAEL of 10 mg/kg bw per day [8] is 3 000 times greater than the estimated dietary exposure to No. 1506 when used as a flavouring agent.	NR	No safety concern

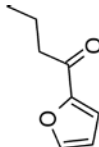
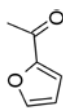
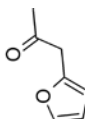
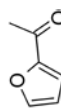
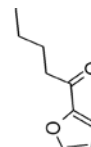
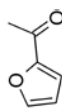
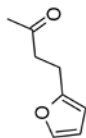
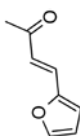
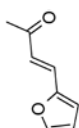
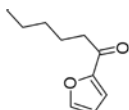
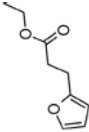
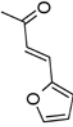
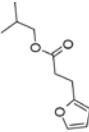
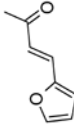
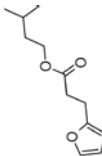
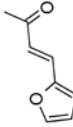
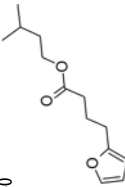
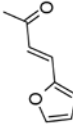
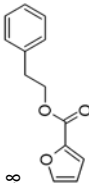
Step 4				Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Flavouring agent	No.	CAS no. and structure	Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*			
2-Butyrylfuran	1507	100113-53-9 	Yes, SPET: 625	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 2 400 times greater than the estimated dietary exposure to No. 1507 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
(2-Furyl)-2-propanone	1508	6975-60-6 	Yes, SPET: 150	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 10 000 times greater than the estimated dietary exposure to No. 1508 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
2-Pentanoylfuran	1509	3194-17-0 	Yes, SPET: 1 000	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 1 500 times greater than the estimated dietary exposure to No. 1509 when used as a flavouring agent.	2-Furyl methyl ketone (No. 1503) 	No safety concern
1-(2-Furyl)butan-3-one	1510	699-17-2 	Yes, SPET: 400	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1510 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	623-15-4 	Yes, SPET: 699	Yes. The NOAEL of 30 mg/kg bw per day [9] is 2 600 times greater than the estimated dietary exposure to No. 1511 when used as a flavouring agent.	NR	No safety concern
Pentyl 2-furyl ketone	1512	14360-50-0 	No, SPET: 15	NR	NR	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?*	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Ethyl 3-(2-furyl)propanoate	1513	10031-90-0 	Yes, SPET: 463	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 3 900 times greater than the estimated dietary exposure to No. 1513 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Isobutyl 3-(2-furan)propanoate	1514	105-01-1 	Yes, SPET: 400	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1514 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Isoamyl 3-(2-furan)propanoate	1515	7779-67-1 	Yes, SPET: 400	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 4 500 times greater than the estimated dietary exposure to No. 1515 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Isoamyl 3-(2-furan)butyrate	1516	7779-66-0 	Yes, SPET: 150	Yes. The NOAEL of 30 mg/kg bw per day [9] for the related substance 4-(2-furyl)-3-buten-2-one (No. 1511) is 12 000 times greater than the estimated dietary exposure to No. 1516 when used as a flavouring agent.	4-(2-Furyl)-3-buten-2-one (No. 1511) 	No safety concern
Phenethyl 2-furoate	1517	7149-32-8 	No, MSDI: 2	NR	NR	No safety concern

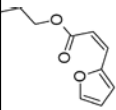
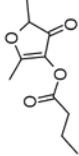
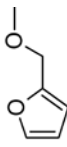
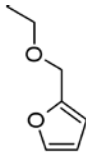
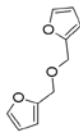
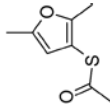
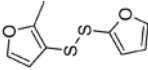
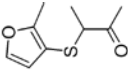
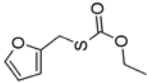
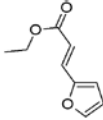
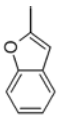
Step 4		Step 5		Conclusion based on current estimated dietary exposure
Flavouring agent	No. CAS no. and structure	Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class?	Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	
Propyl 2-furanacrylate	1518 623-22-3 	No, SPET: 18	NR	No safety concern
2,5-Dimethyl-3-oxo-(2H)-furan-4-yl butyrate	1519 114099-96-6 	Yes, SPET: 1 200	Yes. The NOAEL of 200 mg/kg bw per day [10] for the related substance 2,5-dimethyl-4-hydroxy-3(2H)-furanone (No. 1446) is 10 000 times greater than the estimated dietary exposure to No. 1519 when used as a flavouring agent.	No safety concern
Furfuryl methyl ether	1520 13679-46-4 	Yes, SPET: 200	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 7 500 times greater than the estimated dietary exposure to No. 1520 when used as a flavouring agent.	No safety concern
Ethyl furfuryl ether	1521 6270-56-0 	Yes, SPET: 125	Yes. The NOAEL of 25 mg/kg bw per day [6] for the related substance 2-furyl methyl ketone (No. 1503) is 12 000 times greater than the estimated dietary exposure to No. 1521 when used as a flavouring agent.	No safety concern
Difurfuryl ether	1522 4437-22-3 	No, SPET: 6	NR	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523 55764-22-2 	No, SPET: 2	NR	No safety concern

Table 14 (continued)

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? ^a	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Furfuryl(2-methyl-3-furyl) disulfide	1524	109537-55-5 	No, SPET: 10	NR	NR	No safety concern
3-[(2-Methyl-3-furyl)thio]-2- butanone	1525	61295-44-1 	No, SPET: 0.3	NR	NR	No safety concern
O-Ethyl S-(2-furylmethyl) thiocarbonate	1526	376595-42-5 	No, SPET: 9	NR	NR	No safety concern
(E)-Ethyl 3-(2-furyl)acrylate	2103	53282-12-5 	No, SPET: 20	NR	NR	No safety concern

Flavouring agent	No.	CAS no. and structure	Step 4 Does the higher of the two predicted dietary exposure estimates exceed the threshold of toxicological concern value for the structural class? ^a	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Structural relative name (No.) and Structure	Conclusion based on current estimated dietary exposure
Di-2-furylmethane	2104	1197-40-6 	No, SPET: 40	NR	NR	No safety concern
2-Methylbenzofuran	2105	4265-25-2 	No, SPET: 3	NR	NR	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; no.: number; NOAEL: no-observed-adverse-effect level; NR: not required; SPET: single-portion exposure technique

^a The Committee re-evaluated 39 flavouring agents belonging to the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides and ethers. All 39 flavouring agents have been considered previously by the Committee.

^b Step 1: Genotoxicity data on the flavouring agents in this group do not raise concerns for genotoxicity.

^c Step 2: All 39 flavouring agents belong to structural class III.

^d Step 3: Dietary exposures were estimated using both the SPET and the MSDI method, and the higher of the two values for each flavouring agent is reported. Dietary exposure values are expressed in µg/day.

^e Step 4: The threshold for human dietary exposure for structural class III is 90 µg/day.

Bushy Run Research Center, Export, Pennsylvania, USA; 1987. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

9. Gill MW, Van Miller JP. Fourteen-day dietary minimum toxicity screen (MTS) in albino rats. Study no. 50-528. Unpublished report from Bushy Run Research Center, Export, Pennsylvania, USA to the Flavor and Extract Manufacturers Association, Washington, DC, USA; 1987. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
10. Kelly CM, Bolte HF. ST 07 C99: A 24-month dietary carcinogenicity study in rats. Unpublished report no. 99-2644. Huntingdon Life Sciences, East Millstone, NJ, USA to the Flavor and Extract Manufacturers Association, Washington, DC, USA; 2003. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.

4.1.4 Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters

Introduction

The Committee evaluated two additional flavouring agents belonging to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters. One of the flavouring agents was a linear unsaturated aldehyde, *trans*-6-octenal (No. 2240), and the other was a branched unsaturated alcohol, 2,6-dimethyl-5-heptenol (No. 2241). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee evaluated 42 other members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), reference 137). For 41 of the 42 substances in this group, the Committee concluded that there were no safety concerns at estimated dietary exposures. The evaluation of the remaining substance, ethyl 2-methyl-3,4-pentadienoate (No. 353), was completed at the sixty-eighth meeting ([Annex 1](#), reference 187). The Committee evaluated 20 other members of this group of flavouring agents at its sixty-first meeting ([Annex 1](#), reference 166). The Committee concluded that there were no safety concerns at estimated dietary exposures with respect to all of these 20 flavouring agents. The Committee evaluated nine other members of this group of flavouring agents at its seventy-sixth meeting ([Annex 1](#), reference 211). The Committee concluded that all nine flavouring agents were of no safety concern at estimated dietary exposures.

Both of the additional flavouring agents (Nos 2240 and 2241) in this group have been reported to occur naturally and can be found in ginger [1].

A comprehensive literature search was conducted in PubMed; no additional relevant studies were identified.

Assessment of dietary exposure

The total annual volume of production of the two additional flavouring agents is 0.2 kg [2, 3]. The volume of the annual production in the USA is completely accounted for by *trans*-6-octenal (No. 2240). In Japan, the production volume is completely accounted for by 2,6-dimethyl-5-heptenol (No. 2241).

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 15. The higher estimated daily dietary exposure is for 2,6-dimethyl-5-heptenol (No. 2241), at 300 µg/day, the SPET value obtained from nonalcoholic soft beverages. For *trans*-6-octenal (No. 2240), the estimates of daily dietary exposures via the MSDI method and SPET are 0.03 and 40 µg/day, respectively.

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters has been described in the monographs of the fifty-first, sixty-first, sixty-eighth, and seventy-sixth meetings (Annex 1, references 138, 167, 188, 212).

The aliphatic esters are expected to hydrolyse to their analogous unsaturated aliphatic alcohol and carboxylic acids during passage through the gastrointestinal tract [4, 5, 6]. The resulting linear and branched-chain unsaturated primary alcohols are expected to be absorbed and further oxidized to their analogous aldehydes and acids, and rapidly absorbed [7, 8]. The absorbed aldehydes are oxidized to their analogous unsaturated carboxylic acids. These unsaturated carboxylic acids undergo further enzymatic conversion prior to entry into the β -oxidation pathway, where they are fully metabolized to carbon dioxide and water via the citric acid cycle.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no adequate chemical-specific genotoxicity studies on the additional flavouring agents. However, there are no structural alerts for these compounds. Data from related flavouring agents indicate that these do not show genotoxic potential based on uniformly negative genotoxicity data.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents to *trans*-6-octenal (No. 2240) and 2,6-dimethyl-5-heptenol (No. 2241), the Committee assigned both flavouring agents to structural class I [9].

Steps 3 and 4. Dietary exposures using both the MSDI method and SPET have been determined. The highest estimated dietary exposures of both flavouring agents in structural class I were below the threshold of concern (i.e. 1800 µg/person per day for class I). The Committee therefore concluded that both

flavouring agents (Nos 2240–2241) would not pose a safety concern at current estimated dietary exposures.

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters have low MSDIs (0.01–0.03 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of secondary components

One flavouring agent in this group, 2,6-dimethyl-5-heptenol (No. 2241), has a minimum assay value of less than 95% (see [Annex 3](#)). The major secondary component, 2,6-dimethyl-5-heptenal (No. 349), present at 1–6%, does not present a safety concern at estimated dietary exposures from the use of No. 2241 as a flavouring agent.

Consideration of additional data on previously evaluated flavouring agents

In the previous evaluation of substances in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters, studies of biochemistry, acute toxicity, short-term and long-term toxicity and genotoxicity were available ([Annex 1](#), references 137, 166, 187 and 211). None of the 71 flavouring agents in this group raised safety concerns.

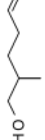
No adequate studies on the additional flavouring agents were available. For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 336, 1269, 1286 and 1640), studies of short-term toxicity (Nos 330, 332 and 333) and studies of genotoxicity (Nos 315, 329, 330, 333, 334, 346, 349, 1272, 1286 and 1637) were available. The studies available for the present evaluation support the conclusions drawn by previous safety evaluations.

Conclusions

The Committee concluded that the two flavouring agents *trans*-6-octenal (No. 2240) and 2,6-dimethyl-5-heptenol (No. 2241), which are additions to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters evaluated previously, do not give rise to any safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

Table 15
Summary of the results of the safety evaluations of two additional flavouring agents in the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of concern?	Step 5 Does a NOAEL exist for the flavouring agent or a structural relative that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
Structural class I					
<i>trans</i> -6-Octenal	2240	63196-63-4 	No, SPET: 40	NR	No safety concern
2,6-Dimethyl-5-heptenol	2241	4234-93-9 	No, SPET: 300	NR	No safety concern

CAS: Chemical Abstracts Service; NOAEL: no-observed-adverse-effect level; NR: not relevant; SPET: single-portion exposure technique

^a Seventy-one flavouring agents in this group were previously evaluated by the Committee (Annex 1, References 137, 166, 187 and 211).

^b Step 2: Two flavouring agents (Nos 2240–2241) are in structural class I.

^c The threshold for human dietary exposure for structural class I is 1800 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure, which was calculated using the SPET.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4: [database]. Zeist, the Netherlands: Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
2. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Gangolli SD, Shilling WH. Hydrolysis of esters by artificial gastric and pancreatic juices. Study no. 11/1968. Unpublished report to the Flavor and Extracts Manufacturers Association, Washington, DC, USA; 1968. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Junge W, Heymann E. Characterization of the isoenzymes of pig-liver esterase: 2. Kinetic studies. *Eur J Biochem.* 1979;95(3):519–25.
6. Heymann E. Carboxylesterases and amidases. In: W. Jakoby, editor. *Enzymatic basis of detoxication*. New York: Academic Press; 1980.
7. Dawson AM, Holdsworth CD, Webb J. Absorption of short chain fatty acids in man. *Proc Soc Exp Biol Med.* 1964;117:97–100.
8. Gaillard D, Derache R. [Metabolism of different alcohols present in alcoholic beverages, in the rat]. *Trav Soc Pharm Montp.* 1965;25:51–62.
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

4.1.5 Maltol and related substances

Introduction

The Committee evaluated two flavouring agents belonging to the group of maltol and related substances: one additional flavouring agent, ethyl maltol isobutyrate (No. 2252), and one flavouring agent, maltol (No. 1480) that was being re-evaluated. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230). Ethyl maltol isobutyrate (No. 2252) has not been previously evaluated by the Committee. Maltol (No. 1480) was re-evaluated because new in vitro and in vivo genotoxicity data had become available.

The Committee previously evaluated seven members of this group of flavouring agents at its sixty-fifth meeting ([Annex 1](#), reference 178). The Committee concluded that all seven flavouring agents were of no safety concern at estimated dietary exposures and maintained the previously established ADIs of 0–1 mg/kg bw for maltol (No. 1480) and 0–2 mg/kg bw for ethyl maltol (No. 2252).

Maltol (No. 1480) was evaluated at the eleventh meeting ([Annex 1](#), reference 14), when a temporary ADI of 0–1 mg/kg bw was established because no long-term studies of toxicity were available. At the eighteenth meeting ([Annex 1](#), reference 35), the Committee withdrew the temporary ADI because the results of long-term studies of toxicity requested at the previous meeting had not been made available. At the twenty-second meeting ([Annex 1](#), reference 47), the Committee evaluated new data on toxicity and established a temporary ADI of 0–0.5 mg/kg bw. At its twenty-fifth meeting, the Committee evaluated additional data and established an ADI of 0–1 mg/kg bw for maltol on the basis of a NOEL of 100 mg/kg bw in rats ([Annex 1](#), reference 56).

Ethyl maltol (No. 1481) was evaluated at the fourteenth meeting ([Annex 1](#), reference 22), when the Committee established an ADI of 0–2 mg/kg bw. At its eighteenth meeting ([Annex 1](#), reference 35), the Committee re-evaluated ethyl maltol and re-affirmed the ADI of 0–2 mg/kg bw.

Maltol (No. 1480) has been reported to occur naturally in a wide variety of foods such as wheat and rye breads, milk, butter, uncured pork, beer, cocoa, coffee, peanuts, soy proteins, beans and clams [1]. During baking (for example, of bread or beans) and roasting (for example, of cocoa, coffee, peanuts), simple sugars are partly converted to maltol [1, 2].

A comprehensive literature search was performed in Scopus; two additional relevant studies were identified.

Assessment of dietary exposure

The total annual volume of production of the additional flavouring agent (ethyl maltol isobutyrate, No. 2252) is 29 kg in Japan [3, 4]. No production volume was reported for Europe, Latin America or the USA.

The total annual volume of production for the flavouring agent presented for re-evaluation, maltol (No. 1480), is approximately 83 200 kg in Europe, 87 600 kg in the USA, 12 500 kg in Japan and 47 600 kg in Latin America [3, 4].

Dietary exposure was estimated using both the SPET and the MSDI method, with the highest values reported in [Table 16](#). The estimated daily dietary exposure is highest for maltol (10 440 µg/day, SPET value for nonalcoholic “soft” beverages). The MSDI values for the four regions range from 2625 to 9091 µg/day. For ethyl maltol isobutyrate (No. 2252), the estimated daily dietary exposures were 8 µg/day (MSDI value) and 400 µg/day (SPET value).

Absorption, distribution, metabolism and excretion

Information on the ADME of flavouring agents belonging to the group of maltol and related substances is described in the monograph of the sixty-fifth meeting ([Annex 1](#), reference 179).

Chemically, maltol is classified as a γ -pyrone. It is a hydroxyl-substituted 4H-pyran-4-one and is anticipated to be metabolized like phenol, which primarily undergoes phase II conjugation of the free hydroxyl substituent. Maltol and ethyl maltol are predominantly metabolized to sulfate and glucuronic acid conjugates, which are then eliminated in the urine [5]. Ethyl maltol isobutyrate (No. 2252) is predicted to be hydrolysed to ethyl maltol and the corresponding simple aliphatic carboxylic acid (isobutyric acid) [6].

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for maltol (No. 1480) or ethyl maltol isobutyrate (No. 2252). Chemical-specific genotoxicity data available for maltol (No. 1480) do not indicate that this flavouring agent has genotoxic potential.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned one flavouring agent (No. 1480) to structural class II and one flavouring agent (No. 2252) to structural class III [7].

Step 3. The highest dietary exposures were estimated using the SPET for both flavouring agents.

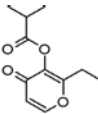
Step 4. The highest estimated dietary exposure for maltol (No. 1480) is above the threshold of toxicological concern (i.e. 540 $\mu\text{g}/\text{day}$ for class II). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

The highest estimated dietary exposure for ethyl maltol isobutyrate (No. 2252) is above the threshold of toxicological concern (i.e. 90 $\mu\text{g}/\text{day}$ for class III). Accordingly, the evaluation of this flavouring agent proceeded to Step 5 of the Procedure.

Step 5. For maltol, the NOAEL of 125 mg/kg bw per day from a 90-day study in dogs [8] provides an MOE of 720 in relation to the estimated daily dietary exposure to No. 1480 (SPET = 10 440 $\mu\text{g}/\text{day}$ or 174 $\mu\text{g}/\text{kg bw per day}$) when used as a flavouring agent. The Committee therefore concluded that maltol (No. 1480) would not pose a safety concern at current estimated dietary exposures.

At its fourteenth meeting ([Annex 1](#), reference 22), the Committee established an ADI of 0–2 mg/kg bw for the structurally related substance ethyl maltol (No. 1481) on the basis of a NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats [8]. This ADI was maintained at the sixty-fifth meeting ([Annex 1](#), reference 178). The NOEL of 200 mg/kg bw per day provides an adequate MOE of 28 570 in relation to estimated daily dietary exposure to No. 2252 (SPET = 400 $\mu\text{g}/\text{day}$ or 7 $\mu\text{g}/\text{kg bw per day}$) when used as a flavouring agent. The Committee concluded that ethyl maltol isobutyrate (No. 2252) would not pose a safety concern at current estimated dietary exposures.

Table 16
Summary of the results of the safety evaluations of maltol and related substances used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4: Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 ^d Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Related structure name (No.) and structure	Conclusion based on current estimated dietary exposure
Structural class I						
Maltol ^e	1480	118-71-8 	Yes, SPET: 10 440	Yes. The NOAEL of 125 mg/kg bw per day from a 90-day study in dogs [8] is 720 times the estimated dietary exposure to No. 1480 when used as a flavouring agent.	Ethyl maltol ^f (No. 1481) 	No safety concern
Structural class III						
Ethyl maltol isobutyrate	2252	852997-28-5 	Yes, SPET: 400	Yes. The NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats for the structurally related substance ethyl maltol (No. 1481) [8] is 28 570 times the estimated dietary exposure to No. 2252 when used as a flavouring agent.	Ethyl maltol ^f (No. 1481) 	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; no.: number; NOAEL: no-observed-adverse-effect level; NOEL: no-observed-effect level; NR: not relevant; SPET: single-portion exposure technique

^a Seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference 178).

^b Step 2: One flavouring agent is in structural class II and one flavouring agent is in structural class III.

^c The threshold of toxicological concern for human dietary exposure for structural class II is 540 µg/day and for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest estimated daily dietary exposures calculated using the SPET.

^d The margin of exposure was calculated based on the highest estimated daily dietary exposure calculated using the SPET.

^e The previously established ADI for maltol was withdrawn by the Committee.

^f The previously established ADI for ethyl maltol of 0–2 mg/kg bw was maintained.

Table 16 summarizes the evaluations of the two flavouring agents (Nos 1480 and 2252) belonging to this group of maltol and related substances.

Consideration of combined intakes from use as flavouring agents

The additional flavouring agent, ethyl maltol isobutyrate (No. 2252), in the group of maltol and related substances has a low MSDI (8 µg/day). The Committee concluded that consideration of combined intakes is not necessary because the additional flavouring agent would not contribute significantly to the combined intake of this flavouring group.

Consideration of secondary components

Ethyl maltol isobutyrate (No. 2252) has a minimum assay of less than 95% (see [Annex 3](#)). The major secondary component in No. 2252, present at 2–3%, is ethyl maltol (No. 1481). This flavouring agent had been previously evaluated by the Committee ([Annex 1](#), reference 178) and did not pose a safety concern at the estimated dietary exposure.

Consideration of additional data on previously evaluated flavouring agents

For two previously evaluated flavouring agents in this group, maltol (No. 1480) and maltol isobutyrate (No. 1482), additional studies of acute toxicity (No. 1482) and genotoxicity (Nos 1480 and 1482) were available for the present evaluation. These additional data raised no safety concerns and support the previous safety evaluations.

However, the Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol at its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies as described in the monograph of that meeting ([Annex 1](#), reference 56). The Committee noted that a NOAEL of 125 mg/kg bw per day was identified in a published 90-day study in dogs [8]. This NOAEL was used to complete the re-evaluation of maltol as a flavouring agent.

Conclusions

In the previous evaluation of flavouring agents in this group of maltol and related substances, biochemical data and studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None of the seven previously evaluated flavouring agents raised safety concerns based on the estimated dietary exposures and the biochemical and toxicological data available ([Annex 1](#), reference 179).

The Committee concluded that the two flavouring agents (Nos. 2252 and 1480) under evaluation, one of which is an addition to the group of maltol

and related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures.

However, the Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting ([Annex 1](#), reference 57). The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the current ADI. The Committee therefore withdrew the ADI for maltol pending review of the appropriate data at a future meeting. The ADI for ethyl maltol was maintained.

An addendum to the monograph was prepared.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
2. Belitz H-D, Grosch W, Schieberle P. Food Chemistry, 4th ed. Berlin Heidelberg, Germany: Springer-Verlag; 2009.
3. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Rennhard HH. The metabolism of ethyl maltol and maltol in the dog. *J Agric Food Chem.* 1971;19(1):152–4.
6. Bennett C. Hydrolysis of maltol propionate via pancreatic secretion. Private communication to the International Organization of the Flavor Industry, Brussels; 1998. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
7. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.
8. Gralla EJ, Stebbins RB, Coleman GL, Delahunt CS. Toxicity studies with ethyl maltol. *Toxicol Appl Pharmacol.* 1969;15(3):604–13.

4.1.6 Menthol and structurally related substances

Introduction

The Committee evaluated seven flavouring agents belonging to the group of menthol and structurally related substances, which was previously evaluated. The Committee re-evaluated menthol (No. 427) and evaluated six additional flavouring agents. These included four menthyl esters (menthyl formate [No. 2246], menthyl propionate [No. 2247], *l*-menthyl butyrate [No. 2248] and

dimenthyl glutarate [No. 2250]), *dl*-isomenthol (No. 2249) and one polyether alcohol, ($\pm$)-2-[(2-*p*-menthoxy)ethoxy]ethanol (No. 2251). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee previously evaluated menthol and 13 other members of this group of flavouring agents at its fifty-first meeting ([Annex 1](#), references 137) and 10 additional members of the group at its sixty-ninth meeting ([Annex 1](#), reference 190). The Committee concluded that all 24 flavouring agents were of no safety concern at estimated dietary exposures.

Menthol (No. 427) was first evaluated by the Committee at the eleventh meeting when an unconditional ADI of 0–0.2 mg/kg bw and a conditional⁴ ADI of 0.2–2 mg/kg bw was established ([Annex 1](#), reference 14). At the eighteenth meeting, an ADI of 0–0.2 mg/kg bw was established for menthol ([Annex 1](#), reference 35). This ADI was maintained at the twentieth meeting. At the twentieth meeting, the Committee requested additional long-term studies of toxicity and carcinogenicity in rats, information on the average and likely maximum dietary exposure to menthol, clinical observations of humans with higher than average dietary exposure to menthol and studies of metabolism ([Annex 1](#), reference 41). At the fifty-first meeting, an ADI of 0–4 mg/kg bw was established on the basis of the NOEL of 380 mg/kg bw per day in a long-term study in rats, applying a safety factor of 100 and rounding to one significant figure ([Annex 1](#), reference 137).

Menthol (No. 427), menthyl formate (No. 2246) and *dl*-isomenthol (No. 2249) have been reported to occur naturally in foods and can be mainly found in peppermint oil and other *Mentha* species oils. Menthol (10–70%) and menthone (No. 429; 7–40%) are the principal constituents of peppermint oil [2, [Annex 1](#), reference 137].

Menthol (No. 427) was re-evaluated because new data had become available since the previous evaluation. Menthol and the other flavourings were evaluated at the request of the Forty-eighth Session of the CCFA [3].

A comprehensive literature search was performed in Scopus; one additional reference was identified.

Assessment of dietary exposure

The total annual volume of production for menthol (No. 427) is 296 000 kg in Europe, 496 000 kg in the USA, 146 000 kg in Japan and 127 000 kg in Latin America [4, 5].

⁴ "Conditional ADI is a term no longer used by JECFA to signify a range above the 'unconditional ADI', which may signify an acceptable intake when special problems, different patterns of dietary intake, and special groups of the population that may require consideration are taken into account." [1]

The total annual volume of production of the six additional flavouring agents in the group of menthol and structurally related substances is 14 900 kg in Europe, 103 937 kg in the USA and 92 kg in Japan. No production volume was reported for Latin America [4, 5].

Of the seven flavouring agents under evaluation by this current Committee, menthol (No. 427) accounts for more than 95% of the total annual production volume in Europe and 82% in the USA. Dimethyl glutarate (No. 2250) accounts for almost all the remaining volume. Menthol (No. 427) accounts for more than 99% of the annual production volume in Japan and 100% in Latin America.

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 17. The highest estimated dietary exposure is for menthol (No. 427; 51 474 µg/day, MSDI value; the SPET value is 42 210 µg/day). For the other flavouring agents, the estimated daily dietary exposures range from 0.03 to 10 784 µg/day (MSDI values) and from 300 to 9000 µg/day (SPET values), with the SPET yielding the highest estimate in all but one case (dimethyl glutarate [No. 2250]).

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of menthol and structurally related substances has previously been described in the monographs of the eleventh, twentieth and fifty-first meetings (Annex 1, references 15, 42 and 138). Additional information was available for the current meeting.

The menthyl esters in this group (Nos 2246–2248 and 2250) can be expected to be readily hydrolysed to menthol and their respective carboxylic acids [6, 7]. Similar to menthol, *dl*-isomenthol (No. 2249) is expected to be conjugated with glucuronic acid and be eliminated in the urine or faeces [8, 9, 10]. The polyether alcohol conjugate of menthol, (±)-2-[(2-*p*-menthoxy)ethoxy] ethanol (No. 2251) is also expected to undergo conjugation with glucuronic acid and subsequent elimination in the urine and faeces.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for these flavouring agents. Chemical-specific genotoxicity data available for menthol (No. 427), *dl*-isomenthol (No. 2249) and (±)-2-[(2-*p*-menthoxy)ethoxy]ethanol (No. 2251) indicate that these flavouring agents are unlikely to be genotoxic based on the weight of evidence.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned six flavouring agents (Nos 427, 2246–2250) to structural class I and one flavouring agent (No. 2251) to structural class III [11].

Step 3. The highest dietary exposures were estimated using the SPET for five of the seven flavouring agents (Nos 2246–2249, 2251) and the MSDI method for menthol (No. 427) and dimethyl glutarate (Nos 2250).

Step 4. The highest estimated dietary exposures for three of the six flavouring agents in structural class I (Nos 2246–2248) are below the threshold of toxicological concern (i.e. 1800 µg/day for class I). The Committee therefore concluded that these flavouring agents would not pose a safety concern at current estimated dietary exposures.

The highest estimated dietary exposures for the remaining three flavouring agents in structural class I (Nos 427, 2249 and 2250) are above the threshold of toxicological concern. Therefore, the evaluation of these flavouring agents proceeded to Step 5.

The highest estimated dietary exposure for the one flavouring agent in structural class III (No. 2251) is above the threshold of toxicological concern (i.e. 90 µg/day for class III). Therefore, the evaluation of this flavouring agent proceeded to Step 5.

Step 5. For menthol (No. 427), an ADI of 0–4 mg/kg bw was allocated on the basis of the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats, applying a safety factor of 100 and rounding to one significant figure ([Annex 1](#), reference 137). The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] provides an adequate MOE of 440 in relation to the estimated dietary exposure to No. 427 (MSDI of 51 474 µg/day). The Committee therefore concluded that menthol (No. 427) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For *dl*-isomenthol (No. 2249), the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] of the structurally related substance menthol (No. 427) provides an adequate MOE of 7600 in relation to the estimated dietary exposure to No. 2249 (SPET value of 3000 µg/day). The Committee therefore concluded that *dl*-isomenthol (No. 2249) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For dimethyl glutarate (No. 2250), the NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] for the structurally related substance menthol (No. 427) provides an adequate MOE of 2100 in relation to the estimated dietary exposure to No. 2250 (MSDI value of 10 784 µg/day). The Committee therefore concluded that dimethyl glutarate (No. 2250) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

For (±)-2-[(2-*p*-methoxy)ethoxy]ethanol (No. 2251), the NOEL of 30 mg/kg bw per day from a 90-day study in rats for the structurally related substance 3-*l*-menthoxypropane-1,2-diol (No. 1408) [13] provides an adequate MOE of 1700 in relation to the estimated dietary exposure to No. 2251 (SPET value of 1000 µg/day). The Committee therefore concluded that (±)-2-[(2-*p*-methoxy)

ethoxy]ethanol (No. 2251) would not pose a safety concern when used as a flavouring agent at current estimated dietary exposures.

Table 17 summarizes the evaluations of the seven flavouring agents belonging to this group of menthol and structurally related substances (Nos 427 and 2246–2251).

Consideration of combined intakes from use as flavouring agents

With the exception of dimethyl glutarate (No. 2250), all additional flavouring agents to the group of menthol and structurally related substances have low MSDI values (range: 0.03–21 µg/day). The highest MSDI value for dimethyl glutarate (No. 2250) is 10 784 µg/day. No production volume for No. 2250 was reported for Japan and Latin America. The Committee concluded that consideration of combined intakes is not necessary for Japan and Latin America because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

In the unlikely event that the flavouring agents with the common metabolite menthol (No. 427) in this group were to be consumed together with menthol (No. 427) on a daily basis, the estimated combined intakes⁵ for the four flavouring agents (Nos 427, 429, 1414 and 2250) with the highest estimated dietary exposures (MSDI values) would be 24 622 µg/day in Europe and 65 627 µg/day in the USA. The estimated combined intake would therefore exceed the human threshold of toxicological concern (1800 µg/day for structural class I). However, the vast majority of the combined intake would be due to menthol alone, for which an ADI of 0–4 mg/kg bw was previously established. The estimated combined intake does not exceed this ADI, which is equal to 240 mg/day for a 60 kg person. Also, as the flavouring agents are likely to be metabolized efficiently, they would not saturate metabolic pathways.

Therefore, the Committee concluded that combined intake would not raise safety concerns.

Consideration of additional data on previously evaluated flavouring agents

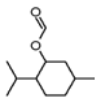
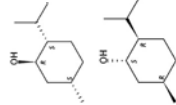
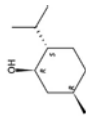
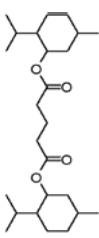
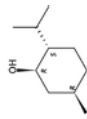
For eight previously evaluated flavouring agents in this group, additional studies of metabolism (No. 429); studies of acute toxicity (Nos 430, 1854 and 1856); and studies of genotoxicity (Nos 429, 430, 431, 445, 1856 and 1857) were available.

Conclusions

In the previous evaluations of substances in this group of menthol and structurally related substances, biochemical data; studies of acute toxicity, short-term toxicity,

⁵ Combined intake was calculated on a molar basis relative to the formation of a common metabolite. In this case, the common metabolite is menthol, with a relative molecular mass of 157.

Table 17
Summary of the results of safety evaluations of menthol and six additional structurally related substances used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c	Step 5 ^d	Related structure name (No.) and structure	Conclusion based on current estimated dietary exposure
			Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?		
Structural class I						
Menthyl formate	2246	2230-90-2 	No, SPET: 400	NR	NR	No safety concern
Menthyl propionate	2247	86014-82-6 	No, SPET: 600	NR	NR	No safety concern
<i>l</i> -Menthyl butyrate	2248	68366-64-3 	No, SPET: 300	NR	NR	No safety concern
<i>dl</i> -Isomenthol	2249	3623-52-7 	Yes, SPET: 3 000	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats for the structurally related substance menthol [12] is 7 600 times the estimated dietary exposure to No. 2249 when used as a flavouring agent.	Menthol (No. 427) 	No safety concern
Dimenthyl glutarate	2250	406179-71-3 	Yes, MSDI: 10 784	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats for the structurally related substance menthol [12] is 2 100 times the estimated dietary exposure to No. 2250 when used as a flavouring agent.	Menthol (No. 427) 	No safety concern

Step 4: ^a		Step 5 ^d		Conclusion based on current estimated dietary exposure
Flavouring agent	No.	Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	
Menthol ^b	427	Yes, MSDI: 51 474	Yes. The NOEL of 380 mg/kg bw per day from a 2-year dietary study in rats [12] is 440 times the estimated dietary exposure to No. 427 when used as a flavouring agent.	No safety concern
Structural class III				
(±)-2-(2- <i>p</i> -Menthoxyl)ethanol	2251	Yes, SPET: 1 000	Yes. The NOEL of 30 mg/kg bw per day from a 90-day study in rats for the structurally related substance 3- <i>l</i> -menthoxypropane-1,2-diol [13] is 1 700 times the estimated dietary exposure to No. 2251 when used as a flavouring agent.	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; NOAEL: no-observed-adverse-effect-level; NOEL: no-observed-effect level; no.: number; NR: not relevant; SPET: single-portion exposure technique

^a Twenty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 137 and 190).

^b Step 2: Six flavouring agents (Nos 427, 2246–2250) are in structural class I and one flavouring agent (No. 2251) is in structural class III.

^c The thresholds for toxicological concern for structural class I and structural class III are 1800 µg/day and 90 µg/day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest daily dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case except for Nos 427 and 2250 where the MSDI value was higher.

^d The margin of exposure was calculated based on the highest estimated daily dietary exposure using either the SPET or the MSDI method.

^e The acceptable daily intake of menthol of 0–4 mg/kg bw established at the fifty-first meeting (Annex 1, reference 137) was maintained.

long-term toxicity and carcinogenicity, genotoxicity, developmental toxicity, immunotoxicity and sensitivity; and human data were available. None of the 24 previously evaluated flavouring agents raised safety concerns based on the estimated dietary exposures and the biochemical and toxicological data available (Annex 1, references 138 and 191).

For the flavouring agent under re-evaluation, menthol (No. 427), additional biochemical data, studies of acute toxicity and genotoxicity and a case study were available. Studies of genotoxicity were available for the *dl*-isomenthol (No. 2249) and $(\pm)$ -2-[(2-*p*-menthoxy)ethoxy]ethanol (No. 2251).

The studies available for the present evaluation raised no safety concerns and support the previous safety evaluations. The additional data on menthol did not indicate a need to revise the ADI of menthol (No. 427).

The Committee concluded that the seven flavouring agents under evaluation, six of which are additions to the group of menthol and structurally related substances evaluated previously, do not give rise to safety concerns at current estimated dietary exposures. The previously established ADI of 0–4 mg/kg bw for menthol was maintained.

An addendum to the monograph was prepared.

References

1. Principles and methods for the risk assessment of chemicals in food. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. Geneva: World Health Organization, International Programme on Chemical Safety; 2009 (Environmental Health Criteria 240).
2. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.4 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>, accessed 15 November 2017).
3. Report of the Forty-eighth Session of the Codex Committee On Food Additives. Xi'an, China, 14–18 March 2016. Rome: Food and Agriculture Organization of the United Nations; and Geneva: World; 2016 (REP16/FA).
4. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
5. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
6. Heymann E. Carboxylesterases and amidases. In: Jakoby WB, editor, Enzymatic basis of detoxification. New York, NY, USA: Academic Press; 1980.
7. Anders MW. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson DH, Caldwell J, Paulson GD, editors. Intermediary xenobiotic metabolism in animals. New York, NY, USA: Taylor and Francis; 1989.

8. Williams RT. Studies in detoxication: The biological reduction of *l*-menthone to *d*-neomenthol and of *d*-isomenthone to *d*-isomenthol in the rabbit. The conjugation of *d*-neomenthol with glucuronic acid. *Biochem J*. 1940;34:690–7.
9. Atzl G, Bertel M, Daxenbichler G, Gleispach H. Determination of etheral oils from the urine by gas-liquid chromatography. *Chromatographia*. 1972;5(4):250–5.
10. Yamaguchi T, Caldwell J, Farmer PB. Metabolic fate of [3H]-*l*-menthol in the rat. *Drug Metab Dispos*. 1994;22(4):616–24.
11. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol*. 1978;16(3):255–76.
12. Bioassay of *DL*-menthol for possible carcinogenicity. US National Cancer Institute Report NCI-CG-TR-98. US National Technical Information Services Report No. PB-288761, Bethesda, Maryland, USA; 1979 [cited in [Annex I](#), reference 138].
13. Wolfe GW. 91-Day subchronic oral toxicity study in rats with D1156.02 (Laboratory project identification HWA, study no. 297-648). Unpublished report from Hazelton Washington, Inc., Vienna, Virginia, USA; 1992. Submitted to WHO by the Flavor and Extract Manufacturers' Association of the United States [cited in [Annex I](#), reference 174].

4.1.7 Miscellaneous nitrogen-containing substances

Introduction

The Committee evaluated three additional flavouring agents belonging to the group of miscellaneous nitrogen-containing substances, which was evaluated previously. The additional flavouring agents included a triazole moiety with a thiopyridine side-chain, 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine (No. 2235); a benzothiadiazine moiety with a piperidinyl side-chain, (S)-1-(3-(((4-Amino-2,2-dioxido-1*H*-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one (No. 2236); and an *N*-pyrazole- and *N*-thiophene-substituted amide, 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(thiophen-2-ylmethyl)acetamide (No. 2237). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230).

The Committee considered whether (S)-1-(3-(((4-amino-2,2-dioxido-1*H*-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one (No. 2236) belonged to the group of aliphatic and aromatic amines and amides. This group includes the structurally related 3[(4-amino-2,2-dioxido-1*H*-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide (No. 2082), which was evaluated at the seventy-sixth meeting ([Annex 1](#), reference 211). The Committee concluded that the additional flavouring agent should remain in this group. All three flavouring agents that were evaluated at this meeting are reported to be flavour modifiers.

The Committee previously evaluated 16 additional members of this group of miscellaneous nitrogen-containing substances at its sixty-fifth meeting

(Annex 1, reference 178); 14 additional members at its sixty-ninth meeting (Annex 1, reference 190); two additional members at its seventy-sixth meeting (Annex 1, reference 211); and two additional members at its seventy-ninth meeting (Annex 1, reference 220). At each meeting, the Committee concluded that all of the evaluated flavouring agents were of no safety concern at estimated dietary exposures.

None of the additional flavouring agents (Nos 2235, 2236 and 2237) in this group have been reported to occur naturally.

A comprehensive literature search on data on the additional flavouring agents was conducted; no additional studies were identified.

Assessment of dietary exposure

The total annual volume of production of the three flavouring agents belonging to the group of miscellaneous nitrogen-containing substances is 0.3 kg in the USA. No data on intake were reported for Europe, Japan or Latin America [1, 2]. Each of these flavouring agents contributes equally to the annual production volume in the USA.

Dietary exposures were estimated using both SPET and the MSDI method. The highest values are reported in Table 18. The estimated daily dietary exposure is highest for 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine (No. 2235), at 800 µg/person per day, the SPET value obtained from soups and broths. For each of the three flavouring agents the estimate from the MSDI method was 0.01 µg/day. For Nos 2236 and 2237 the highest SPET estimates were 750 and 600 µg/person per day, respectively.

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to this group has previously been described in the monographs of the sixty-fifth, sixty-ninth, seventy-sixth and seventy-ninth meetings (Annex 1, references 179, 191, 212 and 221). Additional information was available for this meeting.

Metabolic studies show that Nos 2236 and 2237 have low bioavailability [3, 4]. The data for No. 2235 showed that it was bioavailable with a short half-life in rat plasma. No. 2235 was shown to be metabolized in toxicokinetic and in vivo metabolism studies in the rat [5]. All these additional flavouring agents undergo limited hydrolysis, sulfoxidation or oxidation of the heterocyclic rings or are expected to be excreted unchanged in the faeces or urine [3, 4, 5].

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are no structural alerts for genotoxicity for the three additional flavouring agents (Nos 2235–2237). Chemical-specific genotoxicity data available

for each of these flavouring agents indicate that they lack the potential to be genotoxic.

Step 2. All three flavouring agents (Nos 2235–2237) were assigned to structural class III [6].

Step 3. The highest dietary exposures were estimated using SPET (Table 18).

Step 4. The highest dietary exposure estimates of the three flavouring agents in structural class III were above the threshold of toxicological concern (i.e. 90 µg/person per day for class III). These flavouring agents proceeded to Step 5.

Step 5. For these flavouring agents, the NOAELs of 100 mg/kg bw per day in rats, the highest dose tested in 90-day oral toxicity studies [3, 5], provide adequate margins of exposure (7500, 8000 and 10 000, respectively) relative to the highest estimated dietary exposure to No. 2235 (SPET = 800 µg/day), No. 2236 (SPET = 750 µg/day) and No. 2237 (SPET = 600 µg/day) when used as flavouring agents. The Committee therefore concluded that Nos 2235–2237 would not pose safety concerns at current estimated dietary exposures.

Table 18 summarizes the evaluations of the three flavouring agents belonging to this group of miscellaneous nitrogen-containing substances (Nos 2235–2237).

Consideration of combined intakes from use as flavouring agents

The three additional flavouring agents in this group of miscellaneous nitrogen-containing substances have low MSDIs (0.01 µg/day). The Committee concluded that consideration of combined intakes is not necessary because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of additional data on previously evaluated flavouring agents

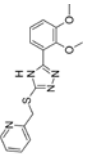
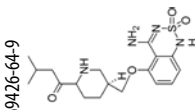
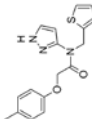
For previously evaluated flavouring agents in this group, studies of acute toxicity (No. 1566 and 1889), genotoxicity (No. 1566) and reproductive/developmental toxicity (No 2161) were available.

Conclusions

In previous evaluations of flavouring agents in this group of miscellaneous nitrogen-containing substances, biochemical data; studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity, genotoxicity and developmental and reproductive toxicity; and thyroid toxicity were available. The estimated dietary exposures and the biochemical and toxicological data available did not raise any safety concerns for the 34 previously evaluated flavouring agents in this group (Annex 1, references 178, 190, 211 and 220).

For the additional flavouring agents in this group, biochemical data (on Nos 2235–2237); studies of short-term toxicity (on Nos 2235–2237); studies on

Table 18
Summary of the results of the safety evaluations of miscellaneous nitrogen-containing substances^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 ^d Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
Structural class III					
2-(((3-(2,3-Dimethoxyphenyl)-1 <i>H</i> -1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	902136-79-2 	Yes, SPET: 800	Yes, The NOAEL of 100 mg/kg bw per day [5] is 7 500 times the estimated dietary exposure to No. 2235 when used as a flavouring agent.	No safety concern
(<i>S</i>)-1-(3-(((4-Amino-2,2-dioxido-1 <i>H</i> -benzol[1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	1469426-64-9 	Yes, SPET: 750	Yes, The NOAEL of 100 mg/kg bw per day [3] is 8 000 times the estimated dietary exposure to No. 2236 when used as a flavouring agent.	No safety concern
2-(4-Methoxyphenoxy)- <i>N</i> -(1 <i>H</i> -pyrazol-3-yl)- <i>N</i> -(thiophen-2-ylmethyl)acetamide	2237	1374760-95-8 	Yes, SPET: 600	Yes, The NOAEL of 100 mg/kg bw per day [4] is 10 000 times the estimated dietary exposure to No. 2237 when used as a flavouring agent.	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; NOAEL: no-observed-adverse-effect level; SPET: single-portion exposure technique

^a Thirty-four flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 178, 190, 211 and 220).
^b Step 2: All three flavouring agents are in structural class III.
^c The threshold for toxicological concern for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using the SPET.
^d The margin of exposure was calculated based on estimated dietary exposure calculated by the SPET.

genotoxicity (on Nos 2235–2237 and a metabolite of 2237); and developmental studies (on Nos 2236 and 2237) were available.

The studies available on the previously evaluated and additional flavouring agents in this group available for the present evaluation raised no safety concerns and support the previous safety evaluations.

The Committee concluded that these three flavouring agents, which are additions to the group of miscellaneous nitrogen-containing substances evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

References

1. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
2. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Arthur AJ, Karanewsky DS, Luksic M, Goodfellow G, Daniels J. Toxicological evaluation of two flavors with modifying properties: 3-((4-amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)-2,2-dimethyl-*N*-propylpropanamide and (S)-1-(3-(((4-amino-2,2-dioxido-1*H*-benzo[*c*][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one. *Food Chem Toxicol*. 2015;76:33–45.
4. Karanewsky DS, Arthur AJ, Liu H, Chi B, Markison S. Toxicological evaluation of a novel cooling compound: 2-(4-methylphenoxy)-*N*-(1*H*-pyrazol-3-yl)-*N*-(2-thienylmethyl)acetamide. *Toxicol Rep*. 2015;2:1291–309.
5. Karanewsky DS, Arthur AJ, Liu H, Chi B, Ida L, Markison S. Toxicological evaluation of a novel umami flavour compound: 2-(((3-(2,3-dimethoxyphenyl)-1*H*-1,2,4-triazol-5-yl)thio)methyl)pyridine. *Toxicol Rep*. 2016;3:501–12.
6. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol*. 1978;16(3):255–76.

4.1.8 Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids

The Committee evaluated two additional flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids that was evaluated previously. The additional flavouring agents were 8-methyldecanal (No. 2238) and 8-methylnonanal (No. 2239). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents ([Annex 1](#), reference 230). Neither of these substances had been previously evaluated.

The Committee evaluated 25 members of this group at its forty-ninth meeting ([Annex 1](#), reference 131). The Committee concluded that all 25 flavouring

agents did not raise any safety concerns at estimated dietary exposures. At its seventy-sixth meeting, the Committee evaluated four additional members of this group of flavouring agents and concluded that all four were of no safety concern at estimated dietary exposures ([Annex 1](#), reference 211).

8-Methylnonanal (No. 2239) was reported to occur naturally in citrus fruits. 8-Methyldecanal (No. 2238) is not reported to occur naturally [1].

A comprehensive literature search was conducted in PubMed; no additional relevant studies were identified.

Assessment of dietary exposure

The total annual volume of production of the two flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids is 0.1 kg in the USA, accounted for by 8-methyldecanal (No. 2238), and 0.1 kg in Japan, accounted for by 8-methylnonanal (No. 2239) [2, 3].

Dietary exposures were estimated using both the SPET and the MSDI method, with highest values reported in [Table 19](#). The highest estimated daily dietary exposure for 8-methyldecanal (No. 2238) is 0.1 µg/day (SPET value). The highest estimated daily dietary exposure of 8-methylnonanal (No. 2239) is 0.03 µg/day (MSDI value).

Absorption, distribution, metabolism and excretion

Information on the ADME of the flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids has previously been described in the monograph of the forty-ninth meeting ([Annex 1](#), references 132). Additional information on the ADME of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids was available for this meeting. The previously described information as well as the additional information available for this meeting, on the ADME of the flavouring agents belonging to this group, are summarized as follows.

The substances in this group share common metabolic pathways, and are expected to be absorbed into the gastrointestinal tract [4, 5]. Shorter branched-chain aliphatic alcohols, aldehydes and acids undergo β -oxidation cleavage, with intermediates metabolized to CO₂ via the tricarboxylic acid cycle. Longer alkyl chain length and branching increases susceptibility to both oxidation and glucuronidation in aliphatic alcohols, as there is an increase in the affinity for UDP-glucuronosyltransferases [6, 7]. Longer and more substituted alcohols produce polar metabolites after undergoing ω -, ω -1 and β -oxidation and selective dehydrogenation and hydration, resulting in chain-shortening [8]. The substances in this group are expected to be metabolized to innocuous products via common metabolic pathways, or be excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. There are neither structural alerts for genotoxicity nor chemical-specific genotoxicity data on the additional flavouring agents. New and previously evaluated data from other related flavouring agents indicate that these flavouring agents are not likely to be genotoxic. Therefore, the weight of evidence indicates that these additional saturated aliphatic acyclic branched-chain primary aldehyde flavouring agents are not likely to be genotoxic.

Step 2. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned both flavouring agents to structural class I [9].

Steps 3 and 4. Dietary exposures using both MSDI method and SPET have been determined. The highest estimated dietary exposures of both flavouring agents in structural class I were below the threshold of concern (i.e. 1800 µg/person per day for Class I). The Committee therefore concluded that both flavouring agents (Nos 2238 and 2239) would not pose a safety concern at current estimated dietary exposures.

Consideration of combined intakes from use as flavouring agents

The two additional flavouring agents in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids have low MSDIs (0.01–0.03 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the exposure to this flavouring group.

Consideration of additional data on previously evaluated flavouring agents

In the previous evaluation of substances in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids, studies of biochemistry, acute toxicity, short-term and long-term toxicity, reproductive and developmental toxicity and genotoxicity were available ([Annex 1](#), references 132 and 211). None of the 29 flavouring agents of this group raised safety concerns.

For the present evaluation, no relevant studies were available for the two additional flavouring agents (Nos 2238 and 2239). For previously evaluated flavouring agents in this group, studies of acute toxicity (Nos 251, 252, 253, 254, 258, 260, 267, 268, 269 and 2176), studies of short-term toxicity (Nos 251, 252, 254, 258, 267, 269, 272 and 275), studies of long-term toxicity (No. 252), studies of genotoxicity (Nos 52, 251, 252, 253, 255, 259, 268, 270, 272 and 275), studies of reproductive and developmental toxicity (Nos 251, 252, 267, 268 and 272) and neurotoxicity (No. 251) were available.

There are positive genotoxicity data, not previously evaluated, for isobutyraldehyde (No. 252) in an in vitro and in vivo chromosomal aberration assay, an in vitro SCE assay and an in vitro forward mutation assay; and for

Table 19
Summary of the results of the safety evaluations of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids used as flavouring agents^{a,b}

Flavouring agent	No.	CAS no. and structure	Step 4 ^c Does the highest dietary exposure estimate exceed the threshold of toxicological concern?	Step 5 Does a NOAEL exist for the flavouring agent or a structurally related substance that provides an adequate margin of exposure?	Conclusion based on current estimated dietary exposure
Structural class I					
8-Methyldecanal	2238	127793-88-8 	No, SPET: 0.1	NR	No safety concern
8-Methylnonanal	2239	3085-26-5 	No, MSDI: 0.03	NR	No safety concern

CAS: Chemical Abstracts Service; MSDI: maximized survey-derived intake; no.: number; NOAEL: no-observed-adverse-effect level; NR: not required; SPET: single-portion exposure technique

^a Twenty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, references 132 and 211).

^b Step 2: Both flavouring agents are in structural class I.

^c The threshold for toxicological concern for structural class I is 1800 µg/day, respectively. All dietary exposure values are expressed in µg per day. The dietary exposure value listed represents the highest daily per capita intake calculated either by SPET or MSDI. The SPET gave the highest estimated dietary exposure for No. 2238 and the MSDI gave the highest estimated dietary exposure for No. 2239.

isobutyric acid (No. 253) in an in vitro forward mutation assay. The bacterial reverse mutation assays, and the in vivo micronucleus assays on isobutyraldehyde (No. 252), were negative. The in vivo chromosomal aberration assay using isobutyraldehyde (No. 252) was only positive at the highest dose, which produced notable signs of cytotoxicity. The negative in vivo results for isobutyraldehyde (No. 252) are consistent with the 2-year inhalational carcinogenicity mouse and rat assays that showed nasal toxicity but no carcinogenicity [10, 11]. Therefore, the weight of evidence indicates that these saturated aldehyde flavouring agents are not likely to be genotoxic. The results of the remainder of the genotoxicity assays on flavouring agents in this group are negative.

The studies available for the present evaluation support the previous safety evaluations.

Conclusions

The Committee concluded that these two flavouring agents, which are additions to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the monograph was prepared.

References

1. Nijssen LM, van Ingen-Visscher CA, Donders JJH. Volatile compounds in food, version 16.3 [database]. Zeist, the Netherlands: TNO Triskelion B.V.; 2017 (<http://www.vcf-online.nl/VcfHome.cfm>).
2. 2015 IOFI Global Poundage Survey. International Organization of the Flavor Industry, Brussels; 2017a. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
3. Interim inquiry on volume use and added use levels for flavoring agents to be presented at the JECFA 86th meeting. Private communication to the International Organization of the Flavor Industry, Brussels; 2017b. Submitted to WHO by the International Organization of the Flavor Industry, Brussels.
4. Dawson AM, Holdsworth CD, Webb J. Absorption of short chain fatty acids in man. *Proc Soc Exp Biol Med.* 1964;117:97–100.
5. Gaillard D, Derache R. [Metabolism of different alcohols present in alcoholic beverages, in the rat.] *Trav Soc Pharm Montp.* 1965;25:51–62.
6. Iwersen S, Schmoldt A. ADH-independent metabolism of aliphatic alcohols: Comparison of oxidation and glucuronidation. *Adv Forensic Sci.* 1995;4:19–22.
7. Jurowich S, Sticht G, Käferstein H. Glucuronidation of aliphatic alcohols in human liver microsomes in vitro. *Alcohol.* 2004;32:187–94.
8. Veenstra G, Webb C, Sanderson H, Belanger SE, Fisk P, Nielsen A et al. Human health risk assessment of long chain alcohols. *Ecotoxicol Environ Saf.* 2009;72(4):1016–30.
9. Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. *Food Cosmet Toxicol.* 1978;16(3):255–76.

10. Abdo KM, Haseman JK, Nyska A. Isobutyraldehyde administered by inhalation (whole body exposure) for up to thirteen weeks or two years was a respiratory tract toxicant but was not carcinogenic in F344/N rats and B6C3F1 mice. *Toxicol Sci.* 1998;42(2):136–51.
11. NTP toxicology and carcinogenesis studies of isobutyraldehyde (CAS No. 78-84-2) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, North Carolina, USA: US Department of Health and Human Services, National Toxicology Program; 1999 (NTP Technical Report 472).

4.2 Specifications of identity and purity of flavouring agents

4.2.1 New and maintained specifications

The Committee received information related to specifications for 20 of the 21 new flavouring agents from the call for data for the present meeting. While carvone (Flavor and Extract Manufacturers Association of the United States [FEMA] No. 2249) was listed in the call for data as a new flavouring agent, the Committee noted that full specifications already existed for (+)-carvone (No. 380.1; *d*-carvone) and (–)-carvone (No. 380.2; *l*-carvone). As no new data on specifications were provided for (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2), the existing specifications were maintained. Revisions were made to the chemical formulae for 3-[(2-methyl-3-furyl)thio]-2-butanone (No. 1525) and *O*-ethyl *S*-(2-furylmethyl)thiocarbonate (No. 1526), both of which remained as full specifications.

Full specifications were prepared for the 20 new flavouring agents for which data were provided. Forty-seven flavouring agents for which full specifications (Nos 427, 973–975, 980–982, 1480, 1491–1526 and 2103–2105) currently exist were considered by the Committee for toxicological re-evaluation at the current meeting (see sections 4.1.1, 4.1.3, 4.1.5 and 4.1.6). Thirty-nine of these flavouring agents (Nos. 1491–1526 and 2103–2105; see section 4.1.3) previously included a statement indicating that the safety evaluation for the flavouring agents had not been completed. As the toxicological evaluations of these 39 flavourings were completed at this meeting and no safety concerns were noted, the text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full.

A statement was added to the existing full specifications for *p*-mentha-1,8-dien-7-al (perillaldehyde; No. 973), (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) indicating that, at the current meeting, the safety evaluations for these flavouring agents were not completed. The specifications for *p*-mentha-1,8-dien-7-al (No. 973), (+)-carvone (No. 380.1) and (–)-carvone (No. 380.2) were maintained as full.

4.2.2 Revised specifications

The Committee received information in support of the revision of full specifications for three flavouring agents that were on the agenda of the present meeting (Nos 433, 619 and 2123).

The Committee revised specifications for *l*-menthyl lactate (No. 433) to reflect the specific isomeric composition of the flavouring agent in commerce. This was accomplished by changing the CAS number from 59259-38-0 to 61597-98-6 and revising the name to *l*-menthyl *l*-lactate.

The Committee revised specifications for *l*-malic acid (No. 619) by removing the specification for specific rotation based on results received, which indicate difficulty in standardizing this measurement for *l*-malic acid.

The Committee revised the specifications for glutamyl-valyl-glycine by revising the melting-point range of the flavouring.



5. Future work and recommendations

Specific food additives (other than flavouring agents)

Anionic methacrylate copolymer

The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid, one of the residual monomers of AMC. Further studies to clarify the in vivo carcinogenic potential are required to complete the evaluation of AMC.

Citric and fatty acid esters of glycerol

The specifications of CITREM were made tentative, pending a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.

The Committee noted that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform.

Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by **December 2019**.

Neutral methacrylate copolymer

The Committee noted that no data were submitted for a suitable method of assay. Tentative specifications for NMC were prepared pending a suitable validated method of assay.

Spirulina extract

The Committee received limited analytical data on spirulina extract. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested by **December 2019**:

- Full compositional characterization of commercial products in both liquid and powder forms;
- Full compositional characterization of the aqueous extract before formulation/standardization;
- Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data); and

- Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).

Modified starches

The Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in Hydroxypropyl starch (INS 1440) and Hydroxypropyl distarch phosphate (INS 1442) in order to consider lowering this limit.

The Committee requested suitable microbiological acceptance criteria and supporting data for all modified starches. Information required in the recommended annexes is summarized in [Table 9](#).

Flavouring agents

Carvone and structurally related substances

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within **3 years**.

For (–)-carvone (No. 380.2), the Committee concluded that toxicological data on (–)-carvone are necessary. Also, data are needed for an exposure assessment for the oral exposure to (–)-carvone from all sources.

Maltol and related substances

The Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol (No. 1480) during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting ([Annex 1](#), reference 57).

The Committee withdrew the ADI for maltol. The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the ADI.

The ADI for ethyl maltol was maintained.

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Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some

- antioxidants (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
 28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
 29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
 30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
 31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocabamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
 32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
 33. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
 34. Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers. FAO Food and Nutrition Paper, No. 4, 1978.
 35. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
 36. Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
 37. Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
 38. Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
 39. Toxicological evaluation of some food colours, thickening agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
 40. Specifications for the identity and purity of certain food additives. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.

41. Evaluation of certain food additives (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 10, 1976.
43. Specifications for the identity and purity of some food additives. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.
46. Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. Evaluation of certain food additives and contaminants (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. Summary of toxicological data of certain food additives and contaminants. WHO Food Additives Series, No. 13, 1978.
49. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 7, 1978.
50. Evaluation of certain food additives (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 14, 1980.
52. Specifications for identity and purity of food colours, flavouring agents, and other food additives. FAO Food and Nutrition Paper, No. 12, 1979.
53. Evaluation of certain food additives (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 15, 1980.
55. Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives). FAO Food and Nutrition Paper, No. 17, 1980.
56. Evaluation of certain food additives (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 16, 1981.
58. Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives). FAO Food and Nutrition Paper, No. 19, 1981.
59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 17, 1982.
61. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 25, 1982.

62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.
64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.
65. Guide to specifications – General notices, general methods, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. Evaluation of certain food additives and contaminants (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.
68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.
72. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
73. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
75. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 37, 1986.
76. Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pes.
77. Evaluation of certain food additives and contaminants (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.
78. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
79. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 38, 1988.
80. Evaluation of certain veterinary drug residues in food (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

81. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988.
83. Evaluation of certain food additives and contaminants (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. Evaluation of certain veterinary drug residues in food (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 25, 1990.
87. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. Evaluation of certain food additives and contaminants (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.
90. Specifications for identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 49, 1990.
91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.
93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, 1991.
96. Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).
97. Evaluation of certain veterinary drug residues in food (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 29, 1991.
99. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, 1991.
100. Guide to specifications – General notices, general analytical techniques, identification tests, test solutions, and other reference materials. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.
101. Evaluation of certain food additives and naturally occurring toxicants (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.

102. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series, No. 30, 1993.
103. Compendium of food additive specifications: addendum 1. FAO Food and Nutrition Paper, No. 52, 1992.
104. Evaluation of certain veterinary drug residues in food (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 31, 1993.
106. Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper, No. 41/5, 1993.
107. Evaluation of certain food additives and contaminants (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 32, 1993.
109. Compendium of food additive specifications: addendum 2. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. Evaluation of certain veterinary drug residues in food (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 33, 1994.
112. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/6, 1994.
113. Evaluation of certain veterinary drug residues in food (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 34, 1995.
115. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/7, 1995.
116. Evaluation of certain food additives and contaminants (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 35, 1996.
118. Compendium of food additive specifications: addendum 3. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. Evaluation of certain veterinary drug residues in food (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 36, 1996.
121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.
122. Evaluation of certain food additives and contaminants (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 37, 1996.

124. Compendium of food additive specifications, addendum 4. FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
125. Evaluation of certain veterinary drug residues in food (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 38, 1996.
127. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/9, 1997.
128. Evaluation of certain veterinary drug residues in food (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 39, 1997.
130. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/10, 1998.
131. Evaluation of certain food additives and contaminants (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.
133. Compendium of food additive specifications: addendum 5. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
134. Evaluation of certain veterinary drug residues in food (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.
135. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 41, 1998.
136. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/11, 1999.
137. Evaluation of certain food additives (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.
138. Safety evaluation of certain food additives. WHO Food Additives Series, No. 42, 1999.
139. Compendium of food additive specifications, addendum 6. FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. Evaluation of certain veterinary drug residues in food (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.
141. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 43, 2000.
142. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/12, 2000.
143. Evaluation of certain food additives and contaminants (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.
144. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 44, 2000.

145. Compendium of food additive specifications, addendum 7. FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. Evaluation of certain veterinary drug residues in food (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.
147. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 45, 2000.
148. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/13, 2000.
149. Evaluation of certain food additives and contaminants (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 901, 2001.
150. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 46, 2001.
151. Compendium of food additive specifications: addendum 8. FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
152. Evaluation of certain mycotoxins in food (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 906, 2002.
153. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper, No. 74, 2001.
154. Evaluation of certain food additives and contaminants (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.
155. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 48, 2002.
156. Compendium of food additive specifications: addendum 9. FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.
157. Evaluation of certain veterinary drug residues in food (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.
158. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 49, 2002.
159. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/14, 2002.
160. Evaluation of certain food additives and contaminants (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.
161. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 50, 2003.
162. Compendium of food additive specifications: addendum 10. FAO Food and Nutrition Paper, No. 52, Add. 10, 2002.
163. Evaluation of certain veterinary drug residues in food (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.
164. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 51, 2003.
165. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/15, 2003.

166. Evaluation of certain food additives and contaminants (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.
167. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 52, 2004.
168. Compendium of food additive specifications: addendum 11. FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.
169. Evaluation of certain veterinary drug residues in food (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.
170. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/16, 2004.
171. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 53, 2005.
172. Compendium of food additive specifications: addendum 12. FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.
173. Evaluation of certain food additives (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.
174. Safety evaluation of certain food additives. WHO Food Additives Series, No. 54, 2005.
175. Compendium of food additive specifications: addendum 13. FAO Food and Nutrition Paper, No. 52, Add. 13 (with Errata), 2005.
176. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.
177. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2006.
178. Evaluation of certain food additives (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934, 2006.
179. Safety evaluation of certain food additives. WHO Food Additives Series, No. 56, 2006.
180. Combined compendium of food additive specifications. FAO JECFA Monographs 1, Volumes 1–4, 2005, 2006.
181. Evaluation of certain veterinary drug residues in food (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006.
182. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 2, 2006.
183. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 57, 2006.
184. Evaluation of certain food additives and contaminants (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940, 2007.
185. Compendium of food additive specifications. FAO JECFA Monographs 3, 2006.
186. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 58, 2007.
187. Evaluation of certain food additives and contaminants (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.

188. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 59, 2008.
189. Compendium of food additive specifications. FAO JECFA Monographs 4, 2007.
190. Evaluation of certain food additives (Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 952, 2009.
191. Safety evaluation of certain food additives. WHO Food Additives Series, No. 60, 2009.
192. Compendium of food additive specifications. FAO JECFA Monographs 5, 2009.
193. Evaluation of certain veterinary drug residues in food (Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 954, 2009.
194. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 61, 2009.
195. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 6, 2009.
196. Evaluation of certain food additives (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.
197. Safety evaluation of certain food additives. WHO Food Additives Series, No. 62, 2010.
198. Compendium of food additive specifications. FAO JECFA Monographs 7, 2009.
199. Evaluation of certain contaminants in food (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959, 2011.
200. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 63/FAO JECFA Monographs 8, 2011.
201. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 9, 2010.
202. Evaluation of certain food additives and contaminants (Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960, 2011.
203. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 64, 2011.
204. Compendium of food additive specifications. FAO JECFA Monographs 10, 2010.
205. Evaluation of certain food additives and contaminants (Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 966, 2011.
206. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 65, 2011.
207. Compendium of food additive specifications. FAO JECFA Monographs 11, 2011.
208. Evaluation of certain veterinary drug residues in food (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 969, 2012.
209. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 66, 2012.
210. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 12, 2012.
211. Evaluation of certain food additives (Seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 974, 2012.
212. Safety evaluation of certain food additives. WHO Food Additives Series, No. 67, 2012.

213. Compendium of food additive specifications. FAO JECFA Monographs 13, 2012.
214. Evaluation of certain food additives and contaminants (Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 983, 2013.
215. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 68, 2013.
216. Compendium of food additive specifications. FAO JECFA Monographs 14, 2013.
217. Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 988, 2014.
218. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 69, 2014.
219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.
220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.
221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.
222. Compendium of food additive specifications. FAO JECFA Monographs 16, 2014.
223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.
224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.
225. Compendium of food additive specifications. FAO JECFA Monographs 17, 2015.
226. Evaluation of certain veterinary drug residues in food (Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 997, 2016.
227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.
228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 18, 2016.
229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.
230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.
231. Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.
232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.
233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1002, 2017.
234. Evaluation of certain food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1007, 2017.
235. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 74, 2018 (in press).
236. Compendium of food additive specifications. FAO JECFA Monographs 20, 2017.
237. Toxicological evaluation of certain food additives. WHO Food Additives Series, No. 75, 2018 (in press).

238. Evaluation of certain veterinary drug residues in food (Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1008, 2018.
239. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 21, 2018.
240. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 76, 2019 (in press).



ANNEX 2

Toxicological information and information on specifications

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Anionic methacrylate copolymer (AMC)	N, T ^a	The Committee was unable to complete the evaluation of AMC. While the copolymer itself is not of health concern, genotoxicity concerns remains for the residual monomer methacrylic acid. The specifications were made tentative pending the completion of the safety evaluation of AMC.
Basic methacrylate copolymer (BMC)	N	The Committee established an ADI “not specified” for basic methacrylate copolymer. The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification. The no-observed-adverse-effect level (NOAEL) for BMC ranged from 750 to 2 000 mg/kg body weight (bw) per day, which were the highest doses tested. The Committee evaluated exposure to BMC for the copolymer and its monomers (<i>n</i>-butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate). Estimated exposures to BMC range from 3.0 to 135 mg/kg bw per day. The total monomeric content of BMC is less than 0.3%. The Committee concluded that the toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.
Erythrosine	R ^b	The Committee concluded that the new data that have become available since the previous evaluation of erythrosine do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw. The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to for all age groups do not present a health concern. erythrosine for all age groups do not present a health concern.
Indigotine	R ^b	The Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies and concluded that there are no reasons to revise the ADI and confirmed the previous ADI of 0–5 mg/kg bw.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper bound of the ADI of 0–5 mg/kg bw. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.
Lutein	R ^{c,d}	Free lutein, lutein esters and free zeaxanthin including <i>meso</i>-zeaxanthin are biochemically and toxicologically equivalent. At the present meeting the Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from <i>Tagetes erecta</i>, synthetic zeaxanthin and <i>meso</i>-zeaxanthin. Free lutein, lutein esters and free zeaxanthin and <i>meso</i>-zeaxanthin are substances of low toxicity for which no adverse effects have been observed in a broad range of toxicological studies in laboratory animals and clinical studies in humans. Based on the absence of toxicity in a wide range of studies, the Committee established a group ADI “not specified” for lutein from <i>Tagetes erecta</i>, lutein esters from <i>Tagetes erecta</i> and zeaxanthin (synthetic). <i>Meso</i>-zeaxanthin was not included in this group ADI, as specifications are not currently available. The group ADI of 0–2 mg/kg bw for lutein from <i>Tagetes erecta</i> and zeaxanthin (synthetic) was withdrawn.
Neutral methacrylate copolymer (NMC)	N, T	The Committee established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative. The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes. The NOAELs for NMC ranged from 454 to 2 000 mg/kg bw per day, and these were the highest doses tested. The Committee evaluated exposure to NMC for the copolymer and its monomers (methyl methacrylate and ethyl acrylate). Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day. The total monomeric content of NMC is less than 0.01%. Toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.
Sorbitol syrup	—	Sorbitol syrup (INS 420(ii)) is currently included in the Codex General Standard for Food Additives (GSFA) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. The Committee was therefore requested to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances, and advise on the need for a separate evaluation of sorbitol syrup or if the ADI “not specified” for sorbitol is also applicable for sorbitol syrup.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		Based on the similarity of the chemical constituents of sorbitol syrup to the previously evaluated sorbitol, maltitol syrup and polyglycitol syrup, the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI “not specified” for sorbitol syrup.
Spirulina extract	N, T	The Committee established a temporary ADI “not specified” for spirulina extract. The ADI was based on the absence of toxicity in repeated-dose animal studies with spirulina extract and dried spirulina. The ADI “not specified” was made temporary due to the tentative nature of the specifications. Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour based on the Budget method and exposure to spirulina extract and dried spirulina from other dietary sources, including food ingredients, dietary supplements, and coatings of food supplements was 190 mg/kg bw for adults (60 kg/person) and 650 mg/kg bw for a child (15 kg/person). The Committee concluded that this dietary exposure does not present a health concern.

—: no specifications prepared; N: new specifications; R: existing specifications revised; T: tentative specifications

^a The specifications were made tentative pending the completion of the safety evaluation of AMC.

^b At the current meeting, high-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

^c The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.

^d At the current meeting, the identity test for melting range was deleted, the identity tests for carotenoids and spectrophotometry were updated, the test for propylene glycol was incorporated verbatim and the previous reference removed, and the method of assay was updated.

Food additives considered for specifications only

Food additive	Specifications
Cassia gum	R ^a
Citric and fatty acid esters of glycerol	R, T ^b
Glycerol ester of wood rosin	R ^c
Modified starches	R ^d , T

R: existing specifications revised; T: tentative specifications

^a The Committee, at its current meeting, received analytical methods and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with *n*-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified. The specifications were revised and the tentative status was removed.

^b The Committee did not receive a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in its specifications monograph. The Committee noted further that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform. Specifications were revised and made tentative pending the availability of data. Specifications will be withdrawn if suitable information is not provided by December 2019.

^c The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species namely *Pinus halepensis* and *Pinus brutia* as source materials. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. Since the specifications monograph for GEWR does not contain an assay, the Committee recommends that the JECFA Secretariat be notified upon the development and validation of an appropriate assay. The existing specifications were revised.

^d The Committee reviewed data on the method of manufacture, identity, and purity of all 16 modified starches. Based on the information received, and available information the Committee noted the following:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered the application of a read-across approach to be appropriate for the toxicological evaluation of these substances.
- All 16 modified starches had been assigned an ADI of “not specified”.

- All modified starches can be additionally bleached or fragmented; therefore revision in the specifications of bleached or fragmented starches would imply the revision of all 16 monographs.
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals impurities content and microbiological considerations). Revision of those common specifications would affect all 16 monographs.
- As a result of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, potentially unavailable, and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.
- Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs should be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture, and the corresponding diversity of impurities. The Committee recommended that all modified starches be included in a modular monograph titled 'Modified Starches' that contains common requirements [General specifications for modified starches] consisting of specifications that apply to all 16 modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The Committee drafted a new modular specifications monograph titled "Modified starches" consisting of an explanatory introduction, "General specifications for modified starches," and eight annexes. The new modular specifications monograph for modified starches is printed in FAO Monograph 22, and will replace the 16 existing individual specifications for modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

The specification for lead included in the General specifications be decreased from 2 mg/kg to 0.2 mg/kg. The limit of lead for starch sodium octenylsuccinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added.

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

A. Alicyclic primary alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Mixture of 1-Vinyl-3- cyclohexenecarbaldehyde and 4-vinyl-1-cyclohexenecarbaldehyde	2253	N	No safety concern
<i>p</i> -Mentha-1,8-dien-7-ol	974	N	No safety concern
<i>p</i> -Mentha-1,8-dien-7-yl acetate	975	N	No safety concern
Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene	980	N	No safety concern
Myrtenol	981	N	No safety concern
Myrtenyl acetate	982	M	No safety concern
Structural class II			
(1-Methyl-2-(1,2,2- trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol	2254	N	No safety concern
Structural class III			
(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester	2255	N	No safety concern
Flavouring agent excluded at Step 1 of the Procedure			
<i>p</i> -Mentha-1,8-dien-7-al (Perillaldehyde)	973	M	Genotoxicity data for <i>p</i> -mentha-1,8-dien-7-al raise concerns for potential genotoxicity

M: existing specifications maintained; N: new specifications

B. Carvone and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Pinocaryyl isobutyrate	2242	N	No safety concern
Carvyl palmitate	2243	N	No safety concern
Structural class III			
6-Hydroxycarvone	2244	N	No safety concern
Flavouring agents not evaluated according to the revised Procedure			
(+)-Carvone	380.1	M	The Committee did not re-evaluate (+)-carvone (No. 380.1) according to the revised Procedure given the lack of information on the oral exposure from all sources and the need to review the ADI. A review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.
(–)-Carvone	380.2	M	The Committee did not re-evaluate (–)-carvone (No. 380.2) according to the revised Procedure given the lack of information on the oral exposure from all sources and the lack of toxicological data.

M: existing specifications maintained; N: new specifications

C. Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-Pentylfuran	1491	M ^a	No safety concern
2-Heptylfuran	1492	M ^a	No safety concern
2-Decylfuran	1493	M ^a	No safety concern
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	M ^a	No safety concern
2,3-Dimethylbenzofuran	1495	M ^a	No safety concern
2,4-Difurfurylfuran	1496	M ^a	No safety concern
3-(2-Furyl)acrolein	1497	M ^a	No safety concern
2-Methyl-3(2-furyl)acrolein	1498	M ^a	No safety concern
3-(5-Methyl-2-furyl)prop-2-enal	1499	M ^a	No safety concern
3-(5-Methyl-2-furyl)butanal	1500	M ^a	No safety concern
2-Furfurylidene-butyraldehyde	1501	M ^a	No safety concern

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
2-Phenyl-3-(2-furyl)prop-2-enal	1502	M ^a	No safety concern
2-Furyl methyl ketone	1503	M ^a	No safety concern
2-Acetyl-5-methylfuran	1504	M ^a	No safety concern
2-Acetyl-3,5-dimethylfuran	1505	M ^a	No safety concern
3-Acetyl-2,5-dimethylfuran	1506	M ^a	No safety concern
2-Butyrylfuran	1507	M ^a	No safety concern
(2-Furyl)-2-propanone	1508	M ^a	No safety concern
2-Pentanylfuran	1509	M ^a	No safety concern
1-(2-Furyl)butan-3-one	1510	M ^a	No safety concern
4-(2-Furyl)-3-buten-2-one	1511	M ^a	No safety concern
Pentyl 2-furyl ketone	1512	M ^a	No safety concern
Ethyl 3-(2-furyl)propanoate	1513	M ^a	No safety concern
Isobutyl 3-(2-furan)propionate	1514	M ^a	No safety concern
Isoamyl 3-(2-furan)propionate	1515	M ^a	No safety concern
Isoamyl 3-(2-furan)butyrate	1516	M ^a	No safety concern
Phenethyl 2-furoate	1517	M ^a	No safety concern
Propyl 2-furanacrylate	1518	M ^a	No safety concern
2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	1519	M ^a	No safety concern
Furfuryl methyl ether	1520	M ^a	No safety concern
Ethyl furfuryl ether	1521	M ^a	No safety concern
Difurfuryl ether	1522	M ^a	No safety concern
2,5-Dimethyl-3-furanthiol acetate	1523	M ^a	No safety concern
Furfuryl 2-methyl-3-furyl disulfide	1524	M ^a	No safety concern
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	M ^a	No safety concern
O-Ethyl-S-(2-furylmethyl)thiocarbonate	1526	M ^a	No safety concern
(E)-Ethyl 3-(2-furyl)acrylate	2103	M ^a	No safety concern
di-2-Furylmethane	2104	M ^a	No safety concern
2-Methylbenzofuran	2105	M ^a	No safety concern

M: existing specifications maintained

^a The text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full.

D. Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
<i>trans</i> -6-Octenal	2240	N	No safety concern
2,6-Dimethyl-5-heptenol	2241	N	No safety concern

N: new specifications

E. Maltol and related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
Maltol	1480	M	No safety concern ^a
Structural class III			
Ethyl maltol isobutyrate	2252	N	No safety concern

M: existing specifications maintained; N: new specifications

^a The previously established ADI for maltol was withdrawn by the Committee.

F. Menthol and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Menthyl formate	2246	N	No safety concern
Menthyl propionate	2247	N	No safety concern
<i>l</i> -Menthyl butyrate	2248	N	No safety concern
<i>d,l</i> -Isomenthol	2249	N	No safety concern
Dimethyl glutarate	2250	N	No safety concern
Menthol	427	M	No safety concern
Structural class III			
(±)-2-[(2- <i>p</i> -Methoxy)ethoxy]ethanol	2251	N	No safety concern

M: existing specifications maintained; N: new specifications

^a The ADI of menthol of 0–4 mg/kg bw established at the fifty-first meeting was maintained.

G. Miscellaneous nitrogen-containing substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-(((3-(2,3-Dimethoxyphenyl)-1 <i>H</i> -1,2,4-triazol-5-yl)thio)methyl)pyridine	2235	N	No safety concern
(<i>S</i>)-1-(3-(((4-Amino-2,2-dioxido-1 <i>H</i> -benzo[<i>c</i>][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one	2236	N	No safety concern
2-(4-Methylphenoxy)- <i>N</i> -(1 <i>H</i> -pyrazol-3-yl)- <i>N</i> -(thiophen-2-ylmethyl)acetamide	2237	N	No safety concern

N: new specifications

H.Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes, and acids

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
8-Methyldecanal	2238	N	No safety concern
8-Methylnonanal	2239	N	No safety concern

N: new specifications

Food additives considered for specifications only

Flavouring agent	No.	Specifications
L-Menthyl lactate	433	R ^a
L-Malic acid	619	R ^b
Glutamyl-valyl-glycine	2123	R ^c

R: existing specifications revised

^a The CAS number was changed from 59259-38-0 to 61597-98-6 and the name to *L*-menthyl *L*-lactate.

^b The specification for specific rotation were removed.

^c The melting point range was revised.

Annex 3

Summary of the safety evaluation of the secondary components for flavouring agents with minimum assay values of less than 95%

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers				
1519	2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	93%	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (1–3%) Butyric acid (No. 87) (1–3%)	The SPET value for No. 1519 is 1 200 µg/day, and 3% of this value is 36 µg/day, which is below the class III threshold of toxicological concern. Butyric acid (No. 87) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
1524	Furfuryl 2-methyl-3-furyl disulfide	90%	Di-(2-methyl-3-furyl) disulfide (6–7%)	The SPET value for No. 1524 is 10 µg/day, and 7% of this value is 0.7 µg/day, which is below the class III threshold of toxicological concern. The major secondary component of No. 1524 is therefore not considered to present a safety concern at estimated dietary exposures from the use of No. 1524 as a flavouring agent.
Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters				
2241	2,6-Dimethyl-5-heptenol	>90%	2,6-Dimethyl-5-heptenal (No. 349) (1–6%)	2,6-Dimethyl-5-heptenal (No. 349) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.
Maltol and related substances				
2252	Ethyl maltol isobutyrate	93%	Ethyl maltol (No. 1481) (2–3%)	Ethyl maltol (No. 1481) has previously been evaluated by the Committee to be of no safety concern at estimated dietary exposures when used as a flavouring agent.



Annex 4

Meeting agenda



Food and Agriculture
Organization of the
United Nations



World Health
Organization

86th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
WHO Headquarters, Geneva 12 – 21 June 2018

Draft Agenda

1. Opening
2. Declarations of Interests (information by the Secretariat on any declared interests and discussion, update by experts)
3. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs
4. Adoption of Agenda
5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives (CCFA)
6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full committee)
7. Evaluations

Food Additives

7.1. Toxicological Evaluation, Exposure Assessment, and Establishment of Specifications:

- Basic methacrylate copolymer (INS 1205)
- Neutral methacrylate copolymer (INS 1206)
- Anionic methacrylate copolymer (INS 1207)
- Lutein from *Tagetes erecta* (INS 161b(i))
- Spirulina extract
- Erythrosine (INS 127)
- Indigotine (INS 132)

7.2. Food additives for revision of specifications and analytical methods:

- Citric and fatty acid esters of glycerol (INS 472 c)
- Glycerol ester of wood rosin (GEWR) (INS445(iii))
- Cassia gum
- Dextrin roasted starch (INS 1400)
- Acid treated starch (INS 1401)
- Alkaline treated starch (INS 1402)
- Bleached starch (INS1403)
- Enzyme-treated starch (INS 1405)
- Monostarch phosphate (INS 1410)
- Distarch phosphate (INS 1412)
- Phosphated distarch phosphate (INS 1413)
- Acetylated distarch phosphate (INS 1414)
- Acetylated distarch adipate (INS 1422)
- Hydroxypropyl starch (INS 1440)
- Hydroxypropyl distarch phosphate (INS 1442)
- Starch sodium octenyl succinate (INS 1450)

Flavourings

7.3 Toxicological evaluation, exposure assessment and establishment of specifications for certain flavourings

- Miscellaneous nitrogen-containing substances
- Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes, and acids
- Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids, and related esters
- Carvone and structurally related substances
- Menthol and structurally related substances
- Maltol and related substances
- Alicyclic primary alcohols, aldehydes, acids and related esters (reevaluation)
- Furan substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers.(reevaluation)

8. Revision of specification for certain flavourings
9. Other matters to be considered (general considerations)
Update of EHC240:
 - Development on guidance on the evaluation of genotoxicity studies
 - Updated guidance on dose-response modelling for the use in risk assessment
 - Steviol glycosides and frame specifications/frame toxicological profiles
10. Other matters as may be brought forth by the Committee during discussions at the meeting.
11. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of certain veterinary drug residues in food

Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1008, 2017 (150 pages)

Safety Evaluation of Certain Food Additives

WHO Food Additives Series, No. 75, 2018 (244 pages)

Evaluation of Certain Food Additives

Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1007, 2017 (92 pages)

Safety Evaluation of Certain Contaminants in Food

WHO Food Additives Series, No. 74, 2018 (897 pages)

Evaluation of Certain Contaminants in Food

Eighty-third Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No.1002, 2017 (166 pages)

Evaluation of Certain Food Additives

Eighty-second Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1000, 2016 (162 pages)

Evaluation of Certain Veterinary Drug Residues in Food

Eighty-first Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 997, 2016 (110 pages)

Toxicological Evaluation of Certain Veterinary Drug Residues in Food

Eighty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 72, 2016 (162 pages)

Evaluation of Certain Food Additives and Contaminants

Eightieth Report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 995, 2016 (114 pages)

Safety Evaluation of Certain Food Additives and Contaminants

Eightieth Meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 71, 2015 (132 pages)

Further information on these and other WHO publications can be obtained from
WHO Press, World Health Organization ■ 1211 Geneva 27, Switzerland ■ www.who.int/bookorders
tel.: +41 22 791 3264; **fax:** +41 22 791 4857; **email:** bookorders@who.int

Evaluation of certain food additives

This report represents the conclusions of a Joint FAO/WHO Expert Committee (JECFA) convened to evaluate the safety of various food additives, including flavouring agents, with a view to concluding on safety concerns and to prepare specifications for the identity and purity of the food additives.

The first part of the report includes updates on the work of the Codex Committee on Food Additives (CCFA) since the eighty-fourth meeting of JECFA and on activities relevant to JECFA with regard to the *Environmental Health Criteria 240: Principles and methods for the risk assessment of chemicals in food* (EHC 240). Following is a summary of the Committee's evaluations of technical, toxicological and dietary exposure data for eight food additives other than flavouring agents – anionic methacrylate copolymer; basic methacrylate copolymer; erythrosine; indigotine; lutein and lutein esters from *Tagetes erecta* and zeaxanthin (synthetic); neutral methacrylate copolymer; sorbitol syrup; and spirulina extract – and eight groups of flavouring agents – alicyclic primary alcohols, aldehydes, acids and related esters; carvone and structurally related substances; furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers; linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters; maltol and related substances; menthol and structurally related substances; miscellaneous nitrogen-containing substances; and saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids.

Specifications and analytical methods were revised for the following food additives other than flavouring agents: cassia gum; citric and fatty acid esters of glycerol (CITREM); glycerol ester of wood rosin (GEWR); and modified starches.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to all of the food additives as well as toxicological information, dietary exposures and information on specifications.

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 7.1

Revision Date 24.02.2023

Print Date 08.05.2023

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking**1.1 Product identifiers**

Product name : L-MENTHOL

Product Number : PHR1116

Brand : Sigma-Aldrich

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

CAS-No. : 2216-51-5

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Chemie GmbH
Industriestrasse 25
CH-9471 BUCHS

Telephone : +41 81 755 2511

Fax : +41 81 756 5449

E-mail address : technischerservice@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +41 43-508-2011 (CHEMTREC)
+41 44-251-5151 (Tox-Zentrum)
145(Tox Info Suisse)

SECTION 2: Hazards identification**2.1 Classification of the substance or mixture****Classification according to Regulation (EC) No 1272/2008**

Skin irritation (Category 2), H315

Eye irritation (Category 2), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements**Labelling according Regulation (EC) No 1272/2008**

Pictogram



Signal Word	Warning
Hazard statement(s)	
H315	Causes skin irritation.
H319	Causes serious eye irritation.
Precautionary statement(s)	
P264	Wash skin thoroughly after handling.
P280	Wear protective gloves/ eye protection/ face protection.
P302 + P352	IF ON SKIN: Wash with plenty of water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332 + P313	If skin irritation occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
Supplemental Hazard Statements	none

Reduced Labeling (<= 125 ml)

Pictogram



Signal Word	Warning
Hazard statement(s)	none
Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	: C ₁₀ H ₂₀ O
Molecular weight	: 156,27 g/mol
CAS-No.	: 2216-51-5
EC-No.	: 218-690-9

Component	Classification	Concentration
(1R,2S,5R)-(-)-menthol		
CAS-No. 2216-51-5 EC-No. 218-690-9	Skin Irrit. 2; Eye Irrit. 2; H315, H319	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.



SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice

Show this material safety data sheet to the doctor in attendance.

If inhaled

After inhalation: fresh air.

In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact

After eye contact: rinse out with plenty of water. Call in ophthalmologist. Remove contact lenses.

If swallowed

After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Carbon dioxide (CO₂) Foam Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

Combustible.

Vapors are heavier than air and may spread along floors.

Forms explosive mixtures with air on intense heating.

Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters

Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information

Prevent fire extinguishing water from contaminating surface water or the ground water system.



SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Avoid inhalation of dusts. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed. Dry.

Store at Room Temperature.

Storage class

Storage class (TRGS 510): 11: Combustible Solids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm



Break through time: 480 min
Material tested: KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested: KCL 741 Dermatril® L

Body Protection

protective clothing

Respiratory protection

Recommended Filter type: Filter A-(P2)

The entrepreneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure

Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	crystalline
b) Color	white
c) Odor	characteristic, aromatic
d) Melting point/freezing point	Melting point/range: > 41,2 - 41,7 °C at 1.013 hPa - OECD Test Guideline 102
e) Initial boiling point and boiling range	212 °C at 1.013,25 hPa
f) Flammability (solid, gas)	No data available
g) Upper/lower flammability or explosive limits	No data available
h) Flash point	94 °C - closed cup - ISO 3679
i) Autoignition temperature	No data available
j) Decomposition temperature	No data available
k) pH	No data available
l) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available



m) Water solubility	0,397 g/l at 20 °C - OECD Test Guideline 105- partly soluble
n) Partition coefficient: n-octanol/water	log Pow: 3,15 at 25 °C - Regulation (EC) No. 440/2008, Annex, A.8 - Bioaccumulation is not expected.
o) Vapor pressure	0,19 hPa at 25 °C - Regulation (EC) No. 440/2008, Annex, A.4
p) Density	0,890 g/cm ³ at 20 °C
Relative density	No data available
q) Relative vapor density	No data available
r) Particle characteristics	No data available
-
s) Explosive properties	No data available
t) Oxidizing properties	none

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity

Forms explosive mixtures with air on intense heating.
A range from approx. 15 Kelvin below the flash point is to be rated as critical.
The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions

Violent reactions possible with:
Strong oxidizing agents

10.4 Conditions to avoid

Strong heating.

10.5 Incompatible materials

No data available

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

Oral: No data available

LC50 Inhalation - Rat - male and female - 4 h - 5.289 mg/l - aerosol



(OECD Test Guideline 403)
Dermal: No data available

Skin corrosion/irritation

Skin - Rabbit
Result: irritating - 4 h
(OECD Test Guideline 404)

Serious eye damage/eye irritation

Eyes - Rabbit
Result: irritating
(OECD Test Guideline 405)

Respiratory or skin sensitization

Local lymph node assay (LLNA) - Mouse
Result: negative
(OECD Test Guideline 429)

Germ cell mutagenicity

No data available

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	static test LC50 - Danio rerio (zebra fish) - 15,6 mg/l - 96 h (Directive 67/548/EEC, Annex V, C.1.)
Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - 26,6 mg/l - 48 h (OECD Test Guideline 202)



Toxicity to algae	static test ErC50 - Desmodesmus subspicatus (green algae) - 21,4 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to bacteria	static test EC50 - activated sludge - 237 mg/l - 3 h (OECD Test Guideline 209)

12.2 Persistence and degradability

Biodegradability aerobic - Exposure time 28 d
Result: 92 % - Readily biodegradable.
(OECD Test Guideline 301D)

Theoretical oxygen demand 2.970 mg/g
Remarks: (Lit.)

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Assessment : The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

No data available

SECTION 14: Transport information

14.1 UN number

ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: - IMDG: - IATA: -



14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: -

14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

No data available

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information**15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture**

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

Other regulations

Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information**Full text of H-Statements referred to under sections 2 and 3.**

H315	Causes skin irritation.
H319	Causes serious eye irritation.



Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); EC_x - Concentration associated with x% response; EL_x - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErC_x - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC₅₀ - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC₅₀ - Lethal Concentration to 50 % of a test population; LD₅₀ - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.8

Revision Date 02.06.2023

Print Date 20.11.2023

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Menthol

Product Number : M2772

Brand : Sigma-Aldrich

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

CAS-No. : 89-78-1

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science Sp.z.o.o.
Szelągowska 30
PL-61-626 POZNAN

Telephone : +48 61 8290-100

Fax : +48 61 8290-120

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(48)-223988029 (CHEMTREC) 112
(numer alarmowy)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008

Skin irritation (Category 2), H315

Eye irritation (Category 2), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008



Pictogram



Signal Word

Warning

Hazard statement(s)

H315

Causes skin irritation.

H319

Causes serious eye irritation.

Precautionary statement(s)

P264

Wash skin thoroughly after handling.

P280

Wear protective gloves/ eye protection/ face protection.

P302 + P352

IF ON SKIN: Wash with plenty of water.

P305 + P351 + P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P332 + P313

If skin irritation occurs: Get medical advice/ attention.

P337 + P313

If eye irritation persists: Get medical advice/ attention.

Supplemental Hazard Statements

none

Reduced Labeling (<= 125 ml)

Pictogram



Signal Word

Warning

Hazard statement(s)

none

Precautionary statement(s)

none

Supplemental Hazard Statements

none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : 2-Isopropyl-5-methylcyclohexanol
Hexahydrothymol

Formula : C₁₀H₂₀O
Molecular weight : 156,27 g/mol
CAS-No. : 89-78-1
EC-No. : 201-939-0

Component	Classification	Concentration



(±)-menthol			
CAS-No.	89-78-1	Skin Irrit. 2; Eye Irrit. 2; H315, H319	<= 100 %
EC-No.	201-939-0		

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice

Show this material safety data sheet to the doctor in attendance.

If inhaled

After inhalation: fresh air.

In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact

After eye contact: rinse out with plenty of water. Call in ophthalmologist. Remove contact lenses.

If swallowed

After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Carbon dioxide (CO₂) Foam Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

Combustible.

Vapors are heavier than air and may spread along floors.

Forms explosive mixtures with air on intense heating.

Development of hazardous combustion gases or vapours possible in the event of fire.



5.3 Advice for firefighters

Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information

Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Avoid inhalation of dusts. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.

For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed. Dry.

Storage class

Storage class (TRGS 510): 11: Combustible Solids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated



SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested:KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested:KCL 741 Dermatril® L

Body Protection

protective clothing

Respiratory protection

required when dusts are generated.

Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.

Recommended Filter type: Filter type P2

The entrepreneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure

Do not let product enter drains.



SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	solid
b) Color	white
c) Odor	characteristic
d) Melting point/freezing point	Melting point/range: 34 - 36 °C - lit.
e) Initial boiling point and boiling range	216 °C - lit.
f) Flammability (solid, gas)	No data available
g) Upper/lower flammability or explosive limits	No data available
h) Flash point	ca.96 °C
i) Autoignition temperature	No data available
j) Decomposition temperature	No data available
k) pH	No data available
l) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: 17 mPa.s at 40 °C - OECD Test Guideline 114
m) Water solubility	0,456 g/l at 25 °C
n) Partition coefficient: n-octanol/water	log Pow: 3,40 at 37 °C - (External MSDS), Bioaccumulation is not expected.
o) Vapor pressure	1 hPa at 55 °C - (External MSDS)
p) Density	0,89 g/mL at 25 °C - lit.
Relative density	0,89
q) Relative vapor density	
r) Particle characteristics	No data available
s) Explosive properties	No data available
t) Oxidizing properties	none

9.2 Other safety information

No data available



SECTION 10: Stability and reactivity

10.1 Reactivity

Forms explosive mixtures with air on intense heating.

A range from approx. 15 Kelvin below the flash point is to be rated as critical.

The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions

Violent reactions possible with:

Strong oxidizing agents

10.4 Conditions to avoid

Strong heating.

10.5 Incompatible materials

No data available

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

LD50 Oral - Rat - male and female - 3.180 mg/kg

Symptoms: Nausea, Vomiting

Remarks: (RTECS)

LC50 Inhalation - Rat - male and female - 4 h - 5,289 mg/l - dust/mist

(OECD Test Guideline 403)

Dermal: No data available

Skin corrosion/irritation

Skin - Rabbit

Result: irritating - 4 h

(OECD Test Guideline 404)

Serious eye damage/eye irritation

Eyes - Rabbit

Result: Eye irritation

(OECD Test Guideline 405)

Respiratory or skin sensitization

Buehler Test - Guinea pig

Result: negative

Remarks: (ECHA)

Germ cell mutagenicity

Test Type: Mutagenicity (mammal cell test): chromosome aberration.



Test system: Chinese hamster ovary cells
Metabolic activation: with and without metabolic activation
Result: negative
Remarks: (ECHA)
Test Type: In vitro mammalian cell gene mutation test
Test system: Mouse lymphoma test
Metabolic activation: with and without metabolic activation
Result: negative
Remarks: (ECHA)
Test Type: Ames test
Test system: Salmonella typhimurium
Metabolic activation: with and without metabolic activation
Result: negative
Remarks: (ECHA)

Test Type: Chromosome aberration test
Species: Mouse
Cell type: Bone marrow
Application Route: Intraperitoneal injection

Result: negative
Remarks: (ECHA)

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: OT0350000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Systemic effects:

gastric spasms



Dizziness
ataxia (impaired locomotor coordination)
Tiredness
depressed respiration
Risk of methaemoglobin formation.

Other dangerous properties can not be excluded.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	static test LC50 - Danio rerio (zebra fish) - 22,3 mg/l - 96 h Remarks: (ECHA)
Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - 26,6 mg/l - 48 h (OECD Test Guideline 202)
Toxicity to algae	static test ErC50 - Desmodesmus subspicatus (green algae) - 16,2 mg/l - 72 h (Directive 67/548/EEC, Annex V, C.3.)
Toxicity to bacteria	static test EC50 - activated sludge - 306 mg/l - 3 h (OECD Test Guideline 209)

12.2 Persistence and degradability

Biodegradability	aerobic - Exposure time 28 d Result: 76 % - Readily biodegradable. (OECD Test Guideline 301D)
Theoretical oxygen demand	2.970 mg/g

12.3 Bioaccumulative potential

Bioaccumulation	Cyprinus carpio (Carp) - 6 Weeks - 0,2 mg/l((±)-menthol)
	Bioconcentration factor (BCF): 0,5 - 15 (OECD Test Guideline 305C)

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Sigma-Aldrich- M2772

Page 9 of 12

The life science business of Merck operates as MilliporeSigma in the US and Canada



Assessment : The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects

Discharge into the environment must be avoided.

SECTION 13: Disposal considerations

13.1 Waste treatment methods

No data available

SECTION 14: Transport information

14.1 UN number

ADR/RID: -

IMDG: -

IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: -

IMDG: -

IATA: -

14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: -

14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

No data available

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

Other regulations

Take note of Dir 94/33/EC on the protection of young people at work.



15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H315	Causes skin irritation.
H319	Causes serious eye irritation.

Full text of other abbreviations

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