Toxicological profile for

Ethyl vanillin

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

3-Ethoxy-4-hydroxybenzaldehyde (PubChem)

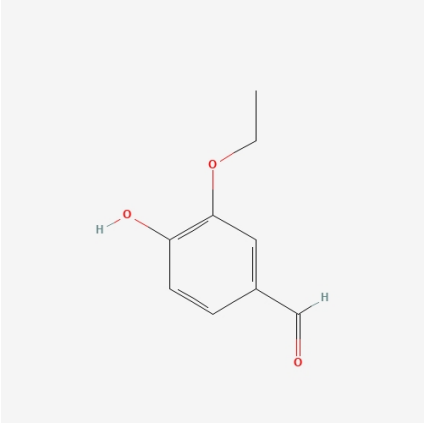
1.2. Synonyms

3-Ethoxy-4-hydroxybenzaldehyde; ETHYL VANILLIN; Ethylvanillin; Bourbonal; Ethylprotal; Ethavan; Ethovan; Vanillal; Vanirom; 4-Hydroxy-3-ethoxybenzaldehyde; Quantrovanil; Rhodiarome; Ethyl protal; Protocatechuic aldehyde ethyl ether; Vanillin, ethyl-; Vanirome; 2-Ethoxy-4-formylphenol; Ethylprotocatechuic aldehyde; 3-Ethoxyprotocatechualdehyde; Arovanillon; Vanbeenol; ethyl-vanillin; EINECS 204-464-7; UNII-YC9ST449YJ; Ethyl protocatechuic aldehyde; Protocatechuic aldehyde 3-ethyl ether; Quantrovanil, Vanillal; thylvanilline; Aethylvanillin; Vanillin,ethyl; Ethyl protocatechualdehyde; Ethoxy, Hydroxybenzaldehyde; 3-ethoxyl-4-hydroxybenzaldhyde; 5-ethoxy-4-hydroxybenzaldehyde; AKOS000119395; (PubChem)

1.3. Molecular formula

$C_9H_{10}O_3$ (PubChem)*1.4. Structural Formula*

1.4. Structural Formula



(PubChem)

1.5. Molecular weight (g/mol)

166.17 (PubChem)*1.6. CAS registration number*

1.6. CAS registration number

121-32-4

1.7. Properties

1.7.1. Melting point

(°C): 74-79 (ChemSpider; EPISuite, 2017; HSDB, 2015)

1.7.2. Boiling point

(°C): 285 (ChemSpider; HSDB, 2015); 294 (EPISuite, 2017); 295 (ChemSpider)

1.7.3. Solubility

2820 mg/L at 25°C (EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 110, 127 or 145 (ChemSpider); 145 (closed cup) (HSDB, 2015)*1.7.6. Flammability limits (vol/vol%)*

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

Not stable; in contact with iron or alkali, it exhibits a red color & loses its flavouring; affected by light (HSDB, 2015)

1.7.10. Vapor pressure

$1.04x10^{-5}$ mmHg at 25°C (EPISuite, 2017; HSDB, 2015)

1.7.11. log Kow

1.58 (EPISuite, 2017; HSDB, 2015)

2. General information

2.1. Exposure

Probable Routes of Human Exposure:

According to the 2012 TSCA Inventory Update Reporting data, 7 reporting facilities estimate the number of persons reasonably likely to be exposed during the manufacturing, processing, or use of ethyl vanillin in the United States may be as low as <10 workers and as high as 99 workers per plant; the data may be greatly underestimated due to confidential business information (CBI) or unknown values(1). [(1) US EPA; Chemical Data Reporting (CDR). Non-confidential 2012 Chemical Data Reporting information on chemical production and use in the United States. Available from, as of June 12, 2015: http://www.epa.gov/cdr/pubs/guidance/cdr_factsheets.html **PEER REVIEWED**

Occupational exposure to ethyl vanillin may occur through dermal contact with this compound at workplaces where ethyl vanillin is produced or used. Use data indicate that the general population may be exposed to ethyl vanillin via ingestion of food and dermal contact with consumer products containing ethyl vanillin(SRC).

Food Survey Values:

Ethyl vanillin was detected in artificial vanilla extracts purchased from local and internet retail stores at concentrations ranging from 0.13 to 2.39 ng/mL(1). Ethyl vanillin was detected in samples of 23 domestic and imported vanilla extract products at concentrations ranging from 0.4 to 2.2 mg/g(2). [(1) Jager et al; Food Chem 107: 1701-9 (2008) (2) Ali L et al; J AOAC Int 91: 383-6 (2008). Available from, as of June 12, 2015: http://www.ncbi.nlm.nih.gov/pubmed/18476352 **PEER REVIEWED**

Milk Concentrations:

Ethyl vanillin was detected in 2 out of 5 powdered milk samples from a local supermarket in Xi'an, China(1). [(1) Ma J et al; J Sep Sci 00: 1-7 (2008)] **PEER REVIEWED** PubMed Abstract

Other Environmental Concentrations:

Ethyl vanillin is a component of tobacco, tobacco smoke, and tobacco substitute smoke(1). [(1) Rodgman A, Perfetti TA; The Chemical Components of Tobacco and Tobacco Smoke. 2nd ed., Boca Raton, FL: CRC Press p. 556, 1496 (2009)] **PEER REVIEWED**

As taken from HSDB, 2015.

OTHER SOURCES OF EXPOSURE			
Cosmetics	Yes (Merck, 1996)	Food	Yes (Burdock, 2010).
Environment	Yes (HSDB, 2015)	Pharmaceuticals	Yes (Martindale, 1993)

OCCURRENCE IN TOBACCO PRODUCTS	
In the burned part	Yes
In tobacco naturally	No evidence (Stedman 1968; Lloyd et al 1976)

“141 volatile flavors including vanillin (in 22 out of 28) and ethyl vanillin (14 out of 28) were detected in 28 e-cig liquid aerosol samples. Other flavors detected include cinnamaldehyde and 3-methyl-1,2-cyclopentanedione (see below). Aldehydes, propylene glycol, and glycerol were also detected. 14/30 e-cigarette liquids showed presence of vanillin and 10/30 samples showed ethyl vanillin using GC–MS. Concentrations up to 3300 µg/ml for vanillin was detected. 4 e-liquid samples were analyzed with GC–MS and various flavors including vanillin and ethyl vanillin were identified. In vitro cultures of lung cells (human bronchial epithelial cells, human lung fibroblasts) were treated with each flavoring chemical and analyzed for pro-inflammatory cytokines, IL-8. Rise in IL-8 and impairment in epithelial barrier function was noted.” As taken from Kaur G et al. 2018. Toxicol. Lett., 288: 143-155. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29481849

Estimated intake based on the MSDI (Maximised Survey-derived Daily Intake) approach is 5400 and 43,000 µg/person/day in the EU and USA, respectively (EFSA, 2008).

Estimated intake from use as a flavouring in the USA is 2.2175 mg/kg bw/day (Burdock GA, 2010).

The following levels in foods have been reported in the USA (Burdock GA, 2010):

Food category	Usual (ppm)	Max (ppm)	Food category	Usual (ppm)	Max (ppm)
Alcoholic beverages	5.04	10.04	Gelatins, puddings	18.08	39.93

Baked goods	42.08	92.97	Hard candy	15.85	30.26
Breakfast cereals	270	330	Meat products	3.9	3.9
Chewing gum	37.46	37.46	Milk products	1398	1403
Condiments, relishes	13	13	Nonalcoholic beverages	17.43	29.72
Confection, frosting	95.94	270.4	Soft candy	56.5	89.64
Fats, oils	0.06	0.15	Sweet sauce	102	172.5
Frozen dairy	12.27	26.61			

Ethyl vanillin is used as a fragrance and a soothing ingredient in cosmetics in the EU. As taken from CosIng (Cosmetic substances and ingredients database).

Ethyl vanillin (CAS RN 121-32-4) is listed as a fragrance ingredient by IFRA and the US EPA (US EPA InertFinder Database, 2023)

Ethyl vanillin (CAS RN 121-32-4) is listed as an ingredient (at given concentrations, where specified) in inside the home (1-5%) and auto products by the CPID. Ethyl vanillin (CAS RN 121-32-4) is used as a flavour enhancer and fragrance ingredient in non-medicinal natural health products. When used as a flavour enhancer, it has an upper limit toxicity restriction of 10 mg/kg bw/day (Health Canada, 2022).

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%
3-ethoxy-4-hydroxy-phenyl formate	40866248	1.24	16594849	0.72
ethyl vanillin	2721890723	82.39	1972952561	86.13
3-ethoxy-4-hydroxy-benzoic acid	429020826	12.99	162894312	7.11
unknown	n.d.	n.d.	33847933	1.48
Total ion chromatogram	3295665161	100	2304840139	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2004) J. Anal. Appl. Pyrolysis, 71, pp. 223-311.

Ingredients CAS Number	Chemical Class	Mol. Wt. (MW) Bp (^{0}C)	Max cig Appln. Level (ppm)	Purity of sample Pyrolysed (%)	Composition of pyrolysate (Compound %)	Max level in smoke (µg)
Ethyl vanillin	Phenol	MW	250	98	Ethylvanillin 99.2	120 0.5

CAS 121-32-4	aldehyde ether	166 Bp 285			Benzofurancarboxaldehyde 0.4 Diethoxybenzaldehyde 0,1 Pyranone 0.1 1 unidentified 0.2	0.1 0.1 0.1

2.3. Ingredient(s) from which it originates

Synthesized from safrole by isomerization to isosafrole and subsequent oxidation to piperonal; the methylene linkage is broken by heating in an alcoholic solution of KOH. The resulting protocatechualdehyde is then reacted with ethyl alcohol. From guaethol by condensation with chloral to yield 3-ethoxy-4-hydroxyphenyl trichloromethyl carinol, which is then boiled with an alcoholic solution of KOH or NaOH, acidified and extracted with chloroform to yield ethyl vanillin.

Ethyl vanillin is not reported to be found in nature.

As taken from Burdock, 2010

3. Status in legislation and other official guidance

States approving use in tobacco	Germany, France, Belgium, UK			
Food	EU	Yes	USA	21CFR 182.60
ADI / TDI	The current JECFA ADI of 0-3 mg/kg bw was set in 1995 and maintained at the 2001 meeting (JECFA, 1996, 2002). At the 2001 meeting, JECFA also concluded that there was “no safety concern” over its use as a food flavouring, based on current intakes from such use, estimated to be 6.2 and 43 mg/day in Europe and the USA, respectively (JECFA, 2002). In the European Commission’s “Synoptic Document” [a provisional list of monomers and additives notified to the European Commission as substances which may be used in the manufacture of plastics intended to come into contact with foodstuffs] updated to July 2003, a column records the SCF’s ADI of 0-5 mg/kg bw. This refers to an earlier value set by JECFA in 1990 at its 35th meeting (Commission 2003). [The 1990 JECFA ADI has now been reduced - see above.]			
Codex Alim.	Not listed			
C of E no.	108		FEMA no.	2464
TLV / OEL	Not listed			
Cosmetics (UK)	Not listed in Schedule 1			

TSCA Requirements:

Section 8(a) of TSCA requires manufacturers of this chemical substance to report preliminary assessment information concerned with production, exposure, and use to EPA as cited in the preamble in 51 FR 41329. Effective date: 9/30/91; Reporting date: 11/27/91. [40 CFR 712.30 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov **PEER REVIEWED**

Pursuant to section 8(d) of TSCA, EPA promulgated a model Health and Safety Data Reporting Rule. The section 8(d) model rule requires manufacturers, importers, and processors of listed chemical substances and mixtures to submit to EPA copies and lists of unpublished health and safety studies. Benzaldehyde, 3-ethoxy-4-hydroxy- is included on this list. Effective date: 9/30/91;

Sunset date: 6/30/98. [40 CFR 716.120 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov **PEER REVIEWED**

FDA Requirements:

Synthetic flavoring substances and adjuvants /for human consumption/ that are generally recognized as safe for their intended use, withn the meaning of section 409 of the Act. Ethyl vanillin is included on this list. [21 CFR 182.60 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov **PEER REVIEWED**

Substances migrating to food from paper and paperboard products used in food packaging that are generally recognized as safe for their intended use, within section 409 of the Act. Ethyl vanillin is included on this list. [21 CFR 182.90 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov **PEER REVIEWED**

Synthetic flavoring substances and adjuvants /for animal drugs, feeds, and related products/ that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act. Ethyl vanillin is included on this list. [21 CFR 582.60 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 20, 2015: http://www.ecfr.gov **PEER REVIEWED**

As taken from HSDB, 2015

“ADI of 0-3 mg/kg bw for ethyl vanillin”

As taken from JECFA, 1996

An EFSA Panel agreed with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substance”, based on the MSDI (Maximised Survey-derived Daily Intake) approach (EFSA, 2008).

There is a REACH dossier on 3-ethoxy-4-hydroxybenzaldehyde (CAS RN 121-32-4) (ECHA, undated).

3-Ethoxy-4-hydroxybenzaldehyde (CAS RN 121-32-4) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

Ethyl vanillin is included on the FDA’s list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and pH control agent, and is generally recognised as safe under 21 CFR sections 182.60 (Synthetic flavoring substances and adjuvants) and 182.90 (Substances migrating to food from paper and paperboard products) (FDA, 2022, 2023a).

Ethyl vanillin is listed on the US EPA InertFinder Database (2023) as approved for non-food and fragrance use pesticide products.

Ethyl vanillin is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule).

US EPA 2020 CDR List. US EPA TSCA inventory

Ethyl vanillin is authorised for use as a flavouring substance in all categories of flavoured foods in the EU under (EU) legislation no 872/2012 (European Commission, 2012).

Ethyl vanillin (CAS RN 121-32-4) is included on the US EPA’s list of Safer Chemical Ingredients with functional use in: fragrances (US EPA, 2023).

Ethyl vanillin has been given GRAS (generally recognized as safe) status by FEMA (Hall and Oser, 1965).

Ethyl vanillin 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:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
ETHYL VANILLIN	ORAL	CAPSULE	121324	YC9ST449YJ	0.64mg	
ETHYL VANILLIN	ORAL	CAPSULE, EXTENDED RELEASE	121324	YC9ST449YJ	NA	
ETHYL VANILLIN	ORAL	PASTE	121324	YC9ST449YJ		7mg
ETHYL VANILLIN	ORAL	SUSPENSION	121324	YC9ST449YJ	0.08mg/5ml	
ETHYL VANILLIN	ORAL	SYRUP	121324	YC9ST449YJ	NA	
ETHYL VANILLIN	ORAL	TABLET, CHEWABLE	121324	YC9ST449YJ	0.14mg	

As taken from FDA, 2023b

Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

ETHYL VANILLIN	
General Information	
synonyms:	BOURBONAL, BOURBONAL ETHYL PROTAL, 3-ETHOXY PROTOCATECHUALDEHYDE
Chemical Names:	3-ETHOXY-4-HYDROXYBENZALDEHYDE
CAS number:	121-32-4
JECFA number:	893
COE number:	108
FEMA number:	2464
Functional Class:	Flavouring Agent FLAVOURING_AGENT
Evaluations	
Evaluation	2019

year:	
ADI:	No safety concern at current levels of intake when used as a flavouring agent
Comments:	Considered for specifications only
Meeting:	87
Specs Code:	R
Evaluation year:	2001
ADI:	0-3 mg/kg bw (1995)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1995 ADI of 0-3 mg/kg bw was maintained at the fifty-seventh meeting (2001).
Meeting:	44
Specs Code:	S
Report:	TRS 909-JECFA 57/84
Tox Monograph:	FAS 48-JECFA 57/273
Specification:	COMPENDIUM ADDENDUM 9/FNP 52 Add.9/146
Previous Years:	1995, TRS 859-JECFA 44/14, COMPENDIUM ADDENDUM 1/FNP 52 Add.1/49 (1992), FAS 35-JECFA 44/141. 0-3 mg/kg bw. FU. S 1992, TRS 828-JECFA 39/13, COMPENDIUM ADDENDUM 1/FNP 52 Add.1/49. 0-5 (TEMPORARY). TE. R 1989, TRS 789-JECFA 35/17, FNP 49-JECFA 35/16 (COM

As taken from WHO, 2021

COE No.:	108
FEMA No.:	2464
JECFA No.:	893
Chemical names:	3-ETHOXY-4-HYDROXYBENZALDEHYDE
Synonyms:	3-ETHOXY PROTOCATECHUALDEHYDE; BOURBONAL ETHYL PROTAL
Functional class:	FLAVOURING AGENT
Latest evaluation:	2001
ADI:	0-3 mg/kg bw (1995)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1995 ADI of 0-3 mg/kg bw was maintained at the fifty-seventh meeting (2001).
Report:	TRS 909-JECFA 57/84
Specifications:	COMPENDIUM ADDENDUM 9/FNP 52 Add.9/146

Tox monograph:	FAS 48-JECFA 57/273
Previous status:	1995, TRS 859-JECFA 44/14, COMPENDIUM ADDENDUM 1/FNP 52 Add.1/49 (1992), FAS 35-JECFA 44/141. 0-3 mg/kg bw. FU. S
	1992, TRS 828-JECFA 39/13, COMPENDIUM ADDENDUM 1/FNP 52 Add.1/49. 0-5 (TEMPORARY). TE. R
	1989, TRS 789-JECFA 35/17, FNP 49-JECFA 35/16 (COMPENDIUM/627), FAS 26-JECFA 35/23. 0-5 (TEMPORARY). TE. R
	1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/25, FAS 68.33/NMRS 44A-JECFA 11/39. 0-10. FU. N

JECFA (2003)

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

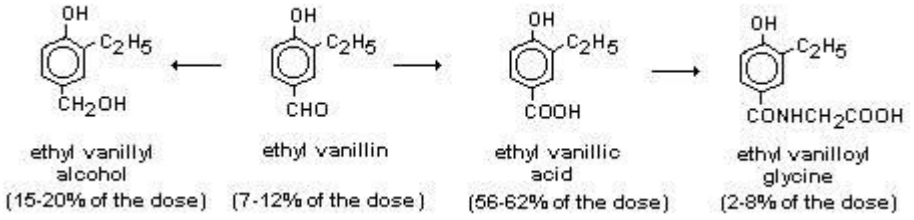
“Early reports indicated that ethyl vanillin was probably metabolized to glucuroethyl vanillin and ethyl vanillic acid, of which some was conjugated with glucuronic and sulfuric acids (Williams, 1959).”

“Ethyl 14C-vanillin was administered to male and female Sprague Dawley CD rats at single oral doses of 50, 100, or 200 mg/kg bw. Rapid metabolism occurred and the principal metabolite at all dose levels was ethyl vanillic acid.”

“Analysis of urine after hydrolysis with glucuronidase and/or sulfatase indicated that the major metabolites were glucuronide or sulfate conjugates of ethyl vanillic acid (56-62%), ethyl vanillyl alcohol (15-20%), and ethyl vanillin (7-12%). A minor proportion of the dose (2-8%) was excreted as the glycine conjugate of vanillic acid (ethyl vanilloyl glycine) (Hawkins et al., 1992).”

“Ethyl vanillic acid was also the major metabolite after dietary administration of ethyl vanillin to rats at doses of 500, 1000 or 2000 mg/kg bw (Hooks et al., 1992a).”

“During urinary organic acid profiling in human subjects, several patients excreted high concentrations of ethyl vanillic acid (3-ethoxy-4-hydroxybenzoic acid) and traces of 3-ethoxy-4-hydroxy-mandelic acid.” As taken from JECFA, 1996



As taken from JECFA, 1996

4.2. Absorption, distribution and excretion

“Ethyl 14C-vanillin was administered to male and female Sprague-Dawley CD rats by gavage in polyethylene glycol solution at single doses of 50, 100, or 200 mg/kg bw. Ethyl vanillin was rapidly absorbed and peak plasma radioactivity occurred within 2 h after dosing at all dose levels, falling rapidly to undetectable levels within 96 h. Plasma radioactivity tended to be higher in female than male rats and it was postulated that this might reflect a lower metabolic capacity of female rats.”

“Urinary excretion of radioactivity was rapid and more than 94% of the dose was excreted by this route within 24 h. Only 1-5% of the dose was excreted in faeces. After 5 days, more than 99% of the administered dose was excreted. No radioactivity was detected in expired air, indicating that the aromatic ring was in a metabolically stable position (Hawkins et al., 1992).”

“Ethyl vanillic acid was identified by GC/MS in the urine of a 9-year old female patient who had received liquid dietary supplementation flavoured with vanilla. Other patients excreting this acid were also known to have consumed foodstuffs flavoured with ethyl vanillin. Eight different urine samples containing more than 50 mg ethyl vanillic acid/g creatinine were also found to contain small amounts of vanillylmandelic acid. Unchanged ethyl vanillin was not detected in any of the urine samples.”

“A healthy adult male volunteer drank a 235 ml aliquot of a liquid dietary supplement containing an unknown quantity of ethyl vanillin. A concentration of 13 mg ethyl vanillic acid/g creatinine was found in a 12-hour urine sample. The compound was not present in urine collected before exposure (Mamer et al., 1985).”

As taken from JECFA, 1996

4.3. Interactions

“Food safety is of extreme importance to human health. Vanillin and ethyl vanillin are the widely used food additives and spices in foods, beverages, cosmetics and drugs. The objective of the present work was to evaluate the impact of vanillin and ethyl vanillin on the activities of CYP2C9, CYP2E1, CYP3A4, CYP2B6 and CYP1A2 in human liver microsomes (HLM) in vitro, and impact on the activities of CYP1A2, CYP2C, CYP3A and CYP2E1 in rat liver microsomes (RLM) in vivo. The in vitro results demonstrated that vanillin and ethyl vanillin had no significant effect on the activity of five human CYP450 enzymes with concentration ranged from 8 to 128 μM. However, after rats were orally administered vanillin or ethyl vanillin once a day for seven consecutive days, CYP2E1 activity was increased and CYP1A2 activity was decreased in RLM. The in vivo results revealed that drug interaction between vanillin/ethyl vanillin and the CYP2E1/CYP1A2-metabolizing drugs might be possible, and also suggested that the application of the above additives in foods and drugs should not be unlimited so as to avoid the adverse interaction” As taken from Chen XM et al. 2012. Fd Chem. Toxic. 50, 1897-1901. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22450566.

Ethyl vanillin …… was reported to enhance the ability of mitomycin C to cause sister chromatid exchanges. [WHO/JECFA; Joint Expert Committee on Food Additives monograph 683. Ethyl vanillin (WHO Food Additives Series 26). Available from, as of June 9, 2015: http://inchem.org/pages/jecfa.html **PEER REVIEWED**

As taken from HSDB, 2015

Introduction: Flavor aldehydes in e-cigarettes, including vanillin, ethyl vanillin (vanilla), and benzaldehyde (berry/fruit), rapidly undergo chemical reactions with the e-liquid solvents, propylene glycol, and vegetable glycerol (PG/VG), to form chemical adducts named flavor aldehyde PG/VG acetals that can efficiently transfer to e-cigarette aerosol. The objective of this study was to compare the cytotoxic and metabolic toxic effects of acetals and their parent aldehydes in respiratory epithelial cells. Aims and methods: Cell metabolic assays were carried out in bronchial (BEAS-2B) and alveolar (A549) epithelial cells assessing the effects of benzaldehyde, vanillin, ethyl vanillin, and their corresponding PG acetals on key bioenergetic parameters of mitochondrial function. The potential cytotoxic effects of benzaldehyde and vanillin and their corresponding PG acetals were analyzed using the LIVE/DEAD cell assay in BEAS-2B cells and primary human nasal epithelial cells (HNEpC). Cytostatic effects of vanillin and vanillin PG acetal were compared using Click-iT EDU cell proliferation assay in BEAS-2B cells. Results: Compared with their parent aldehydes, PG acetals diminished key parameters of cellular energy metabolic functions, including

basal respiration, adenosine triphosphate production, and spare respiratory capacity. Benzaldehyde PG acetal (1-10 mM) increased cell mortality in BEAS-2B and HNEpC, compared with benzaldehyde. Vanillin PG acetal was more cytotoxic than vanillin at the highest concentration tested while both diminished cellular proliferation in a concentration-dependent manner. Conclusions: Reaction products formed in e-liquids between flavor aldehydes and solvent chemicals have differential toxicological properties from their parent flavor aldehydes and may contribute to the health effects of e-cigarette aerosol in the respiratory system of e-cigarette users. Implications: With no inhalation toxicity studies available for acetals, data from this study will provide a basis for further toxicological studies using in vitro and in vivo models. This study suggests that manufacturers' disclosure of e-liquid ingredients at time of production may be insufficient to inform a comprehensive risk assessment of e-liquids and electronic nicotine delivery systems use, due to the chemical instability of e-liquids over time and the formation of new compounds. As taken from Jabba SV et al. 2020. Nicotine Tob. Res. 22(Suppl 1), S25-S34. PubMed, 2021 available at

5. Toxicity

5.1. Single dose toxicity

Non-Human Toxicity Values:

LD50 Dog iv 760 mg/kg [Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V5 911] **PEER REVIEWED**

LD50 Rat sc 1800 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 1729] **PEER REVIEWED**

LD50 Rabbit oral 3000 mg/kg [Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V5 911] **PEER REVIEWED**

LD50 Rat oral >2000 mg/kg [Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V5 911] **PEER REVIEWED**

LD50 Rat oral 1590 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 1729] **PEER REVIEWED**

LD50 Mouse ip 750 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 1729] **PEER REVIEWED**

LD50 Guinea pig ip 1140 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 1729] **PEER REVIEWED**

As taken from HSDB, 2015

LD50(oral, rat): 3500 mg/kg bw.

LD50 (dermal, rabbit): > 7940 mg/kg bw.

As taken from Monsanto, 1991.

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
LD50 -	Oral	Rodent -	1590	Details of	FAONAU FAO Nutrition Meetings

Lethal dose, 50 percent kill		rat	mg/kg	toxic effects not reported other than lethal dose value	Report Series. (Rome, Italy) No.?-57, 1948-77. Discontinued. Volume(issue)/page/year: 44A,39,1967
LDLo - Lowest published lethal dose	Subcutaneous	Rodent - rat	1800 mg/kg	Details of toxic effects not reported other than lethal dose value	JAPMA8 Journal of the American Pharmaceutical Association, Scientific Edition. (Washington, DC) V.29-49, 1940-60. For publisher information, see JPMSAE. Volume(issue)/page/year
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - mouse	750 mg/kg	Details of toxic effects not reported other than lethal dose value	CTOXAO Clinical Toxicology. (New York, NY) V.1-18, 1968-81. For publisher information, see JTCTDW. Volume(issue)/page/year: 10,61,1977
LDLo - Lowest published lethal dose	Intravenous	Mammal - dog	760 mg/kg	Details of toxic effects not reported other than lethal dose value	COREAF Comptes Rendus Hebdomadaires des Seances, Academie des Sciences. (Paris, France) V.1-261, 1835-1965. For publisher information, see CRASEV. Volume(issue)/page/year: 238,2576,1954
LDLo - Lowest published lethal dose	Oral	Rodent - rabbit	3 gm/kg	Details of toxic effects not reported other than lethal dose value	JAPMA8 Journal of the American Pharmaceutical Association, Scientific Edition. (Washington, DC) V.29-49, 1940-60. For publisher information, see JPMSAE. Volume(issue)/page/year: 29,425,1940
LD50 - Lethal dose, 50 percent kill	Administration onto the skin	Rodent - rabbit	>7940 mg/kg	Details of toxic effects not reported other than lethal dose value	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. Volume(issue)/page/year: OTS0534355
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - guinea pig	1140 mg/kg	Details of toxic effects not reported other than lethal dose value	COREAF Comptes Rendus Hebdomadaires des Seances, Academie des Sciences. (Paris, France) V.1-261, 1835-1965. For publisher information, see CRASEV. Volume(issue)/page/year: 238,2576,1954

As taken from RTECS, 2018

Oral LD_{50} rat >2 g/kg bw (BIBRA, 1988)

“When groups of 6 rabbits were given ethyl vanillin by gavage, a dose of 150 mg/kg bw caused no adverse effects. At 2500 mg/kg bw, only a transient increase in respiration rate was observed. The minimum oral lethal dose was reported to be 3000 mg/kg bw (Deichmann & Kitzmuller, 1940).”

As taken from JECFA, 1996

SPECIES	ROUTE	DOSE DATA
Mouse	Intraperitoneal	LD50: 750 mg/kg bw
Rat	Oral	
Rat	Oral	LD50: 1590 mg/kg bw
Rat	Subcutaneous	LD50: > 1200 mg/kg bw
Guinea pig	Intraperitoneal	LD50: 1140 mg/kg bw
Dog	Intravenous	LD50: 760 mg/kg bw

LD50 values taken from EFSA (2012):

Rat (M, F) Gavage: > 2000 (Jenner et al., 1964) Rat (M) Gavage: 4470 (Rhone-Poulenc Inc., 1992b) Rat (M, F) Oral: 3500 (Monsanto Co., 1991a, b) Rabbit NR Gavage: 2000 (Deichmann and Kitzmiller, 1940)

5.2. Repeated dose toxicity

Doses of 300 mg/kg bw were administered to rats by gavage twice weekly for 14 weeks without any adverse effects. In another experiment, groups of 16 rats were fed 20 mg/kg bw daily for 18 weeks without adverse effect but 64 mg/kg bw daily for 10 weeks reduced growth rate and caused myocardial, renal, hepatic, lung, spleen and stomach injuries. [WHO/JECFA; Joint Expert Committee on Food Additives monograph 683. Ethyl vanillin (WHO Food Additives Series 26). Available from, as of June 2, 2015: http://inchem.org/pages/jecfa.html **PEER REVIEWED**

Neither 20,000 and 50,000 ppm of ethyl vanillin fed to male rats in the diet for 1 year, nor 5000, 10,000, and 20,000 ppm fed to male and female rats in the diet for 2 years produced any effects. [Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V5 930] **PEER REVIEWED**

As taken from HSDB, 2015

No adverse effects were observed in studies in which rats were fed up to 2.5 g/kg bw/day for 1 year, or up to 1 g/kg bw/day in the diet for 2 years (BIBRA, 1988).

Type of Test	Route of Exposure	Species Observed	Dse Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	4480 mg/kg/70D (intermittent)	Cardiac - other changes Blood - changes in spleen Nutritional and Gross Metabolic - weight loss or decreased weight gain	JAPMA8 Journal of the American Pharmaceutical Association, Scientific Edition. (Washington, DC) V.29-49, 1940-60. For publisher information, see JPMSAE. Volume(issue)/page/year: 29,425,1940
TDLo - Lowest published	Oral	Rodent - rat	28 gm/kg/2W (intermittent)	Behavioral - food intake (animal) Liver - changes in liver weight	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific &

toxic dose				Nutritional and Gross Metabolic - weight loss or decreased weight gain	Technical Information. Volume(issue)/page/year: OTS0540113
TDLo - Lowest published toxic dose	Oral	Rodent - rat	182 gm/kg/13W (continuous)	Liver - liver function tests impaired Liver - changes in liver weight Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol)	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. Volume(issue)/page/year: OTS0540703
TDLo - Lowest published toxic dose	Oral	Rodent - rat	4480 mg/kg/70D (intermittent)	Nutritional and Gross Metabolic - weight loss or decreased weight gain	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskie soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year: -,399,1994
TDLo - Lowest published toxic dose	Oral	Rodent - rat	21 mg/kg/7D (intermittent)	Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - hepatic microsomal mixed oxidase (dealkylation, hydroxylation, etc.)	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20-1982- Volume(issue)/page/year: 50,1897,2012

As taken from RTECS, 2018

“Doses of 300 mg ethyl vanillin/kg bw were administered to rats by gavage twice weekly for 14 weeks without any adverse affects. In another experiment, groups of 16 rats were fed ethyl vanillin at a dose of 20 mg/kg bw/day for 18 weeks without adverse effect. However, 64 mg/kg bw/day for 10 weeks reduced growth rate and caused myocardial, renal, hepatic, lung, spleen and stomach injuries (nature not specified) (Deichmann & Kitzmuller, 1940).”

“Sixteen rats were given 30 mg ethyl vanillin weekly for 7 weeks without adverse effect on growth, food intake or protein utilization (Spore, 1960).”

“Groups of CD Sprague-Dawley BR rats (20/sex/group) were fed ethylvanillin of > 99.9% purity (nature of diet e.g., semi-synthetic/chow diet, not specified) at dose levels of 0, 500, 1000 or 2000 mg/kg bw/day for 13 weeks. The study was designed in accordance with toxicological principles for the safety assessment of food additives established by the US FDA (FDA, 1982). The diet was prepared weekly and showed stability for up to 18 days at room temperature. The achieved mean dose over the 13-week period was within 1.5% of the nominal value. Food consumption and body weight were recorded weekly. Ophthalmoscopy was done before treatment and at termination of the study. Detailed haematological and clinical chemical examinations were carried out at week 6 and 13. At termination, all animals were necropsied and organ weights recorded (adrenals, brain,

heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, spleen, testes, thyroids gland, uterus). A complete histological examination was performed on rats in the control and top-dose groups (adrenals, alimentary tract, aorta, brain, eyes, femur, Harderian gland, heart, kidneys, larynx and pharynx, liver, lungs, cervical and mesenteric lymph nodes, mammary gland, ovaries, pancreas, pituitary gland, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spleen, sternum, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina). The examination was extended to the low and intermediate dosage groups where treatment-related effects were suspected. No clinical signs or treatment-related deaths of toxicological significance were observed in treated animals during the study. Food intake was statistically significantly reduced in females at the highest dose group at week 1, and in treated male groups at weeks 1-4; thereafter there were no significant differences in food intake between controls and treated animals. Water intake, measured accurately during week 12 of treatment, did not differ notably from controls. Body-weight gain in males and females in the high-dose group was significantly reduced compared to control throughout the study; significant lower body-weight gain was also apparent in males of the intermediate- and low-dose groups during the first 4 weeks of treatment. The authors considered these differences from control not to be treatment-related since the differences were not dosage-related in magnitude, and because of intra-group variability noted in feeding patterns of all groups of male rats. Impaired food efficiency was noted for both male and female rats at the highest dose level. There were no treatment-related differences from control in haematological parameters at week 6 or at termination. Clinical biochemical analyses showed statistically significant higher values in the high-dose group compared to control for ALAT, ALP, cholesterol and total plasma protein. Cholesterol levels were significantly increased in males at the intermediate-dose group at week 6 only. The authors considered the alteration of the clinical biochemical parameters secondary to the hepatic changes seen histologically. Other sporadic differences from control values were generally within normal ranges for the strain and were not considered of toxicological significance. At autopsy, enlarged cervical lymph nodes were noted in males at the intermediate-dose group, and in both sexes at the highest dose group. In addition, there was a reduction in adipose tissue in rats of both sexes at the highest dose group. Absolute liver weights were similar to controls but relative liver weights were increased in the intermediate- and high-dose animals. Absolute and relative spleen weights were increased in the intermediate- and high-dose groups. Although relative spleen weights were increased in the low-dose males, the absolute organ weights were unaffected, and in the absence of histopathological changes this observation was considered by the authors to be of no toxicological significance. Histological examination revealed a dose-related increased incidence of hepatic peribiliary inflammatory change in both males and females of the intermediate- and high-dose groups, and minor bile duct hyperplasia affecting 1/20 intermediate- and 4/20 high-dose males. There were no changes observed in the liver parenchyma and no degenerative or inflammatory changes of the bile duct epithelium. Increased white pulp cellularity and prominence of germinal centres in the spleen, and increased prominence of germinal centres and lymphoid proliferation in cervical lymph nodes were seen in the intermediate- and high-dose groups. The authors considered the findings of the lymphoid tissue to be associated reactive changes to the hepatic peribiliary inflammatory observations. The authors concluded that no treatment-related changes were observed at 500 mg/kg bw/day which was considered to be the NOEL in this study (Hooks et al., 1992b).” “Single rabbits were given ethyl vanillin orally in 10% aqueous glycerine at doses of 15 mg/kg bw/day for 13 or 26 days; 32 mg/kg bw/day for 15 days; 41 mg/kg bw/day for 26 days; or 49 mg/kg bw/day for 43 days. At the highest close level, anaemia, diarrhoea and lack of weight gain were observed but no toxic signs were reported at any of the lower doses (Deichmann & Kitzmuller, 1940).”

“Subcutaneous injection of ethyl vanillin to rabbits at doses of 148-154 mg/kg bw/day for 6 days did not elicit any observed adverse effects. Similarly, oral intubation of ethyl vanillin in a milk vehicle at a dose of 240 mg/kg bw during 25 days (observation period 56 days), or during 54 days (observation period 126 days) did not produce any observed effects (the parameters observed were not specified in any of these studies) (Deichmann & Kitzmuller, 1940).”

“The maximum tolerated dose for ethyl vanillin in strain A mice when administered i.p. 3 times/week for 2 weeks was reported to be 75 mg/kg bw. Administration of ethyl vanillin i.p. at doses of 15 or 75 mg/kg bw, 3 times/week for 8 weeks resulted in mortalities of 8/20 and 10/20 animals, respectively. Control animals receiving i.p. injections of the vehicle tricaprylin, had survival rates of 77/80 males and 77/80 females. In the control group, 28% of males and 23% of females developed lung tumours whereas in the treated groups only one animal, in the higher dose group, exhibited a single lung nodule. It was concluded that ethyl vanillin did not potentiate the pulmonary tumour response in strain A mice (Stoner et al., 1973).”

“Vanillin and ethyl vanillin were given as a solution in milk to one rabbit at 240 mg/kg bw per day for 56 days and to two rabbits at the same dose for 126 days. Both substances were also administered as a solution in 10% glycerol, vanillin at a dose of 83 mg/kg bw per day for 14 days or 103 mg/kg bw per day for 61 days and ethyl vanillin at a dose of 15 mg/kg bw per day for 15 days, 15 mg/kg bw per day for 31 days, 32 mg/kg bw per day for 17 days, 41 mg/kg bw per day for 31 days, or 49 mg/kg bw per day for 49 days. Appearance, behaviour, and body-weight gain were not significantly affected. There were no gross or histopathological alterations in test animals. In rabbits receiving the substances in glycerol solution, anaemia, diarrhoea, and lack of weight gain were observed at the highest dose; no toxic effects were seen at lower doses. Glycerol poisoning, evidenced by restlessness, tremor, convulsions, and coma, was observed in rabbits given 83 mg/kg bw per day of vanillin for 14 days and in those given 15 mg/kg bw per day of ethyl vanillin for 15 days (Deichmann & Kitzmiller, 1940).”

“In the recent 13-week toxicity study in which rats were fed ethyl vanillin at 500, 1000 or 2000 mg/kg bw/day, treated males showed a transient reduction in body-weight gain compared with controls during the first 4 weeks of treatment. Since this effect was only transient and associated with reduced food intake, probably due to impaired palatability, the Committee concluded that the NOEL was 500 mg/kg bw/day.”

As taken from JECFA, 2002

Safety Evaluation

Quantitative Risk Type	Quantitative Risk Value	Product Use	Safety Evaluation Owner	POD Method	POD Value	POD Owner
Not calculated	Not calculated	Not specified	EU SCC	LOAEL	56.1	COSMOS TTC (NON-CANCER)

Adjustment factors used in calculations:
Adjustment factor: Study: Dose Duration: 3 (3)
Adjustment factor: Study: LOEL-NOEL Extrapolation: 3.0 (3)

Critical study: RAT (Reproductive/Developmental Toxicity) Oral - Dietary exposure for 1 GEN

NOEL/LEL Owner	Original NOEL	Original LEL	Critical Sites	Critical Effects
US FDA CFSAN	Not established	505.0 mg/kg bw/day	• SPLEEN	• SPLEEN - WEIGHT CHANGES; PATHOLOGY

Safety Evaluation Comments: PAFA database is a hazard identification resource, not a safety assessment source. | Murno (oral TTC dataset) and PAFA database (a hazard identification resource, not a safety assessment source)

Source Document: no source document available

POD Method	POD Value	POD Owner
NOEL	1441.0	MUNRO

Lowest-observed effect

Owner	Type	Value	Sites	Effects
MUNRO	LOEL	Not established	• NO EFFECTS	• NO EFFECTS

No-observed effect: MUNRO: NOEL: 1441.0 mg/kg bw/day

Adjustment factors: >Critical study: Target Organ Toxicity > Chronic Toxicity (Rat, Oral - Dietary exposure) for 730 day

As taken from the COSMOS database available at https://ng.cosmosdb.eu

5.3. Reproduction toxicity

“Animals given 200, 1000, or 2000 mg/kg bw per day of ethyl vanillin (No. 893) had a slight, non-significant increase in body weight and a statistically significant ($p < 0.05$) decrease in food consumption at the low dose, while those at the two higher doses had an increased mortality rate, gross lesions, clinical signs, and depressed body-weight gain and food consumption. [...]In view of the lack of adverse effects on offspring at all doses and on dams at the low dose of each substance, the authors concluded that the compounds had no reproductive or developmental effects.” As taken from JECFA, 2002.

SPECIES	TEST CONDITIONS	EFFECTS	REFERENCE
Rat, strain CD Sprague-Dawley BR (20/sex/group)	Though not a study of reproductive or developmental toxicity, the reproductive tissues were examined in rats fed 0, 500, 1000 or 2000 mg/kg bw/day for 13 weeks. Organ weights were recorded (including ovaries, testes and uterus) and tissues were examine microscopically (including ovaries, seminal vesicles, uterus and vagina) in top-dose groups and in other groups where treatment-related effects were suspected.	None	Hooks et al. 1992
Saccharomyces cerevisiae (recombinant, human oestrogen receptor DNA)	In vitro assay for oestrogenic activity. Cells treated with serial dilutions (concentration range unspecified).	None	Miller et al. 2001

Female Sprague-Dawley rats (10/group) were treated orally with ethyl vanillin at 0, 200, 1000 or 2000 mg/kg bw/day from at least one week pre-mating to four days post-partum. There were no adverse effects observed on the offspring. Maternal toxicity was seen from the lowest dose; critical effects were on growth and food consumption (Vollmuth et al. 1990).

5.4. Mutagenicity

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which ethyl vanillin was part of the test mixture include EMT960820 and EMT000305 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

Ethyl vanillin did not induce micronuclei in vivo. Similarly, whilst it did not induce chromosome aberrations or sister chromatid exchanges in Chinese hamster cells in vitro directly, it did enhance the ability of mitomycin C to induce sister chromatid exchanges (BIBRA, 1988).

Ethyl vanillin was not mutagenic in Ames tests, both in the presence and absence of a metabolic activation system, and it did not induce heritable mutations in fruit flies (BIBRA, 1988).

“From the SCE studies with human lymphocytes the authors concluded that benzaldehyde derivatives, including ethyl vanillin, were probably direct acting SCE inducers and the aldehyde moiety was of primary importance (Jansson et al., 1988). This contrasts with the negative effect in CHO cells (Sasaki et al., 1987).”

“In a study on the anti-mutagenic potential of flavourings, ethyl vanillin was reported to show marked anti-mutagenic activity against mutagenicity induced by 4-nitroquinoline 1-oxide, furylfuramide, captan or methylglyoxal in Escherichia coli WP2s but was ineffective against mutations induced by Trp-P-2 or IQ in Salmonella typhimurium TA98. It was proposed that the anti-mutagenic activity was due to enhancement of an error-free recombinant repair system (Ohta et al., 1986; Watanabe et al., 1988).”

As taken from JECFA, 1996

TEST SYSTEM	**TEST OBJECT**	**CONCENTRATION**	**RESULTS**
Micronucleus test	Mouse	2x0-1000 mg/kg bw	Negative
Ames test	Salmonella Typhimurium TA92, TA94, TA98, TA100, TA1535,TA1537	0-10 mg/plate	Negative
Ames test	S. Typhimurium TA98, TA100,TA1535, TA1537	0-10 mg/plate	Negative
Ames test	S. Typhimurium TA98, TA100, A1535, TA1537, TA1538	0-3.6 mg/plate	Negative
Chromosomal	Chinese Hamster Ovary cells	0-0.25 mg/mL	Negative
Aberrations	(CHO) in vitro		Negative
Sister chromatid	Chinese Hamster Ovary cells	0-100 M	Negative
Exchange (SCE)	(CHO) in vitro		Negative
Sister chromatid	Human lymphocytes in vitro	0-2 M	Positive
Heritable mutations	Drosophilia Melanogaster 50 mM		Negative

Ethyl vanillin was found to be negative when tested for mutagenicity using the Salmonella/microsome preincubation assay, using the standard protocol approved by the National Toxicology Program (NTP). Ethyl vanillin was tested in as many as 5 Salmonella typhimurium strains (TA1535, TA1537, TA97, TA98, and TA100) in the presence and absence of rat and hamster liver S-9, at doses of 0.100, 0.333, 1.000, 3.333, 6.000, and 8.000 mg/plate. The highest ineffective dose tested without appreciable toxicity in any S. typhimurium strain was 6.000 mg/plate in strains

TA1537 and TA98 without activation. At this dose, strains TA100 and TA1535 exhibited some clearing of the background bacterial lawn when tested without activation. Slight to total clearing of the background lawn was observed at the high dose. [Mortelmans K et al; Environ Mutagen 8:1-119 (1986)] **PEER REVIEWED**

As taken from HSDB, 2015

Genotoxicity	[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]

In vivo

SPECIES	TEST CONDITIONS	ENDPOINT	RESULT	REFERENCE
Mice (5 males per group)	Given by intraperitoneal injection at 0, 47, 94, 187, 375 and 750 mg/kg bw/day for 3 days, bone marrow examined for micronucleated cells 24 hr later.	Chromosome damage	-ve high quality study	NTP, 1994, 1996
Mice (5 male strain BDF_1 per group)	Intraperitoneal injection of a single unspecified dose followed by sacrifice of the animals 24 hr later. Assessed (presumably bone marrow or peripheral blood) for micronuclei induction (no further details given in brief report)	Chromosome damage	-ve	Ohuchida et al. 1989
Groups of 4 NMRI mice	Two intraperitoneal injections of 0, 0.33, 0.67 or 1 g/kg bw, 24 hr apart. Mice killed at 30 hr and bone marrow scored for micronucleated cells.	Chromosome damage	-ve	Wild et al. 1983
Drosophila melanogaster	Basc test for induction of sex-linked recessive lethal mutations. Males were fed a 50 mM solution of ethyl vanillin for 3 days and allowed to mate with untreated females to produce 3 successive broods.	Germ cell mutation	-ve	Wild et al. 1983

IN VITRO

TEST SYSTEM	TEST CONDITIONS	ENDPOINT	ACTIVATION	RESULT	REFERENCES
Mouse lymphoma cells	Mutation assay, only published as an abstract, no further details.	Mutation	With and without S9	+ve (with S9)	Heck et al. 1989
Chinese hamster lung cells	Incubated for 48 hr at concentrations up to 0.25 mg/ml, cells examined for chromosome aberrations and polyploidy.	Chromosome damage and changes in chromosome number	Without	-ve (chromo-some aberrations) +ve (at the top dose, marked increase in polyploid cells)	Ishidate et al. 1984

Human white blood cells	Incubated at concentrations up to 2 mM, examined for sister chromatid exchanges.	Chromosome effects	Without	+ve	Jansson et al. 1988
Chinese hamster ovary cells	Incubated at concentrations up to 0.1 M, cells examined for sister chromatid exchanges.	Chromosome effects	Without	-ve (limited study, not tested with S9)	Sasaki et al. 1987
Chinese hamster ovary cells	Cells pretreated with mitomycin C exposed to concentrations up to 0.1 M, and scored for sister chromatid exchanges.	Chromosome effects	Without	enhanced ability of mitomycin C to induce SCE	Sasaki et al. 1987
Salmonella typhimurium TA100, TA1535, TA1537, TA98 and Escherichia coli WP2uvrA	Ames test with amounts up to 5 mg/plate.	Mutation	With and without S9	-ve probably a good quality study	JETOC, 1997
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test, only published as an abstract, no details given.	Mutation	With and without S9	-ve	Heck et al. 1989
Salmonella typhimurium TA92, TA94, TA100, TA1535 and TA1537 (and possibly TA2637)	Ames test with amounts up to 10 mg/plate	Mutation	With and without S9	-ve probably a good quality study	Ishidate et al. 1984
Salmonella typhimurium TA1535, TA100, TA1537, TA98	Ames test, up to 8 mg/plate	Mutation	With and without S9 from rat and hamster liver	-ve high quality study	Mortelmans et al. 1986; NTP, 1982
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test with amounts up to 3.6 mg/plate.	Mutation	With and without S9	-ve	Wild et al. 1983

Bacillus subtilis H17 and M45 strains.	Tested for differential killing ability (rec assay) at 21 µg/disk.	DNA damage (indicative test)	Not clear from Japanese paper (JECFA, 2001 says with and without S9)	-ve	Oda et al. 1978

Test system:	Ames salmonella typhimurium
Strain indicator:	Ta97
Metabolic activation:	None
Method:	Preincubation
Dose:	0.01-1 mg/plate (test material solvent: dmso)
Results:	Negative
Reference:	[fujita,h and sasaki,m; mutagenicity test of food additives with salmonella typhimurium ta97 and ta102. Ii.; kenkyu nenpo - tokyo-toritsu eisei kenkyusho 38:423-430, 1987]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta102
Metabolic activation:	None
Method:	Preincubation
Dose:	0.01-1 mg/plate (test material solvent: dmso)
Results:	Negative
Reference:	[fujita,h and sasaki,m; mutagenicity test of food additives with salmonella typhimurium ta97 and ta102. Ii.; kenkyu nenpo - tokyo-toritsu eisei kenkyusho 38:423-430, 1987]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta97
Metabolic activation:	Rat, liver, s-9, aroclor 1254
Method:	Preincubation
Dose:	0.01-1 mg/plate (test material solvent: dmso)
Results:	Negative
Reference:	[fujita,h and sasaki,m; mutagenicity test of food additives with salmonella typhimurium ta97

	and ta102. Ii.; kenkyu nenpo - tokyo-toritsu eisei kenkyusho 38:423-430, 1987]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta102
Metabolic activation:	Rat, liver, s-9, aroclor 1254
Method:	Preincubation
Dose:	0.01-1 mg/plate (test material solvent: dmso)
Results:	Negative
Reference:	[fujita,h and sasaki,m; mutagenicity test of food additives with salmonella typhimurium ta97 and ta102. Ii.; kenkyu nenpo - tokyo-toritsu eisei kenkyusho 38:423-430, 1987]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta98
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta98
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium

Strain indicator:	Ta100
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta100
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta1535
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium

Strain indicator:	Ta1535
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta1537
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta1537
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium

Strain indicator:	Ta102
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta102
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium
Strain indicator:	Ta104
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	Ames salmonella typhimurium

Strain indicator:	Ta104
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	E. Coli
Strain indicator:	Wp2uvra
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	E. Coli
Strain indicator:	Wp2uvra
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	E. Coli

Strain indicator:	Wp2uvra/pkm101
Metabolic activation:	None
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]
Test system:	E. Coli
Strain indicator:	Wp2uvra/pkm101
Metabolic activation:	Rat, liver, s-9, phenobarbital and beta-naphthoflavone
Method:	Preincubation
Dose:	0.0763-5000 ug/plate (test material solvent: dmso)
Results:	Negative
Reference:	[japan chemical industry ecology- toxicology and information center, japan; mutagenicity test data of existing chemical substances based on the toxicity investigation of the industrial safety and health law; (suppl), 1997]

As taken from CCRIS, 2006

Type of Test	**Route of Exposure**	**Species Observed**	**Dose Data**	**Reference**
Sister chromatid exchange		Human Lymphocyte	1 mmol/L	MUREAV Mutation Research. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1964- Volume(issue)/page/year: 206,17,1988
Cytogenetic analysis		Rodent - hamster Fibroblast	250 mg/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 22,623,1984

As taken from RTECS, 2018

Ethyl vanillin …… was reported to enhance the ability of mitomycin C to cause sister chromatid exchanges. [WHO/JECFA; Joint Expert Committee on Food Additives monograph 683. Ethyl vanillin (WHO Food Additives Series 26). Available from, as of June 9, 2015: http://inchem.org/pages/jecfa.html **PEER REVIEWED**

As taken from HSDB, 2015

“The Ministry of Health, Labour and Welfare has carried out genotoxicity tests for food additives used in Japan in cooperation with the Japan Food Additives Association since 1979. Hayashi et al. summarized these data and published a list of 337 designated additives (Shitei-tenkabutsu in Japanese) with genotoxicity test data in 2000. Thereafter, 29 items were eliminated, and 146 items were newly added. Currently, 454 designated additives are allowed to be used as food additives in Japan. This report, based on the Hayashi report, covers the addition of newly derived genotoxicity test data. Routinely, the bacterial reverse mutation test (Ames test), mammalian cell chromosomal aberration test, and in vivo rodent bone marrow micronucleus test have been used for the evaluation of genotoxicity of food additives. In addition to the data from these tests being updated in this report, it newly includes results of transgenic rodent somatic and germ cell gene mutation assays (TGR assays), incorporated in the Organisation for Economic Co-operation and Development (OECD) test guidelines after 2000. We re-evaluated the genotoxicity of 13 designated food additives considering their TGR data.” As taken from Yamada M and Honma M. 2018. Genes and Environment 40, 27. Available at https://genesenvironment.biomedcentral.com/articles/10.1186/s41021-018-0115-2

“This paper evaluates use of the Threshold of Toxicological Concern (TTC) approach to assess safety of botanical preparations that may contain potentially genotoxic constituents, based on estimation of the fraction that may be genotoxic. A database of 107 chemical constituents of botanicals was compiled and their potential for genotoxicity evaluated from published data. Forty-three constituents met the criteria for potential genotoxicity. Concentration data on their occurrence in plants provided 2878 data points; the majority were in the low ppm level (range 0.00001-139,965 ppm, by dry weight). Weibull models of the quantitative distribution data were used to calculate 95th percentile values for chemical concentrations, analysing the dataset according to their presence in botanicals (i) as a single chemical, (ii) as two or more chemicals from the same chemical group, or (iii) as two or more chemicals from different chemical groups. The highest 95th percentile concentration value from these analyses was 1.8%. Using the TTC value of 0.15 µg/person per day for potentially genotoxic substances proposed in 2004, this value of 1.8% was used to derive an adjusted TTC value of 10 µg of plant material on a dry weight basis/person per day for assessment of potentially genotoxic substances in botanicals.” As taken from Mahony C et al. 2020. Food Chem. Toxicol. 138, 111182. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32058013/

5.5. Cytotoxicity

Type of Test	Exposure	Species Observed	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - skin	>2000 umol/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TXAPA9 Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- Volume(issue)/page/year: 245,281,2010
IC50 - Inhibitor Concentration 50	In vitro	Human - skin	161.7 umol/L/48H	In Vitro Toxicity Studies - other assays	TXAPA9 Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- Volume(issue)/page/year: 245,281,2010

IC25	In vitro	Human - leukemia cells	569.5 mg/L/24H	In Vitro Toxicity Studies - cell viability (dye exclusion): trypan blue assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 26,1150,2012
IC30 - Inhibitor Concentration 30	In vitro	Human - lymphocyte	66 mg/L/45H	In Vitro Toxicity Studies - cell viability (dye exclusion): trypan blue assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,901,2015

As taken from RTECS, 2018

“Increased aggregation of β-amyloid (Aβ) peptides induces oxidative stress, which is considered a major contributor in the development of Alzheimer's disease (AD). Prevention of Aβ-induced neurotoxicity is proposed as a possible modality for treatment of AD. The present study aimed to elucidate possible effects of ethyl vanillin (EVA), an analog of vanillin isolated from vanilla beans, on the Aβ1-42-induced oxidative injury in PC12 cells. EVA restrained the decrease in PC12 cell viability and apoptosis induction caused by treatment with Aβ1-42. In addition, EVA markedly alleviated intracellular lipid peroxidation as demonstrated by malondialdehyde levels and reactive oxygen species production in Aβ1-42-treated PC12 cells. In addition, the reduction in the activity levels of the antioxidative enzymes superoxide dismutase, catalase and glutathione peroxidase was detected in Aβ1-42-treated PC12 cells. This effect was partially reversed by treatment with EVA. Furthermore, the results indicated that EVA attenuated Aβ1-42-induced caspase-3 activation and the increase noted in the apoptosis regulator Bcl-2/apoptosis regulator Bax ratio of PC12 cells. These results indicated that EVA could be used as an efficient and novel agent for the prevention of neurodegenerative diseases via inhibition of oxidative stress and cell apoptosis.” As taken from Zhong L et al. 2019. Exp. Ther. Med. 17(4), 2666-2674. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30930969

“We identified the most popular electronic cigarette (EC) refill fluids using an Internet survey and local and online sales information, quantified their flavor chemicals, and evaluated cytotoxicities of the fluids and flavor chemicals. “Berries/Fruits/Citrus” was the most popular EC refill fluid flavor category. Twenty popular EC refill fluids were purchased from local shops, and the ingredient flavor chemicals were identified and quantified by gas chromatography-mass spectrometry. Total flavor chemical concentrations ranged from 0.6 to 27.9 mg/ml, and in 95% of the fluids, total flavor concentration was greater than nicotine concentration. The 20 most popular refill fluids contained 99 quantifiable flavor chemicals; each refill fluid contained 22 to 47 flavor chemicals, most being esters. Some chemicals were found frequently, and several were present in most products. At a 1% concentration, 80% of the refill fluids were cytotoxic in the MTT assay. Six pure standards of the flavor chemicals found at the highest concentrations in the two most cytotoxic refill fluids were effective in the MTT assay, and ethyl maltol, which was in over 50% of the products, was the most cytotoxic. These data show that the cytotoxicity of some popular refill fluids can be attributed to their high concentrations of flavor chemicals.” As taken from Hua M et al. 2019. Scientific Reports 9, 2782. Available at https://www.nature.com/articles/s41598-019-38978-w.pdf

High-throughput Assay Data

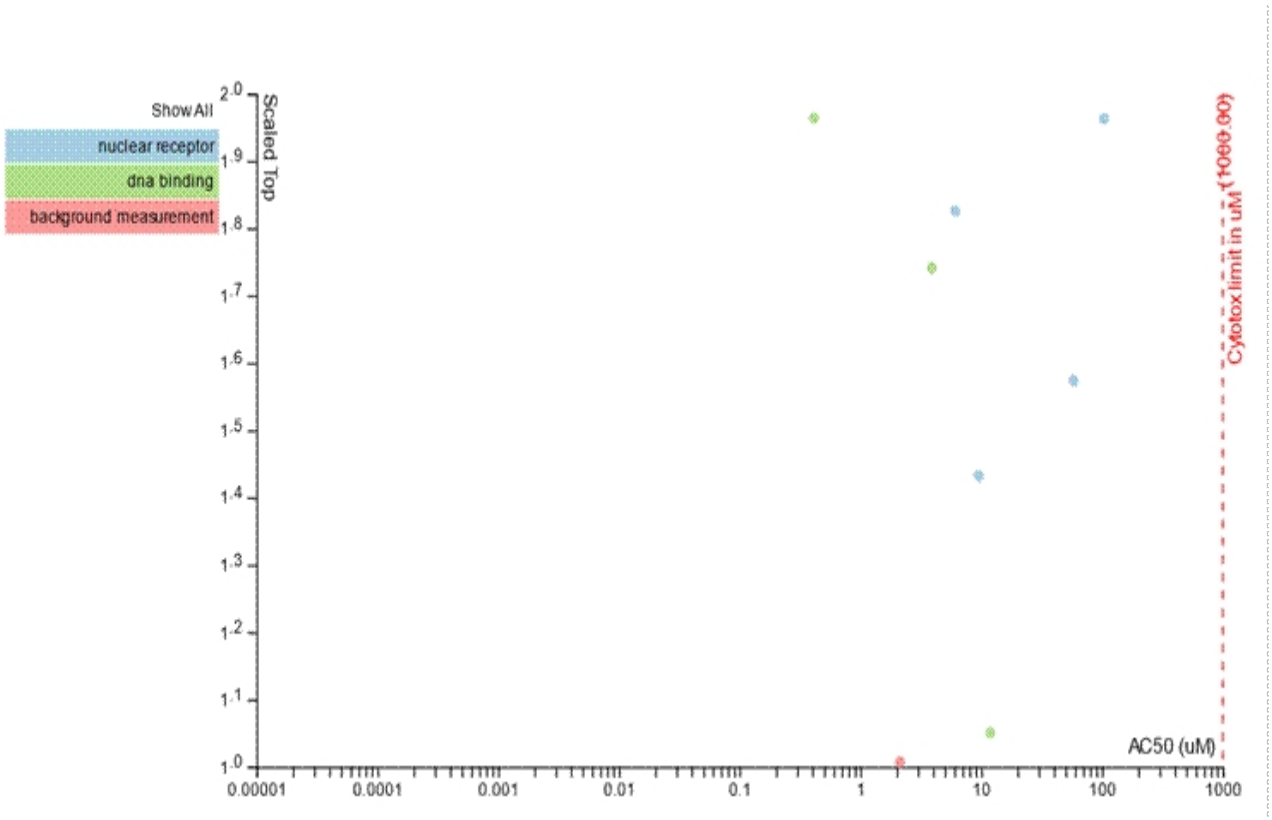
The US Environmental Protection Agency (EPA) evaluated 3-ethoxy-4-hydroxybenzaldehyde (CAS RN 121-32-4) in a series of high-throughput assays, which are publicly available on the US EPA’s

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 3-ethoxy-4-hydroxybenzaldehyde 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



“Flavor chemicals in electronic cigarette (EC) fluids, which may negatively impact human health, have been studied in a limited number of countries/locations. To gain an understanding of how the composition and concentrations of flavor chemicals in ECs are influenced by product sale location, we evaluated refill fluids manufactured by one company (Ritchy LTD) and purchased worldwide. Flavor chemicals were identified and quantified using gas chromatography/mass spectrometry (GC/MS). We then screened the fluids for their effects on cytotoxicity (MTT assay) and proliferation (live-cell imaging) and tested authentic standards of specific flavor chemicals to identify those that were cytotoxic at concentrations found in refill fluids. A total of 126 flavor chemicals were detected in 103 bottles of refill fluid, and their number per/bottle ranged from 1-50 based on our target list. Two products had none of the flavor chemicals on our target list, nor did they have any nontargeted flavor chemicals. A total of 28 flavor chemicals were present at concentrations ≥1 mg/mL in at least one product, and 6 of these were present at concentrations ≥10 mg/mL. The total flavor chemical concentration was ≥1 mg/mL in 70% of the refill fluids and ≥10 mg/mL in 26%. For sub-brand duplicate bottles purchased in different countries, flavor chemical concentrations were similar and induced similar responses in the *in vitro* assays (cytotoxicity and cell growth inhibition). The levels

of furaneol, benzyl alcohol, ethyl maltol, ethyl vanillin, corylone, and vanillin were significantly correlated with cytotoxicity. The margin of exposure calculations showed that pulegone and estragole levels were high enough in some products to present a nontrivial calculated risk for cancer. Flavor chemical concentrations in refill fluids often exceeded concentrations permitted in other consumer products. These data support the regulation of flavor chemicals in EC products to reduce their potential for producing both cancer and noncancer toxicological effects." As taken from Omaiye EE et al. 2020. Chem. Res. Toxicol. 33(12), 2972-2987. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33225688/

"Electronic cigarettes (e-cigarettes) were introduced in the United States in 2007 and by 2014 they were the most popular tobacco product amongst youth and had overtaken use of regular tobacco cigarettes. E-cigarettes are used to aerosolize a liquid (e-liquid) that the user inhales. Flavorings in e-liquids is a primary reason for youth to initiate use of e-cigarettes. Evidence is growing in the scientific literature that inhalation of some flavorings is not without risk of harm. In this review, 67 original articles (primarily cellular in vitro) on the toxicity of flavored e-liquids were identified in the PubMed and Scopus databases and evaluated critically. At least 65 individual flavoring ingredients in e-liquids or aerosols from e-cigarettes induced toxicity in the respiratory tract, cardiovascular and circulatory systems, skeletal system, and skin. Cinnamaldehyde was most frequently reported to be cytotoxic, followed by vanillin, menthol, ethyl maltol, ethyl vanillin, benzaldehyde and linalool. Additionally, modern e-cigarettes can be modified to aerosolize cannabis as dried plant material or a concentrated extract. The U.S. experienced an outbreak of lung injuries, termed e-cigarette, or vaping, product use-associated lung injury (EVALI) that began in 2019; among 2,022 hospitalized patients who had data on substance use (as of January 14, 2020), 82% reported using a delta-9-tetrahydrocannabinol (main psychoactive component in cannabis) containing e-cigarette, or vaping, product. Our literature search identified 33 articles related to EVALI. Vitamin E acetate, a diluent and thickening agent in cannabis-based products, was strongly linked to the EVALI outbreak in epidemiologic and laboratory studies; however, e-liquid chemistry is highly complex, and more than one mechanism of lung injury, ingredient, or thermal breakdown product may be responsible for toxicity. More research is needed, particularly with regard to e-cigarettes (generation, power settings, etc.), e-liquids (composition, bulk or vaped form), modeled systems (cell type, culture type, and dosimetry metrics), biological monitoring, secondhand exposures and contact with residues that contain nicotine and flavorings, and causative agents and mechanisms of EVALI toxicity." As taken from Stefaniak AB et al. 2021. Pharmacol. Ther. 224, 107838. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33746051/

"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/

"Our goal was to evaluate the effects of EC refill fluids and EC exhaled aerosol residue (ECEAR) on cultured human keratinocytes and MatTek EpiDerm™, a 3D air liquid interface human skin model. Quantification of flavor chemicals and nicotine in Dewberry Cream and Churrios refill fluids was done using GC-MS. The dominant flavor chemicals were maltol, ethyl maltol, vanillin, ethyl vanillin, benzyl alcohol, and furaneol. Cytotoxicity was determined with the MTT and LDH assays, and inflammatory markers were quantified with ELISAs. Churrios was cytotoxic to keratinocytes in the MTT assay, and both fluids induced ROS production in the medium (ROS-Glo™) and in cells (CellROX). Exposure of EpiDerm™ to relevant concentrations of Dewberry Cream and Churrios for 4 or 24 h caused secretion of inflammatory markers (IL-1α, IL-6, and MMP-9), without altering

EpiDerm™ histology. Lab made fluids with propylene glycol (PG) or PG plus a flavor chemical did not produce cytotoxic effects, but increased secretion of IL-1α and MMP-9, which was attributed to PG. ECEAR derived from Dewberry Cream and Churrios did not produce cytotoxicity with Epiderm™, but Churrios ECEAR induced IL-1α secretion. These data support the conclusion that EC chemicals can cause oxidative damage and inflammation to human skin."

Khachatoorian C et al. (2021) E-cigarette fluids and aerosol residues cause oxidative stress and an inflammatory response in human keratinocytes and 3D skin models.

5.6. Carcinogenicity

No evidence of carcinogenicity was observed in studies in which rats were fed up 1 g/kg bw/day in the diet for 2 years (BIBRA, 1988). However, this study would not meet current regulatory guidelines.

"Groups of 5 male rats were fed 0, 2%, or 5% ethyl vanillin in the diet for 1 year without any adverse effects (Hagan et al., 1967)."

"Groups of Osborne-Mendel rats (12/sex/group) were fed diets containing 0, 0.5, 1 or 2% ethyl vanillin for 2 years, and 2% or 5% for 1 year. Haematological examinations (RBC, WBC, haemoglobin and haematocrit) were performed at 3, 6, 12 and 22 months and at termination in the 2-year study. All animals were necropsied and liver, kidney, spleen, heart and testes weights recorded. Histological examinations were performed on these organs and remaining thoracic and abdominal viscera, bone and bone marrow, and muscle. No adverse effects on growth, haematology, organ weights or histology of major tissues were reported (Hagan et al., 1967)."

As taken from JECFA, 1996

"Vanillin or ethyl vanillin was dissolved in corn oil and added to the diet of five male weanling Osborne-Mendel rats at concentrations calculated to provide an average daily intake of 1000 or 2500 mg/kg bw for 1 year. Ten male and 10 female rats were fed a diet containing 3% corn oil as a control. Weekly measurements of body weight and food intake and observations of general condition showed no differences between test and control groups. No differences in haematological parameters were seen at necropsy. The NOEL for vanillin and ethyl vanillin was 2500 mg/kg bw per day (Hagan et al., 1967)."

"Four groups of eight young albino rats were fed vanillin or ethyl vanillin as a 4% solution in milk at an estimated daily intake of either 20 mg/kg bw for 126 days or 64 mg/kg bw for 70 days. In the 70-day study, half the animals were killed and the other half were put on a recovery diet for 8 more weeks. Additionally, 12 rats were given a dose of 300 mg/kg bw of vanillin or ethyl vanillin as a 4% solution in olive oil orally by gavage twice per week for 14 weeks. Observation of appearance, behaviour, and body-weight gain showed a reduced growth rate and myocardial, renal, hepatic, lung, spleen, and stomach injuries at the dose of 64 mg/kg bw (nature not specified) (Deichmann & Kitzmiller, 1940)."

"Vanillin or ethyl vanillin dissolved in propylene glycol was added to the diet of groups of 12 male and 12 female rats at a concentration estimated to provide an average daily intake of 250, 500, or 1000 mg/kg bw, for 2 years. Twenty control rats were fed 3% propylene glycol. Weekly measurements of body weight and food intake and observations of general condition failed to show any differences between test and control groups. Haematological examinations at necropsy showed no effects in any of the animals at any concentration. The NOEL for vanillin and ethyl vanillin was 1000 mg/kg bw per day (Hagan et al., 1967)."

As taken from JECFA, 2002

SPECIES	TEST CONDITIONS	EVIDENCE OF CARCINOGENICITY	REFERENCE

Rat, Osborne-Mendel, 12 per sex per group	Fed 0, 0.5, 1.0, 2.0% in the diet for 2 years (top dose equivalent to about 1 g/kg bw/day). It is not clear whether tissues other than the major organs were examined. [Limited study, current protocols recommend 50 animals/sex]	None	Hagan et al. 1967
Mice, two groups of 20 females, strain A/He	Mice (of a strain that is highly susceptible to lung tumour induction) were given intraperitoneal injections of ethyl vanillin at up to a maximum tolerated dose of 75 mg/kg bw, thrice weekly for 8 wk. Examined at 24 wk for lung tumours and for “abnormalities” of liver, spleen, kidneys, thymus, intestine and endocrine glands.	None	Stoner et al. 1973

5.7. Irritation/immunotoxicity

Skin, Eye and Respiratory Irritations:

A human skin irritant. [Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 1610] **PEER REVIEWED**

... Highly irritating action on the eyes and mucous membranes of the respiratory tract. /Aldehydes/ [Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 84] **PEER REVIEWED**

Human Toxicity Excerpts:

... A maximization test was carried out on 25 volunteers. The material /ethyl vanillin/ was tested at a concentration of 2% in petrolatum and produced no sensitization reactions. [Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V5 930] **PEER REVIEWED**

... When tested as 2% in petrolatum, ethyl vanillin produced mild irritation after a 48 hr closed-patch test in 25 human subjects. [Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V5 930] **PEER REVIEWED**

In a volunteer study, ethyl vanillin (EV) demonstrated no sensitizing potential but was a skin irritant. [BIBRA working group; Toxicity profile. The British Industrial Biological Research Association; 4 (1967)] **PEER REVIEWED**

As taken from HSDB, 2015

The present work aimed to assess novel pharmacological properties of ethyl vanillin (EVA) which is used as a flavoring agent for cakes, dessert, confectionary, etc. EVA exhibited an inhibitory activity in the chorioallantoic membrane angiogenesis. Anti-inflammatory activity of EVA was convinced using the two in vivo models, such as vascular permeability and air pouch models in mice. Antinociceptive activity of EVA was assessed using acetic acid-induced writhing model in mice. EVA suppressed production of nitric oxide and induction of inducible nitric oxide synthase in the lipopolysaccharide (LPS)-activated RAW264.7 macrophage cells. However, EVA could not suppress induction of cyclooxygenase-2 in the LPS-activated macrophages. EVA diminished reactive oxygen species level in the LPS-activated macrophages. EVA also suppressed enhanced matrix metalloproteinase-9 gelatinolytic activity in the LPSactivated RAW264.7 macrophage cells. EVA at the used concentrations couldn't diminish viability of the macrophage cells. Taken together, the anti-angiogenic, anti-inflammatory and anti-nociceptive properties of EVA are based on its suppressive effect on the production of nitric oxide possibly via decreasing the reactive oxygen species level. As taken from Jung HJ et al Pharm Res. 2010, Feb; 33(2):309-16. PubMed, 2010

available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=20195833&dopt=AbstractPlus

In human volunteer studies, when tested at 2 % in petrolatum, ethyl vanillin was a mild irritant, but it failed to induce any sensitisation reactions. Covered contact also caused an irritant reaction in a dermatitis patient (BIBRA, 1988).

10 mg exposure during 48h on human skin produced only a mild irritant effect (Anon (1975). Food and Cosmetics Toxicology 13,103).

As taken from RTECS, 2018.

“In a 24-hour closed patch test in 25 subjects, ethyl vanillin tested at 2% in petrolatum produced a mild irritation. No sensitization reactions occurred when ethyl vanillin was used at 2% in petrolatum in a maximization test on 25 volunteers (Kligman, 1970).”

“People previously sensitized to balsam of Peru, benzoin, rosin, benzoic acid, orange peel, cinnamon and cloves have been reported to cross-react with hydroxybenzaldehydes such as vanillin or ethyl vanillin. A patient with contact dermatitis showed strong reactions to balsam of Peru, cassia oil and ethyl vanillin, it was not known whether the dermatitis was a response to occupational exposure to ethyl vanillin in a candy factory or to rubber (Rudzki & Grzwa, 1976).”

As taken from JECFA, 1996

No skin irritation was seen in six rabbits given a 24-hour dermal application of 0.5 g ethavan moistened with water.

Finely ground ethavan was not irritating to six rabbits when 100 mg instilled into the eye for 24 hours.

As taken from Monsanto, 1991.

Sensitization

No skin sensitization reactions were observed in a maximization test carried out on 25 volunteers using a concentration of 2% in petrolatum (Kligman, 1970).

A maximization test (Kligman 1966) using 2% in petrolatum did not induce any skin sensitisation reactions in 25 volunteers (Kligman 1970).

In a two-centre study on a total of 200 patients with contact dermatitis, 5% in petrolatum was not irritating or sensitizing in (probably 24/48-hr, covered) patch tests (Frosch et al. 1995).

Basketter et al. (2001) observed no activity in the murine lymph node assay and reported ethyl vanillin to be a non-sensitizer in humans, despite its extensive commercial use.

The SCCS (2011) described ethyl vanillin as a possible sensitizer, assessing the structural alert as “complex”.

“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 and the reodorant but also to several ingredients of the latter product, including “Vanillal S10026”, 5% pet” (SCCS, 2011, Annex I).

IMMUNOTOXICITY

Possible effects on cell-mediated immunity were investigated in an assay to measure host resistance to bacterial challenge. Groups of 20 female CD1 mice were orally dosed with 0, 750, 1500 or 3000 mg/kg bw on 5 consecutive days and challenged with an intravenous injection with Listeria monocytogenes following the third day of dosing. A decrease in survival time (monitored for 10 days after challenge) was seen at the highest dose (Gaworski et al. 1994). [The authors

concluded that immune system modulation resulted only at concentrations that produced overt toxicity and were of limited relevance in terms of immunotoxicity.]

In the same report, no effect on humoral immunity was detected in groups of 10 female CD1 mice orally dosed with 0, 750, 1500 or 3000 mg/kg bw on 5 consecutive days in an assay for antibody plaque-forming response to sheep erythrocytes (Gaworski et al. 1994).

The present work aimed to assess novel pharmacological properties of ethyl vanillin (EVA) which is used as a flavoring agent for cakes, dessert, confectionary, etc. EVA exhibited an inhibitory activity in the chorioallantoic membrane angiogenesis. Anti-inflammatory activity of EVA was convinced using the two in vivo models, such as vascular permeability and air pouch models in mice. Antinociceptive activity of EVA was assessed using acetic acid-induced writhing model in mice. EVA suppressed production of nitric oxide and induction of inducible nitric oxide synthase in the lipopolysaccharide (LPS)-activated RAW264.7 macrophage cells. However, EVA could not suppress induction of cyclooxygenase-2 in the LPS-activated macrophages. EVA diminished reactive oxygen species level in the LPS-activated macrophages. EVA also suppressed enhanced matrix metalloproteinase-9 gelatinolytic activity in the LPSactivated RAW264.7 macrophage cells. EVA at the used concentrations couldn't diminish viability of the macrophage cells. Taken together, the anti-angiogenic, anti-inflammatory and anti-nociceptive properties of EVA are based on its suppressive effect on the production of nitric oxide possibly via decreasing the reactive oxygen species level. As taken from Jung HJ et al. Arch Pharm Res. 2010, Feb; 33(2):309-16. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=20195833&dopt=AbstractPlus

“E-cigarette flavorings have not been thoroughly evaluated for inhalational toxicity. We have shown that the flavoring chemical cinnamaldehyde impairs human neutrophils, macrophages, and natural killer cells. Here we investigated the effects of other common e-liquid flavoring chemicals on phagocytosis and oxidative burst in neutrophils. We demonstrate that cinnamaldehyde and ethyl vanillin dose-dependently decrease oxidative burst and that benzaldehyde and benzaldehyde propylene glycol acetal dose-dependently impair phagocytosis. Isoamyl acetate did not affect either measure of neutrophil function. These data suggest that inhaling aromatic aldehydic flavoring chemicals, such as cinnamaldehyde, benzaldehyde, benzaldehyde propylene glycol acetal, or ethyl vanillin, could impair neutrophil function.” As taken from Hickman E et al. 2019. Chem. Res. Toxicol. 32(6), 982–985. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31117350/

“There has been significant progress in recent years in the development and application of alternative methods for assessing the skin sensitization potential of chemicals. The pathways involved in skin sensitization have been described in an OECD adverse outcome pathway (AOP). To date, a single non-animal test method is not sufficient to address this AOP so numerous approaches involving the use of 2 or more assays are being evaluated for their performance. The 2 out of 3 approach is a simple approach that has demonstrated very good sensitivity, specificity and overall accuracy numbers for predicting the skin sensitization potential of chemicals. Chemicals with at least two positive results in tests addressing Key events 1-3 are predicted sensitizers, while chemicals with none or only one positive outcome are predicted non-sensitizers. In this report we have thoroughly reviewed the discordant results of 29 chemicals with 1 out of 3 positive results to understand better what led to the results observed and how this information might impact our hazard assessments of these chemicals. We initially categorized each chemical using a weight of evidence approach as positive, negative or indeterminate based on review of available human and animal data as well as what skin sensitization alerts were triggered using two versions of OECD Toolbox and DEREK Nexus. We determined that 4 of the 29 chemicals should be classified as indeterminate and not included in analysis of method performance based on insufficient, borderline and/or conflicting data to confidently categorized the chemicals as allergens or non-allergens. Of the 29 chemicals included in this analysis, 17 were classified as negative and would be correctly identified using a 2 out of 3 approach while 8 chemicals were classified as positive in vivo and

would be false-negative with this approach. For some of these chemicals, the outcomes observed can be explained by in vitro borderline results (13 chemicals) or in some instances there is mechanistic understanding of why a chemical is positive or negative in a particular assay (9 chemicals). Thus, when comparing the performance of different defined approaches, one should attempt to only include chemicals which demonstrate clear evidence to be categorize as allergens or non-allergens. Finally, when interpreting the results obtained for an individual unknown chemical it is critical that the in vitro skin sensitization data is reviewed critically and there is a good understanding of the variance and applicability domain limitations for each assay being used." As taken from Kolle SN et al. 2019. Regul. Toxicol. Pharmacol. 106, 352-368. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31112722/

List of flavouring compounds in e-cigarettes with human health concerns

Chemical	CAS number	Concern	References
Ethyl vanillin	121-32-4	Respiratory irritation	(Vardavas et al. 2017)

As taken from NICNAS, 2019

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Ethyl vanillin 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 Ethyl vanillin 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	1563	JTI KB Study Report(s)
In vitro cytotoxicity	1563	JTI KB Study Report(s)

"Preservatives could be part of an effective intervention strategy for the control of Cronobacter species in foods, but few compounds with the desired antimicrobial properties have been identified to date. We examined the antibacterial activity of vanillin, ethyl vanillin, and vanillic acid against seven Cronobacter spp. in quarter-strength tryptic soy broth with 5 g/liter yeast extract (TSBYE) adjusted to pH 5.0, 6.0, and 7.0 at 10, 21, and 37°C. All compounds exhibited pH- and temperature-dependant bacteriostatic and bactericidal activity. MICs of vanillin and ethyl vanillin consistently increased with decreasing pH and temperature, but vanillic acid had little activity at pH values of 6.0 and 7.0. The MICs for all temperatures, pH values, and bacterial strains tested were 2 mg/ml ethyl vanillin, 3 mg/ml vanillin, and >8 mg/ml vanillic acid. MBCs also were influenced by pH, although significantly higher concentrations were needed to inactivate the bacteria at 21°C than at 10 or 37°C. Survivor curves for Cronobacter sakazakii strains at the MBCs of each compound revealed that all treatments resulted in immediate loss of cell viability at 37°C. Measurements of propidium iodide uptake indicated that the cell membranes were damaged by exposure to all three compounds. The thermal resistance of C. sakazakii was examined at 58°C in TSBYE supplemented with MBCs of each compound at pH 5.0 and 6.0. D-values at pH 5.0 were reduced from 14.56 ± 0.60 min to 0.93 ± 0.01, 0.63 ± 0.01, and 0.98 ± 0.02 min for vanillin, ethyl vanillin, and vanillic acid, respectively. These results suggest that vanillin, ethyl vanillin, and vanillic acid may be useful for the control of Cronobacter spp. in food during preparation and storage". As taken from Yemis GP et al. 2011. J. Food Protec. 74, 2062-2069. PubMed, 2014. Available at http://www.ncbi.nlm.nih.gov/pubmed/22186046.

"Humans have ~400 intact odorant receptors, but each individual has a unique set of genetic variations that lead to variation in olfactory perception. We used a heterologous assay to determine how often genetic polymorphisms in odorant receptors alter receptor function. We identified

agonists for 18 odorant receptors and found that 63% of the odorant receptors we examined had polymorphisms that altered in vitro function. On average, two individuals have functional differences at over 30% of their odorant receptor alleles. To show that these in vitro results are relevant to olfactory perception, we verified that variations in OR10G4 genotype explain over 15% of the observed variation in perceived intensity and over 10% of the observed variation in perceived valence for the high-affinity in vitro agonist guaiacol but do not explain phenotype variation for the lower-affinity agonists vanillin and ethyl vanillin." As taken from Mainland JD et al. 2014. Nat. Neurosci. 17(1), 114-20. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/24316890

"Vanilla flavour is familiar to consumers through foods, cosmetics, household products and some medicines. Vanilla flavouring agents typically contain vanillin or its analogue ethyl vanillin. Our previous study revealed that the inhalation of eugenol, which contains a vanillyl group, has an appetite-enhancing effect, and the inhalation of aroma compounds containing the vanillyl group or its analogues led to increased food intake in mice. Here, we found that vanillin, ethyl vanillin and eugenol showed appetite-enhancing effects, whereas isoeugenol and safrole did not. These results suggest that the appetite-enhancing effects could be attributable to the vanillyl group and could be affected by the position of the double bond in the aliphatic chain. Furthermore, the results of intraperitoneal administration of eugenol and vanillin suggest that their appetite-enhancing effects could occur via stimulation of olfactory receptors." As taken from Ogawa K et al. 2018. J. Nat. Med. 72(3), 798-802. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29569223

"An interesting finding from our study was that certain flavorants may actually inhibit the formation of radicals. Ethyl vanillin PG acetal and ethyl vanillin, both used in fragrances, foods, and beverages to impart a vanilla characteristic, showed similar inhibitions of radicals. While the decrease in radicals by ethyl vanillin PG acetal was not significant, decreases observed with higher concentrations of the unacetalated ethyl vanillin were significant. This differential effect may indicate that the aldehyde group present in ethyl vanillin but not in ethyl vanillin PG acetal may play a role in its antioxidant potential. A recent study found that ethyl vanillin and vanillin both can act as a strong antioxidants in vitro and in vivo further suggesting a role in radical inhibition [54]. This study also suggests the importance of the aldehyde group found on both ethyl vanillin and vanillin as the antioxidant properties were not observed with vanillyl alcohol or vanillic acid, both of which lack the aldehyde group [54]. The radical inhibition effects of ethyl vanillin suggest its possible use an additive in e-liquids reduce free radical production during aerosol formation. Further tests will be needed to determine if there are any toxic compounds formed during the aerosolizing process of e-liquids." As taken from Bitzer ZT et al. 2018. Free Radic. Biol. Med. 120: 72-79. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29548792

Type of Test	Exposure	Species Observed	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - skin	161.7 umol/L/48H	In Vitro Toxicity Studies - other assays	TXAPA9 Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- Volume(issue)/page/year: 245,281,2010
IC50 - Inhibitor Concentration 50	In vitro	Human - lymphocyte	66 mg/L/45H	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,901,2015

As taken from RTECS, 2018

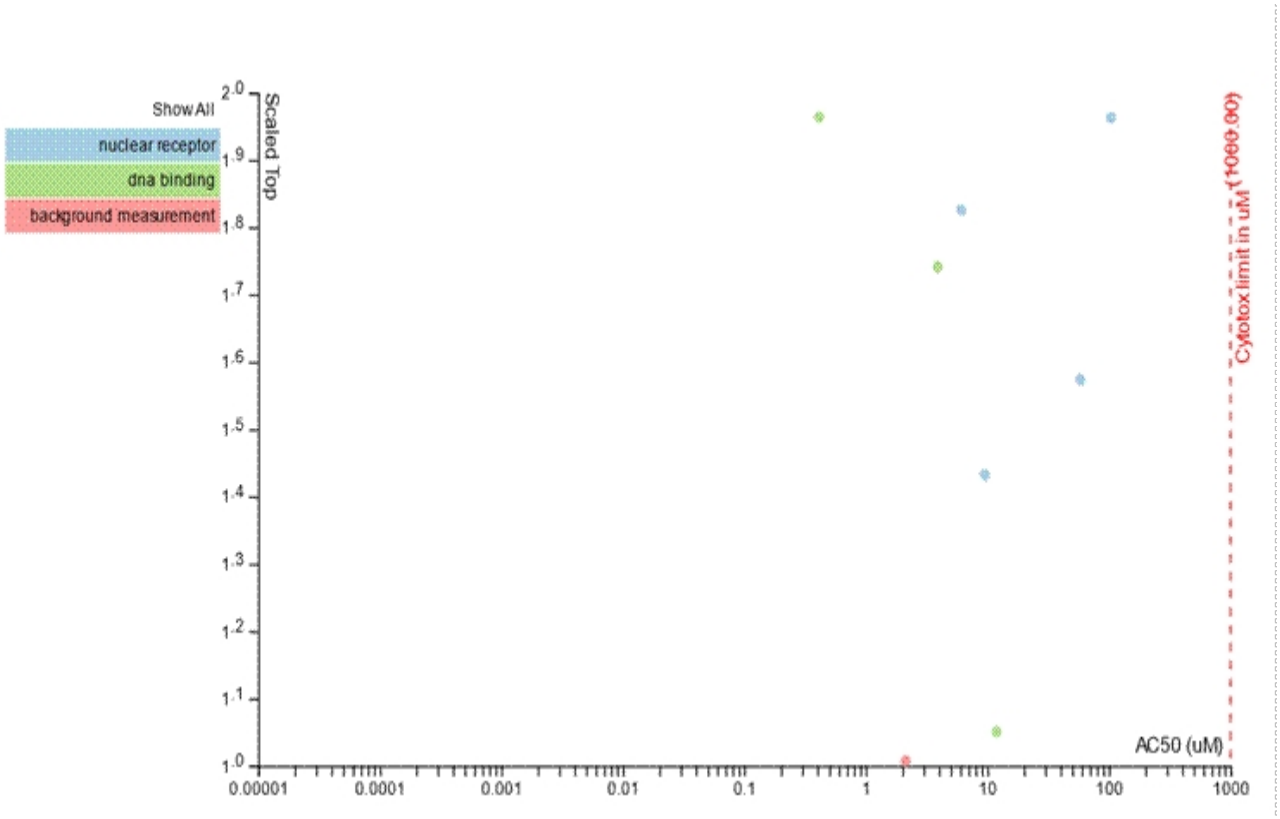
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 3-ethoxy-4-hydroxybenzaldehyde (CAS RN 121-32-4) 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 3-ethoxy-4-hydroxybenzaldehyde 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



6. Functional effects on

6.1. Broncho/pulmonary system

... Highly irritating action on the eyes and mucous membranes of the respiratory tract. /Aldehydes/ [Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 84] **PEER REVIEWED**

As taken from HSDB, 2015

“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 flavoring 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. Toxicol. Lett. 288, 143-155. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29481849

6.2. Cardiovascular system

“Vanillin (VA) and vanillyl alcohol (VAA), components of natural vanilla, and ethyl vanillin (EtVA; synthetic analog) are used as flavoring agents and/or as additives by the food, cosmetic, or pharmaceutic industries. VA, VAA, and EtVA possess antioxidant and anti-inflammatory properties, but their vascular effects have not been determined. Therefore, we compared in isolated porcine coronary and basilar arteries the changes in isometric tension caused by VA, VAA, and EtVA. VA and its analogs caused concentration-dependent relaxations of both preparations during contractions from U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F2α, a thromboxane A2 receptor agonist), and of coronary arteries contracted with KCl or endothelin-1. The order of potency was VAA <VA <EtVA. The relaxations were not inhibited by endothelium removal, by inhibitors of NO synthases (N(ω)-nitro-l-arginine methyl ester hydrochloride), cyclooxygenases (indomethacin), soluble guanylyl cyclase (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one [ODQ]), KCa (1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole [TRAM-34], 6,12,19,20,25,26-hexahydro-5,27:13,18:21,24-trietheno-11,7-metheno-7H-dibenzo[b,n][1,5,12,16]tetraazacyclotricosine-5,13-diium ditrifluoroacetate hydrate [UCL-1684], or iberiotoxin), by KATP (glibenclamide), by Kir (BaCl2), by transient receptor potential receptor vanilloid 3 (TRPV3) channels (ruthenium red), or by antioxidants (catalase, apocynin, tempol, N-acetylcysteine, tiron). VA and its analogs inhibited contractions induced by Ca(2+) reintroduction in coronary arteries, and by an opener of L-type Ca(2+)-channels (methyl 2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-1,4-dihydropyridine-3-carboxylate [Bay K8644]) in coronary and basilar arteries. They inhibited contractions of coronary rings induced by the protein kinase C activator phorbol 12,13-dibutyrate to the same extent as the removal of extracellular Ca(2+) or incubation with nifedipine. Thus, in porcine arteries, relaxation from VA (and its analogs) is due to inhibition of L-type Ca(2+) channels. Hence, these compounds could be used to relieve coronary or cerebral vasospasms due to exaggerated Ca(2+) influx, but therapeutic efficacy would require exposures that far exceed the current levels obtained by the use of vanillin additives.” As taken from Raffai G et al. 2015. J. Pharmacol. Exp. Ther.352(1), 14-22. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25344384.

6.3. Nervous system

“The nonselective cation channel transient receptor potential ankryn subtype family 1 (TRPA1) is expressed in neurons of dorsal root ganglia and trigeminal ganglia and also in vagal afferent neurons that innervate the lungs and gastrointestinal tract. Many TRPA1 agonists are reactive electrophilic compounds that form covalent adducts with TRPA1. Allyl isothiocyanate (AITC), the common agonist used to identify TRPA1, contains an electrophilic group that covalently binds with

cysteine residues of TRPA1 and confers a structural change on the channel. There is scientific motivation to identify additional compounds that can activate TRPA1 with different mechanisms of channel gating. We provide evidence that ethyl vanillin (EVA) is a TRPA1 agonist. Using fluorescent calcium imaging and whole-cell patch-clamp electrophysiology on dissociated rat vagal afferent neurons and TRPA1-transfected COS-7 cells, we discovered that EVA activates cells also activated by AITC. Both agonists display similar current profiles and conductances. Pretreatment with A967079, a selective TRPA1 antagonist, blocks the EVA response as well as the AITC response. Furthermore, EVA does not activate vagal afferent neurons from TRPA1 knockout mice, showing selectivity for TRPA1 in this tissue. Interestingly, EVA appears to be pharmacologically different from AITC as a TRPA1 agonist. When AITC is applied before EVA, the EVA response is occluded. However, they both require intracellular oxidation to activate TRPA1. These findings suggest that EVA activates TRPA1 but via a distinct mechanism that may provide greater ease for study in native systems compared with AITC and may shed light on differential modes of TRPA1 gating by ligand types." As taken from Wu SW et al. 2017. J. Pharmacol. Exp. Ther. 362(3), 368-377. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/28620120

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

"We systematically evaluated the antioxidant activity of ethyl vanillin, a vanillin analog, as compared with the activities of vanillin and other vanillin analogs using multiple assay systems. Ethyl vanillin and vanillin exerted stronger antioxidant effects than did vanillyl alcohol or vanillic acid in the oxygen radical absorbance capacity (ORAC) assay, although the antioxidant activities of vanillyl alcohol and vanillic acid were clearly superior to those of ethyl vanillin and vanillin in the three model radical assays. The antioxidant activity of ethyl vanillin was much stronger than that of vanillin in the oxidative hemolysis inhibition assay, but was the same as that of vanillin in the ORAC assay. Oral administration of ethyl vanillin to mice increased the concentration of ethyl vanillic acid, and effectively raised antioxidant activity in the plasma as compared to the effect of vanillin. These data suggest that the antioxidant activity of ethyl vanillin might be more beneficial than has been thought in daily health practice" (Tai et al, 2011. Bioscience, Biotechnology and Biochemistry, 75, 2346-2350. Available at https://www.jstage.jst.go.jp/article/bbb/75/12/75_110524/_pdf).

"Diabetes-induced oxidative stress and apoptosis is regarded as a critical role in the pathogenesis of diabetic nephropathy (DN). Treating diabetes-induced kidney damage and renal dysfunction has been thought a promising therapeutic option to attenuate the development and progression of DN. In this study, we investigated the renoprotective effect of ethyl vanillin (EVA), an active analogue of vanillin isolated from vanilla beans, on streptozotocin- (STZ-) induced rat renal injury model and high glucose-induced NRK-52E cell model. The EVA treatment could strongly improve the deterioration of renal function and kidney cell apoptosis in vivo and in vitro. Moreover, treating with EVA significantly decreased the level of MDA and reactive oxygen species (ROS) and stabilized antioxidant enzyme system in response to oxidative stress by enhancing the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in vivo and in vitro. Furthermore, EVA also markedly suppressed cleaved caspase-3, Bax, and nuclear transcription factor erythroid 2-related factor (Nrf2) expression in STZ-induced rats. Therefore, these results of our investigation provided that EVA might protect against kidney injury in DN by inhibiting oxidative stress and cell apoptosis." As taken from Tong Y et al. 2019. Oxid. Med. Cell Longev. 2019, 2129350. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31781325/

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 ethyl vanillin 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 ethyl vanillin. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	166	Carmines, 2002 & Rustemeier et al., 2002
	2,920	Baker et al., 2004a
	6.5 195 3900 3400 (Cigar)	JTI KB Study Report(s)
	8,020	Gaworski et al., 2011 & Coggins et al., 2011e
	84	Roemer et al, 2014
In vitro genotoxicity	166	Carmines, 2002 & Roemer et al., 2002
	2,920	Baker et al., 2004c
	6.5	Renne et al., 2006
	6.5 195 400 3400 (Cigar)	JTI KB Study Report(s)
	2,720	fGLH Study Report (2010)
	8,020	Gaworski et al., 2011 & Coggins et al., 2011e
	84	Roemer et al, 2014
In vitro cytotoxicity	166	Carmines, 2002 & Roemer et al., 2002
	2,920	Baker et al., 2004c
	6.5 195 400	JTI KB Study Report(s)

	3400 (Cigar)	
	2,720	fGLH Study Report (2010)
	8,020	Gaworski et al., 2011 & Coggins et al., 2011e
	84	Roemer et al, 2014
Inhalation study	848	Gaworski et al., 1998
	166	Carmines, 2002 & Vanscheeuwijck et al., 2002
	2,920	Baker et al., 2004c
	6.5	Renne et al., 2006
	6.5 195 400	JTI KB Study Report(s)
	8,020	Gaworski et al., 2011 & Coggins et al., 2011e
	84	Schramke et al, 2014
Skin painting	848	Gaworski et al., 1999
	6.5 195	JTI KB Study Report(s)
In vivo genotoxicity	84 3400 (Cigar)	Schramke et al, 2014 JTI KB Study Report(s)

“Abstract Context: Waterpipe smoke causes DNA damage in peripheral blood leukocytes and in buccal cells of smokers. OBJECTIVE: To determine the exposure effect of waterpipe smoke on buccal cells and peripheral blood leukocytes in regard to DNA damage using comet assay. MATERIALS AND METHODS: The waterpipe smoke condensates were analyzed by gas chromatography-mass spectrometry (GC-MS). The study was performed on 20 waterpipe smokers. To perform comet assay on bucaal cells of smokers, 10 µl of cell suspension was mixed with 85 µl of pre-warmed 1% low melting agarose, applied to comet slide and electrophoresed. To analyze the effect of smoke condensate in vitro, 1 ml of peripheral blood was mixed with 10 µl of smoke condensate and subjected for comet assay. RESULTS: The GC-MS analysis revealed the presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4on, nicotine, hydroxymethyl furancarboxaldehyde and 3-ethoxy-4-hydroxybenzaldehyde in the smoke condensates. Waterpipe smoking caused DNA damage in vivo in buccal cells of smokers. The tail moment and tail length in buccal cells of smokers were 186 ± 26 and 456 ± 71, respectively, which are higher than control. The jurak and moassel smoke condensates were found to cause DNA damage in peripheral blood leukocytes. The moassel smoke condensate was more damaging. DISCUSSION: There is wide misconception that waterpipe smoking is not as harmful as cigarette smoking. This study demonstrated that waterpipe smoke induced DNA damage in exposed cells. CONCLUSION: Waterpipe smokes cause DNA damage in buccal cells. The smoke condensate of both jurak and moassel caused comet formation suggesting DNA damage in peripheral blood leukocytes.” As taken from Al-Amrah HJ et al. 2014. Inhal. Toxicol.26(14), 891-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25357232.

Transfer studies:

In a pyrolysis study, 100% of ethyl vanillin added to cigarettes was transferred intact to the smoke (Purkis et al. 2011)

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Ethyl vanillin 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 Ethyl vanillin 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	1563	JTI KB Study Report(s)
In vitro cytotoxicity	1563	JTI KB Study Report(s)

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Ethyl vanillin 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	4,500	Logic (2019) Labstat International Inc. (2021)
In vitro genotoxicity	4,500	Logic (2019) Labstat International Inc. (2022)
In vitro cytotoxicity	4,500	Logic (2019) Labstat International Inc. (2022)
In vivo genotoxicity	19.0	Logic (2019)
Inhalation study	19.0	Logic (2019)

Aerosol from heated tobacco stick(s) containing Ethyl vanillin 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	0.14	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	0.14	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	0.14	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

“Introduction: "Vaping" electronic cigarettes (e-cigarettes) is increasingly popular with youth, driven by the wide range of available flavors, often created using flavor aldehydes. The objective of this study was to examine whether flavor aldehydes remain stable in e-cigarette liquids or whether they undergo chemical reactions, forming novel chemical species that may cause harm to the user.

Methods: Gas chromatography was used to determine concentrations of flavor aldehydes and reaction products in e-liquids and vapor generated from a commercial e-cigarette. Stability of the detected reaction products in aqueous media was monitored by ultraviolet spectroscopy and nuclear magnetic resonance spectroscopy, and their effects on irritant receptors determined by fluorescent calcium imaging in HEK-293T cells. **Results:** Flavor aldehydes including benzaldehyde, cinnamaldehyde, citral, ethylvanillin, and vanillin rapidly reacted with the e-liquid solvent propylene glycol (PG) after mixing, and upward of 40% of flavor aldehyde content was converted to flavor aldehyde PG acetals, which were also detected in commercial e-liquids. Vaping experiments showed carryover rates of 50%-80% of acetals to e-cigarette vapor. Acetals remained stable in physiological aqueous solution, with half-lives above 36 hours, suggesting they persist when inhaled by the user. Acetals activated aldehyde-sensitive TRPA1 irritant receptors and aldehyde-insensitive TRPV1 irritant receptors. **Conclusions:** E-liquids are potentially reactive chemical systems in which new compounds can form after mixing of constituents and during storage, as demonstrated here for flavor aldehyde PG acetals, with unexpected toxicological effects. For regulatory purposes, a rigorous process is advised to monitor the potentially changing composition of e-liquids and e-vapors over time, to identify possible health hazards. **Implications:** This study demonstrates that e-cigarette liquids can be chemically unstable, with reactions occurring between flavorant and solvent components immediately after mixing at room temperature. The resulting compounds have toxicological properties that differ from either the flavorants or solvent components. These findings suggest that the reporting of manufacturing ingredients of e-liquids is insufficient for a safety assessment. The establishment of an analytical workflow to detect newly formed compounds in e-liquids and their potential toxicological effects is imperative for regulatory risk analysis." As taken from Erythropel HC et al. 2019. Nicotine Tob. Res. 21(9), 1248–1258. PubMed, 2020 available at

https://pubmed.ncbi.nlm.nih.gov/30335174/

"The widespread use of electronic cigarettes (e-cig) is a serious public health concern; however, mechanisms by which e-cig impair the function of airway epithelial cells-the direct target of e-cig smoke-are not fully understood. Here we report transcriptomic changes, including decreased expression of many ribosomal genes, in airway epithelial cells in response to e-cig exposure. Using RNA-seq we identify over 200 differentially expressed genes in air-liquid interface cultured primary normal human bronchial epithelial (NHBE) exposed to e-cig smoke solution from commercial e-cig cartridges. In particular, exposure to e-cig smoke solution inhibits biological pathways involving ribosomes and protein biogenesis in NHBE cells. Consistent with this effect, expression of corresponding ribosomal proteins and subsequent protein biogenesis are reduced in the cells exposed to e-cig. Gas chromatography/mass spectrometry (GC/MS) analysis identified the presence of five flavoring chemicals designated as 'high priority' in regard to respiratory health, and methylglyoxal in e-cig smoke solution. Together, our findings reveal the potential detrimental effect of e-cig smoke on ribosomes and the associated protein biogenesis in airway epithelium. Our study calls for further investigation into how these changes in the airway epithelium contribute to the current epidemic of lung injuries in e-cig users."

Park HR et al. (2021) Electronic cigarette smoke reduces ribosomal protein gene expression to impair protein synthesis in primary human airway epithelial cells.

10. Ecotoxicity

10.1. Environmental fate

Environmental Fate/Exposure Summary:

Ethyl vanillin's production and use as a flavoring agent and in perfumery may result in its release to the environment through various waste streams. If released to air, a vapor pressure of 1X10-5 mm Hg at 25 deg C indicates ethyl vanillin will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase ethyl vanillin 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 12 hours. Particulate-phase ethyl vanillin will be removed from the atmosphere by wet and dry deposition. If released to soil, ethyl vanillin is expected to have moderate mobility based upon an estimated Koc of 180. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's Law constant of 8.1X10-10 atm-cu m/mole. Ethyl vanillin is not expected to volatilize from dry soil surfaces based upon its vapor pressure. A 52.9% of the theoretical BOD was achieved in 5 days with a sewage inoculum, suggesting that biodegradation may be rapid in the environment. If released into water, ethyl vanillin is expected to adsorb to suspended solids and sediment in the water column based upon the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. An estimated BCF of 10 suggests bioconcentration in aquatic organisms is low. Occupational exposure to ethyl vanillin may occur through inhalation and dermal contact with this compound at workplaces where ethyl vanillin is produced or used. The general population may be exposed to ethyl vanillin via dermal contact with perfumes and ingestion of food products that contain this compound as a flavorant. (SRC) **PEER REVIEWED**

Artificial Pollution Sources:

Ethyl vanillin's production and use as a flavoring agent(1) and in perfumery(2) may result in its release to the environment through various waste streams(SRC). [(1) Fenaroli's Handbook of Flavor Ingredients Volume 2. Furia TE, Bellanca N Eds. 2nd ed. Cleveland,OH: The Chemical Rubber Co (1975) (2) Budvari S; Merck Index, 12th ed, Whitehouse Station, NJ Merck & Co. p 654 (1996)] **PEER REVIEWED**

Environmental Fate:

TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 180(SRC), determined from a log Kow of 1.61(2) and a regression-derived equation(3), indicates that ethyl vanillin is expected to have moderate mobility in soil(SRC). Volatilization of ethyl vanillin from moist soil surfaces is not expected to be an important fate process(SRC) given an estimated Henry's Law constant of 8.1X10-10 atm-cu m/mole(SRC), determined from its experimental values for vapor pressure, 1X10-5 mm Hg at 25 deg C(4), and water solubility, 2,822 mg/l at 25 deg C(2). Ethyl vanillin is not expected to volatilize from dry soil surfaces(SRC) based upon its measured vapor pressure(4). A 52.9% of the theoretical BOD was achieved in 5 days with a sewage inoculum(5), suggesting that biodegradation may be rapid in the environment(SRC). [(1) Swann RL et al; Res Rev 85: 23 (1983) (2) Jin L et al; Chemosphere 35: 2707-12 (1997) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 4-9 (1990) (4) Yaws CL; Handbook of Vapor Pressure Vol 3 C8-C28 Compounds. Houston,TX: Gulf Publ Co (1994)(5) Babeu L, Vaishnav DD; J Indust Microb 2: 107-15 (1987)] **PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 180(SRC), determined from a log Kow of 1.61(2) and a regression-derived equation(3), indicates that ethyl vanillin is expected to adsorb to suspended solids and sediment in water(SRC). Volatilization from water surfaces is not expected(3) based upon an estimated Henry's Law constant of 8.1X10-10 atm-cu m/mole(SRC) determined from its experimental values for vapor pressure, 1X10-5 mm Hg at 25 deg C(4), and water solubility, 2,822 mg/l at 25 deg C(2). According to a classification scheme(5), an estimated BCF of 10(3,SRC), from its log Kow(2) suggests the potential for bioconcentration in aquatic organisms is low(SRC). A 52.9% of the theoretical BOD was achieved

in 5 days with a sewage inoculum(6), suggesting that biodegradation may be rapid in the environment(SRC). [(1) Swann RL et al; Res Rev 85: 23 (1983) (2) Jin L et al; Chemosphere 35: 2707-12 (1997) (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) Yaws CL; Handbook of Vapor Pressure Vol 3 C8-C28 Compounds. Houston,TX: Gulf Publ Co (1994) (5) Franke C et al; Chemosphere 29: 1501-14 (1994) (6) Babeu L, Vaishnav DD; J Indust Microb 2: 107-15 (1987)] **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), ethyl vanillin, which has a vapor pressure of 1X10-5 mm Hg at 25 deg C(2), is expected to exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase ethyl vanillin 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 12 hours(SRC) from its estimated rate constant of 3.3X10-11 cu cm/molecule-sec at 25 deg C(SRC), determind by a fragment constant estimation method(3). Particulate-phase ethyl vanillin may be removed from the air by wet and dry deposition(SRC). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Yaws CL; Handbook of Vapor Pressure Vol 3 C8-C28 Compounds. Houston,TX: Gulf Publ Co (1994) (3) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993)] **PEER REVIEWED**

Environmental Biodegradation:

A 52.9% of the theoretical BOD was achieved in 5 days with a sewage inoculum(1), suggesting that biodegradation may be rapid in the environment(SRC). [(1) Babeu L, Vaishnav DD; J Indust Microb 2: 107-15 (1987)] **PEER REVIEWED**

Environmental Abiotic Degradation:

The rate constant for the vapor-phase reaction of ethyl vanillin with photochemically-produced hydroxyl radicals has been estimated as 3.3X10-11 cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 12 hours at an atmospheric concentration of 5X10+5 hydroxyl radicals per cu cm(1). Ethyl vanillin is not expected to undergo hydrolysis in the environment due to the lack of hydrolyzable functional groups(2). [(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**

Environmental Bioconcentration:

An estimated BCF of 10 was calculated for ethyl vanillin(SRC), using a log Kow of 1.61(1) and a regression-derived equation(2). According to a classification scheme(3), this BCF value suggests the potential for bioconcentration in aquatic organisms is low(SRC). [(1) Jin L et al; Chemosphere 35: 2707-12 (1997) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 5-4, 5-10 (1990) (3) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

Soil Adsorption/Mobility:

The Koc of ethyl vanillin is estimated as approximately 180(SRC), using a log Kow of 1.61(1) and a regression-derived equation(2). According to a classification scheme(3), this estimated Koc value suggests that ethyl vanillin is expected to have moderate mobility in soil(SRC). [(1) Jin L et al; Chemosphere 35: 2707-12 (1997) (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**

Volatilization from Water/Soil:

The Henry's Law constant for ethyl vanillin is estimated as 8.1X10-10 atm-cu m/mole(SRC) from its experimental values for vapor pressure, 1X10-5 mm Hg(1), and water solubility, 2,822 mg/l(2). This

Henry's Law constant indicates that ethyl vanillin is expected to be essentially nonvolatile from water surfaces(3). Ethyl vanillin's estimated Henry's Law constant(1,2,SRC) indicates that volatilization from moist soil surfaces is not expected(SRC). Ethyl vanillin is not expected to volatilize from dry soil surfaces(SRC) based upon its vapor pressure(1). [(1) Yaws CL; Handbook of Vapor Pressure Vol 3 C8-C28 Compounds. Houston,TX: Gulf Publ Co (1994) (2) Jin L et al; Chemosphere 35: 2707-12 (1997) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington,DC: Amer Chem Soc pp. 15-1 to 15-29 (1990)] **PEER REVIEWED**

Sediment/Soil Concentrations:

Ethyl vanillin was identified, not quantified, in suspended sediment from Singletary Lake, NC(1). [(1) Christman RF et al; Sci Total Environ 47: 195-210 (1985)] **PEER REVIEWED**

As taken from HSDB, 2003

EPISuite provides the following data:

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

Bond Method :	1.10E-010 atm-m3/mole (1.11E-005 Pa-m3/mole)
Group Method:	3.79E-009 atm-m3/mole (3.84E-004 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 2.235E-008 atm-m3/mole (2.264E-003 Pa-m3/mole) VP: 0.000293 mm Hg (source: MPBPVP) WS: 2.87E+003 mg/L (source: WSKOWWIN)

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

Log Kow used:	1.58 (exp database)
Log Kaw used:	-8.347 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	9.927
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	1.2008 1.0000 2.8525 (weeks) 3.9214 (days) 0.9631 0.9566 0.8775
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	0.00459 Pa (3.44E-005 mm Hg)
Log Koa (Koawin est):	9.927
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa)	0.000654

model:	0.00207

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.0231
Mackay model:	0.0497
Octanol/air (Koa) model:	0.142

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	32.6848 E-12 cm3/molecule-sec
Half-Life =	0.327 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	3.927 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Reaction With Nitrate Radicals May Be Important!	
Fraction sorbed to airborne particulates (phi): 0.0364 (Junge-Pankow, Mackay avg) 0.142 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	16.97 L/kg (MCI method)
Log Koc:	1.230 (MCI method)
Koc :	62.02 L/kg (Kow method)
Log Koc:	1.793 (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: 3.79E-009 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	1.991E+005 hours (8298 days)
Half-Life from Model Lake:	2.173E+006 hours (9.052E+004 days)

Removal In Wastewater Treatment:

Total removal:	2.00 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.91 percent

Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0713	7.85	1000
Water	28.3	360	1000
Soil	71.5	720	1000
Sediment	0.072	3.24e+003	0

Persistence Time: 630 hr

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzaldehyde, 3-ethoxy-4-hydroxy- (CAS RN 121-32-4) is not persistent in the environment:

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.9566
TOPKAT probability of biodegradation	0.926
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	0.3272

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

#28: Biodegradation in water: screening tests

MATERIALS AND METHODS			
Test material identity			
	Identifier	:	CAS number
	Identity	:	121-32-4
	Identifier	:	Common name
	Identity	:	3-ethoxy-4-hydroxybenzaldehyde
	Identifier	:	Common name
	Identity	:	n+ôn+ìpé¿pâêpé¡pé+n+ìn+ön+ìpâÆpâëpâ¡pé¡pé+pâÖpâ¦pé¦péópâ½pâçpâÆpâë

Study design			
	Details on inoculum	:	- Concentration of sludge:30mg/L
	Duration	:	2
	Unit	:	wk
Initial test substance concentration			
	Initial concentration	:	100
	Unit	:	mg/L
Parameter followed for biodegradation estimation			
	Details on study design	:	Standard type
Reference substance			
RESULTS AND DISCUSSION			
% Degradation of test substance			
	% degradation	:	88
	Parameter	:	other:
	Parameter	:	BOD
	% degradation	:	97
	Parameter	:	other:
	Parameter	:	TOC
	% degradation	:	100
	Parameter	:	other:
	Parameter	:	HPLC

As taken from the Japanese National Institute for Technology and Evaluation (NITE's) Japan Chemicals Collaborative Knowledge (J-CHECK) database, accessed May 2017. Available at http://www.safe.nite.go.jp/jcheck/template.action?ano=4044&mno=3-1201&cno=121-32-4&request_locale=en

10.2. Aquatic toxicity

According to the Ecological Categorization List from the Canadian Domestic Substances List, benzaldehyde, 3-ethoxy-4-hydroxy- (CAS RN 121-32-4) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	84.3
Experimental result iT (mg/l)	84.3
Test species iT (Latin)	Pimephales promelas
Test species iT (Common)	Fathead minnow
Final EndPoint iT	EC50
Exposure duration iT (hours)	48
Reference iT	First Quarterly Report, U.S.EPA Cooperative Agreement No.CR 809234-01-0, Center for Lake Superior Environ.Stud., Univ.of Wisconsin, Superior, WI :52 p.(Publ in Part As 12447, 12448, 12858, 12859, 3217) (Author Communication Used)
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	47.5
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	46.214
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	29.8571
Toxicity to fish (LC50 in mg/l) as predicted by Aster	43.667875
Toxicity to fish (LC50 in mg/l) as predicted by PNN	6.29694
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	2
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	206.454
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics	3.26E+000

QSAR in Ecosar v0.99g	

ROBUST STUDY SUMMARY - Inherent Toxicity

Item	Yes	No
Reference: Call, D.J., L.T. Brooke, N. Ahmad and Q.D. Vaishnav (1981). Aquatic pollutant hazard assessments and development of a hazard prediction technology by quantitative structure-activity relationships. First Quarterly Progress Report to EPA. U.S. EPA Cooperative Agreement No. CR 809234010. University of Wisconsin-Superior. 52p.		
Test Substance (CAS # and name): 121-32-4 3-ethoxy-4-hydroxybenzaldehyde		
***Chemical composition of the substance (including purity, by-products)**	**X**	
Persistence/stability of test substance in aquatic solution		X
Method		
References	X	
***OECD, EU, national, or other standard method**?		**X**
Justification of the method/protocol if not a standard method was used		X
***GLP (Good Laboratory Practice)**	**n/a**	
Test organisms (specify common and Latin names) Pimephales promelas (fathead minnow)		
Latin or both Latin and common names reported?	X	
Life cycle age / stage of test organism	X	
Sex	n/a	
Length and weight of test organisms	X	
Number of test organisms per replicate	X	
Food type / feeding periods (acclimation/during test)	X	
Test design / conditions		
Test type – acute or chronic (specify, but do **not** assess this item): Acute		
Experiment type (laboratory or field) specified?	X	
System type (static, semi-static, flow through)?	X	
Negative or positive controls (specify)?	X	
Number of replicates (including controls) and concentrations		X
Exposure pathways (food, water, both)	X	

Exposure duration	X	
***Measured concentrations reported?**		**X**
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	X	
Was pH within 6-9 range? (do **not** assess this item)	X	
Was temperature within 5-28 °C range? (do **not** assess this item)	X	
Photoperiod and light intensity	X	
Stock and test solution preparation	X	
Use of emulgators / solubilizers (especially for poorly soluble / unstable substances)		X
Analytical monitoring intervals	X	
Statistical methods used		X
Results		
Toxicity values (LC_{50}, EC_{50}, or IC_{50} - specify, do **not** assess this item): EC50 (48h) = 84.3 mg/L		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, do **not** assess this item):		
***Was toxicity value below the chemical's water solubility**?	**X**	
Other adverse effects (carcinogenicity, mutagenicity, etc. Do **not** assess this item)		X
Score: Major items: **2/4** Overall score: 17/23 **74%**		
EC Reliability code: 2		
Reliability category (high, satisfactory, low): **Satisfactory**		
Comments:		

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

ECOSAR Version 1.11 reports the following aquatic toxicity data for CAS RN 121-32-4:

Values used to Generate ECOSAR Profile Log Kow: 1.546 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 2820 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Aldehydes (Mono)

Phenols

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)

Aldehydes (Mono) :	Fish	96-hr	LC50	16.417
Aldehydes (Mono) :	Daphnid	48-hr	LC50	33.226
Aldehydes (Mono) :	Green Algae	96-hr	EC50	45.746
Aldehydes (Mono) :	Fish		ChV	2.972
Aldehydes (Mono) :	Daphnid		ChV	4.449 !
Aldehydes (Mono) :	Green Algae		ChV	14.969
Aldehydes (Mono) :	Fish (SW)	96-hr	LC50	23.277
Aldehydes (Mono) :	Fish (SW)		ChV	2.622 !
Phenols :	Fish	96-hr	LC50	63.894
Phenols :	Daphnid	48-hr	LC50	15.716
Phenols :	Green Algae	96-hr	EC50	75.547
Phenols :	Fish		ChV	6.035
Phenols :	Daphnid		ChV	2.992
Phenols :	Green Algae		ChV	35.695
Phenols :	Fish (SW)	96-hr	LC50	31.288
Neutral Organic SAR :	Fish	96-hr	LC50	349.069
(Baseline Toxicity) :	Daphnid	48-hr	LC50	190.067
	Green Algae	96-hr	EC50	119.051
	Fish		ChV	32.474
	Daphnid		ChV	16.495
	Green Algae		ChV	28.398

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

NOTE: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu.

Record for 3-ethoxy-4-hydroxybenzaldehyde (CAS RN 121-32-4):

Spec. Sci. Name Spec. Common Name	Exp. Type Chem. Anal.	Media Type Loc	Resp. Site Obs. Dur. (Days)	Endpoint BCF	Trend Eff%	Effect Effect Meas.	Conc (Standardized) Appl. Rate	Stat. Signif. Sig. Level
Fish; Standard Test Species								
Pimephales promelas	F	FW		EC50	DEC	BEH	A 90600 ug/L	
Fathead Minnow	M	LAB	1			EQUL/		
Pimephales promelas	F	FW		EC50	DEC	BEH	A 84300 ug/L	
Fathead Minnow	M	LAB	2			EQUL/		
Pimephales promelas	F	FW		LC50	INC	MOR	A 98000 ug/L	
Fathead Minnow	M	LAB	1			MORT		
Pimephales promelas	F	FW		LC50	INC	MOR	A 90400 ug/L	
Fathead Minnow	M	LAB	2			MORT		
Pimephales promelas	F	FW		LC50		MOR	A 87600 (81400-94300) ug/L	
Fathead Minnow	M	LAB	4			MORT		
Fish; Standard Test Species; U.S. Exotic/Nuisance Species								
Cyprinus carpio	GV	FW				MOR/	F (205-222) mg/kg bdwt	
Common Carp	U	LAB	1.833			MORT		

As taken from the EPA ECOTOX database.

LC50 values to fishes, mg/l : 87.6 96 hr, Pimephales promelas, Brooke et al. 1984

EC50 values to fishes, mg/l : 91.7 96 hr, bhv, Pimephales promelas, Brooke et al. 1984

As taken from The Finnish Environment Institute. Available at http://wwwp.ymparisto.fi/scripts/Kemrek/Kemrek_uk.asp?Method=MAKECHEMdetailsform&txtChemId=2664

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR Version 1.11 reports the following terrestrial toxicity data for CAS RN 121-32-4:

Values used to Generate ECOSAR Profile

Log Kow: 1.546 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 2820 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Phenols

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Phenols :	Earthworm	14-day	LC50	236.709
Phenols :	Lemna gibba	7-day	EC50	51.144

10.5. All other relevant types of ecotoxicity

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.709 (BCF = 5.123 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-1.1325 days (HL = 0.0737 days)
Log BCF Arnot-Gobas method (upper trophic):	0.564 (BCF = 3.663)
Log BAF Arnot-Gobas method (upper trophic):	0.564 (BAF = 3.663)
log Kow used:	1.58 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzaldehyde, 3-ethoxy-4-hydroxy- (CAS RN 121-32-4) is not bioaccumulative in the environment:

Empirical Log Kow	1.58
Log Kow predicted by KowWin	1.55
Log BAF T2MTL predicted by Gobas	0.523569929420154
Log BCF 5% T2LTL predicted by Gobas	0.460569840601777

Log BCF Max predicted by OASIS	1.46512127747714
Log BCF predicted by BCFWIN	0.517

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

11. References

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12. Other information

- Data are available from the RepDose database, a “database for the analysis of relationship between chemical function groups/categories and target organs in repeated dose studies”. The website carries the following comments: “Permission is granted to download or print material published on this site for personal use only. Its use for any other purpose, and in particular its commercial use or distribution, are strictly forbidden in the absence of prior written approval.” These data have, therefore, not been included in the current update. The website is available at: Fraunhofer ITEM (2014). RepDose Database. Available at: http://fraunhofer-repdose.de/
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Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats

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A series of in vitro and in vivo studies evaluated the potential effects of tobacco flavoring and casing ingredients. Study 1 utilized as a reference control cigarette a typical commercial tobacco blend without flavoring ingredients, and a test cigarette containing a mixture of 165 low-use flavoring ingredients. Study 2 utilized the same reference control cigarette as used in study 1 and a test cigarette containing eight high-use ingredients. The in vitro Ames *Salmonella typhimurium* assay did not show any increase in mutagenicity of smoke condensate from test cigarettes designed for studies 1 and 2 as compared to the reference. Sprague-Dawley rats were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 wk to smoke from the test or reference cigarettes already described, or to air only, and necropsied after 13 wk of exposure or following 13 wk of recovery from smoke exposure. Exposure to smoke from reference or test cigarettes in both studies induced increases in blood carboxyhemoglobin (COHb) and plasma nicotine, decreases in minute volume, differences in body or organ weights compared to air controls, and a concentration-related hyperplasia, squamous metaplasia, and inflammation in the respiratory tract. All these effects were greatly decreased or absent following the recovery period. Comparison of rats exposed to similar concentrations of test and reference cigarette smoke indicated no difference at any concentration. In summary, the results did not indicate any consistent differences in toxicologic effects between smoke from cigarettes containing the flavoring or casing ingredients and reference cigarettes.

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Flavoring ingredients are added to tobacco during the manufacture of many types of commercial cigarettes, and humectants such as glycerol are added to increase the moisture-holding capacity of the tobacco. There has been much speculation about the effect of these added ingredients on the toxicity of the resultant smoke. Wynder and Hoffman (1967) hypothesized that adding

nontobacco ingredients might increase or decrease the toxic effects of inhaled tobacco smoke, and later publications (LaVoie et al., 1980; Hoffman and Hoffman, 1997, 2001; World Health Organization, 2001) supported that hypothesis. Recently published research results (Gaworski et al., 1998; Paschke et al., 2002; Rodgman, 2002a, 2002b; Rodgman and Green, 2002; Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002; Baker et al., 2004) have presented data from in vitro, and in vivo toxicity studies that indicate the addition of ingredients to tobacco does not increase the toxicity of the smoke. Baker et al. (2004), using a pyrolysis technique that mimics closely the combustion conditions inside burning cigarettes (Baker and Bishop, 2004), studied the effects of pyrolysis on the chemistry, in vitro genotoxicity and cytotoxicity, and inhalation toxicity in rodents of 291 single ingredients added to cigarettes.

The studies described herein were designed to evaluate the potential influence of low-use flavoring ingredients and high-use mixed casing or flavoring ingredients on the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against an identical reference cigarette respectively produced without flavors or casings.

MATERIALS AND METHODS

Cigarette Design

In study 1, 165 low-use flavoring ingredients were added to a single test cigarette and compared to a reference cigarette without these ingredients. In study 2, eight high-use flavoring or casing ingredients were added to a single test cigarette and compared to the same reference cigarette that was used in study 1. Thus, the design covered these ingredients as well as possible interactions between them and/or their combustion or pyrolysis products. The prototype cigarettes were designed to be representative of commercial, full flavor filter cigarettes. Test and reference cigarettes were constructed with conventional commercial equipment.

The ingredients selected for evaluation in these studies comprise low-use and high-use ingredients normally utilized in the manufacture of commercial cigarettes. The point of addition was chosen to mimic actual process conditions. Study 1 and study 2 ingredients were incorporated into a flavoring or casing system at levels exceeding their normal use. Table 1 outlines the tobacco components of the blend used to construct the cigarettes in both study 1 and study 2. The blends were cased with a mixture of glycerin and water (at a ratio of 2:1) to provide the necessary moisture for standard processing. In preparation of study 1 cigarettes, the ingredients were applied at a rate of 10 kg/1000 kg leaf blend, that is, at 1% on the test cigarettes, and the casing was applied at a rate of 30 kg/1000 kg leaf blend. The study 2 ingredient system was applied at a rate of 31 kg/1000 kg leaf blend (3.1%). The 165 ingredients included in the study 1 mixture appear listed in order of descending application rate in Table 2,

TABLE 1
Blend composition of prototype cigarettes

	Percent of blend component in cigarettes	
Blend components	Tobacco wet weight	Tobacco dry weight
Burley	24	22.9
Virginia	28	25.7
Oriental	14.8	13.6
Reconstituted sheet	23.4	20.1
Expanded tobacco	9.7	8.8

along with the corresponding CAS-Number, regulatory identifiers (where applicable) and application rate. The seven casings and one flavoring included in the study 2 mixture appear listed in order of descending application rate in Table 3. Cellulose acetate filters with 32% average air dilution were used in all cigarettes. Monogram inks were not subject to these studies.

Cigarette Performance

A preliminary cigarette performance evaluation was carried out prior to the toxicology studies. Prior to characterization, the cigarettes were conditioned for a minimum of 48 h at a temperature of $22 \pm 1°C$ and a relative humidity (RH) of $60 \pm 2\%$, in accordance with ISO Standard 3402. Subsequently, the cigarettes were smoked on a 20-port Borgwaldt smoking machine under the conditions stipulated in ISO Standard 3308. Therefore, the puffing regime for mainstream smoke used a 35 ± 0.3 ml puff volume, with 2.0 ± 0.05 s puff duration once every 60 ± 0.5 s. Smoke samples were respectively collected in accordance with the analytical method.

In Vitro Study Design

The mutagenicity of total particulate matter (TPM) in study 1 and 2 cigarettes was investigated using an Ames assay protocol that conformed to OECD Guideline 471. For this purpose, prototype cigarettes containing a mixture of ingredients, reference cigarettes without these ingredients, and 2R4F cigarettes (a standard reference cigarette developed and validated by the University of Kentucky) were smoked on a Borgwaldt RM200 rotary smoking machine under the ISO standard 3308 condition. TPM was collected in a standard fiberglass (Cambridge) trap with dimethyl sulfoxide (DMSO), and the DMSO solution was stored in the dark at $-80°C$ prior to performance of the Ames assay. Each sample was tested with and without S9 metabolic activation in five strains of *Salmonella typhimurium*: TA98, TA100, TA102, TA1535, and TA1537. Evaluation of the Ames assay data was carried out in terms of the mutagenic response, taking into consideration the reproducibly dose-related increase in number of revertants, even if the increase was less than twofold. The mutagenic response to TPM from the reference and test cigarettes was compared using the linear portion of the slope (revertants/mg TPM).

TABLE 2
Ingredients added to test cigarettes in study 1

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Benzyl alcohol	100-51-6	2137	172.515	58c	260
2	Immortelle extract	8023-95-8	2592	182.20	225n	156
3	Coriander oil	8008-52-4	2334	182.20	154n	65
4	Balsam peru resinoid	8007-00-9	2117	182.20	298n	65
5	Anise star oil	8007-70-3	2096	N.A.	238n	65
6	Celery seed oil	89997-35-3	2271	182.20	52n	65
7	Vanillin	121-33-5	3107	182.60	107c	65
8	Potassium sorbate	24634-61-5	2921	182.3640	N.A.	39
9	Propyl *para*-hydroxybenzoate	94-13-3	2951	172.515	N.A.	39
10	Benzoin resinoid	9000-05-9	2133	172.510	439n	26
11	Cedarwood oil	8000-27-9	N.A.	N.A.	252n	26
12	Clary extract	8016-63-5	2321	182.20	415n	26
13	Methylcyclopentenolone	80-71-7	2700	172.515	758c	26
14	Phenethyl alcohol	60-12-8	2858	172.515	68c	26
15	Piperonal	120-57-0	2911	182.60	104c	26
16	Tea extract	84650-60-2	N.A.	182.20	451n	26
17	Vanilla oleoresin	8024-06-4	3106	182.20	474n	26
18	Brandy	N.A.	N.A.	N.A.	N.A.	26
19	*trans*-Anethole	4180-23-8	2086	182.60	183c	19.5
20	Coffee extract	84650-00-0	N.A.	182.20	452n	19.5
21	5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	698-10-2	3153	N.A.	2300c	19.5
22	Propionic acid	79-09-4	2924	184.1081	3c	13
23	Acetic acid	64-19-7	2006	184.1005	2c	13
24	Amyl formate	638-49-3	2068	172.515	497c	13
25	Angelica root oil	8015-64-3	2088	182.20	56n	13
26	Beeswax absolute	8012-89-3	2126	184.1973	N.A.	13
27	Benzyl benzoate	120-51-4	2138	172.515	262c	13
28	Benzyl propionate	122-63-4	2150	172.515	413c	13
29	Cardamom oil	8000-66-6	2241	182.20	180n	13
30	beta-Carotene	7235-40-7	N.A.	184.1245	N.A.	13
31	Ethyl acetate	141-78-6	2414	182.60	191c	13
32	Ethyl butyrate	105-54-4	2427	182.60	264c	13
33	Ethyl levulinate	539-88-8	2442	172.515	373c	13
34	Eucalyptol	470-82-6	2465	172.515	182c	13
35	Geranium oil	8000-46-2	2508	182.20	324n	13
36	Labdanum resinoid	8016-26-0	2610	172.510	134n	13
37	Lavandin oil	8022-15-9	2618	182.20	257n	13
38	Maltol	118-71-8	2656	172.515	148c	13
39	Spearmint oil	8008-79-5	3032	182.20	285n	13
40	Ethyl hexanoate	123-66-0	2439	172.515	310c	10.4
41	Acetylpyrazine	22047-25-2	3126	N.A.	2286c	9.1
42	Ethylmaltol	4940-11-8	3487	172.515	692c	9.1
43	Chamomile oil, Roman	8015-92-7	2275	182.20	48n	6.5
44	Citronella oil	8000-29-1	2308	182.20	39n	6.5
45	delta-Decalactone	705-86-2	2361	172.515	621c	6.5
46	gamma-Decalactone	706-14-9	2360	172.515	2230c	6.5
47	Ethyl phenylacetate	101-97-3	2452	172.515	2156c	6.5

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
48	Ethyl valerate	539-82-2	2462	172.515	465c	6.5
49	Ethyl vanillin	121-32-4	2464	182.60	108c	6.5
50	Fennel sweet oil	8006-84-6	2485	182.20	200n	6.5
51	Glycyrrhizin ammoniated	53956-04-0	N.A.	184.1408	N.A.	6.5
52	gamma-Heptalactone	105-21-5	2539	172.515	2253c	6.5
53	3-Hexen-1-ol	928-96-1	2563	172.515	750c	6.5
54	3-Hexenoic acid	1577-18-0	3170	N.A.	2256c	6.5
55	Hexyl alcohol	111-27-3	2567	172.515	53c	6.5
56	Isoamyl phenylacetate	102-19-2	2081	172.515	2161c	6.5
57	Methyl phenylacetate	101-41-7	2733	172.515	2155c	6.5
58	Nerol	106-25-2	2770	172.515	2018c	6.5
59	Nerolidol	142-50-7	2272	172.515	67c	6.5
60	Peruvian (bois de rose) oil	8015-77-8	2156	182.20	44n	6.5
61	Phenylacetic acid	103-82-2	2878	172.515	672c	6.5
62	Pyruvic acid	127-17-3	2970	172.515	19c	6.5
63	Rose absolute	8007-01-0	2988	182.20	405n	6.5
64	Sandalwood oil	8006-87-9	3005	172.510	420n	6.5
65	Sclareolide	564-20-5	3794	N.A.	N.A.	6.5
66	Triethyl citrate	77-93-0	3083	184.1911	N.A.	6.5
67	2,3 5-Trimethylpyrazine	14667-55-1	3244	N.A.	735c	6.5
68	Olibanum absolute	8016-36-2	2816	172.510	93n	6.5
69	delta-Octalactone	698-76-0	3214	N.A.	2195c	6.5
70	2-Hexenal	6728-26-3	2560	172.515	748c	5.2
71	Ethyl octadecanoate	111-61-5	3490	N.A.	N.A.	5.2
72	4-Hydroxy-3-pentenoic acid lactone	591-12-8	3293	N.A.	731c	3.9
73	Methyl 2-pyrrolyl ketone	1072-83-9	3202	N.A.	N.A.	3.9
74	Methyl linoleate (48%) methyl linolenate (52%) mixture	112-63-0 301-00-8	3411	N.A.	713c	3.9
75	Petitgrain mandarin oil	8014-17-3	2854	182.20	142n	3.9
76	Propenylguaethol	94-86-0	2922	172.515	170c	3.9
77	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-2-en-4-one	23696-85-7	3420	N.A.	N.A.	3.9
78	2-Propionyl pyrrole	1073-26-3	3614	N.A.	N.A.	3.9
79	Orange essence oil	8008-57-9	2825	182.20	143n	2.6
80	Benzyl phenylacetate	102-16-9	2419	172.515	232c	2.6
81	2,3-Butanedione	431-03-8	2370	184.1278	752c	1.95
82	2,3,5,6-Tetramethylpyrazine	1124-11-4	3237	N.A.	734c	1.95
83	Hexanoic acid	142-62-1	2559	172.515	9c	1.56
84	Cinnamaldehyde	104-55-2	2286	182.60	102c	1.3
85	Acetophenone	98-86-2	2009	172.515	138c	1.3
86	2-Acetylthiazole	24295-03-2	3328	N.A.	N.A.	1.3
87	Amyl alcohol	71-41-0	2056	172.515	514c	1.3
88	Amyl butyrate	540-18-1	2059	172.515	270c	1.3
89	Benzaldehyde	100-52-7	2127	182.60	101c	1.3
90	Butyl butyrate	109-21-7	2186	172.515	268c	1.3
91	Butyric acid	107-92-6	2221	182.60	5c	1.3
92	Cinnamyl alcohol	104-54-1	2294	172.515	65c	1.3

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TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
93	DL-Citronellol	106-22-9	2309	172.515	59c	1.3
94	Decanoic acid	334-48-5	2364	172.860	11c	1.3
95	para-Dimethoxybenzene	150-78-7	2386	172.515	2059c	1.3
96	3,4-Dimethyl-1,2-cyclopentanedione	13494-06-9	3268	N.A.	2234c	1.3
97	Ethylbenzoate	93-89-0	2422	172.515	261c	1.3
98	Ethyl heptanoate	106-30-9	2437	172.515	365c	1.3
99	Ethyl isovalerate	108-64-5	2463	172.515	442c	1.3
100	Ethyl myristate	124-06-1	2445	172.515	385c	1.3
101	Ethyl octanoate	106-32-1	2449	172.515	392c	1.3
102	Ethyl palmitate	628-97-7	2451	N.A.	634c	1.3
103	Ethyl propionate	105-37-3	2456	172.515	402c	1.3
104	2-Ethyl-3-methylpyrazine	15707-23-0	3155	N.A.	548c	1.3
105	Genet absolute	8023-80-1	2504	172.510	436n	1.3
106	Geraniol	106-24-1	2507	182.60	60c	1.3
107	Geranyl acetate	105-87-3	2509	182.60	201c	1.3
108	gamma-Hexalactone	695-06-7	2556	172.515	2254c	1.3
109	Hexyl acetate	142-92-7	2565	172.515	196c	1.3
110	Isoamyl acetate	123-92-2	2055	172.515	214c	1.3
111	Isoamyl butyrate	106-27-4	2060	172.515	282c	1.3
112	3,7-Dimethyl-1,6-octadiene-3-ol	78-70-6	2635	182.60	61c	1.3
113	Menthyl acetate	89-48-5	2668	172.515	206c	1.3
114	Methyl isovalerate	556-24-1	2753	172.515	457c	1.3
115	Methyl salicylate	119-36-8	2745	175.105	433c	1.3
116	3-Methylpentanoic acid	105-43-1	3437	N.A.	N.A.	1.3
117	gamma-Nonalactone	104-61-0	2781	172.515	178c	1.3
118	Oakmoss absolute	9000-50-4	2795	172.510	194n	1.3
119	Orris absolute	8002-73-1	N.A.	172.510	241n	1.3
120	Palmitic acid	57-10-3	2832	172.860	14c	1.3
121	Phenethyl phenylacetate	102-20-5	2866	172.515	234c	1.3
122	3-Propylidenephthalide	17369-59-4	2952	172.515	494c	1.3
123	Sage oil	8022-56-8	3001	182.20	61n	1.3
124	alpha-Terpineol	98-55-5	3045	172.515	62c	1.3
125	Terpinyl acetate	80-26-2	3047	172.515	205c	1.3
126	gamma-Undecalactone	104-67-6	3091	172.515	179c	1.3
127	gamma-Valerolactone	108-29-2	3103	N.A.	757c	1.3
128	3-Butylidenphthalide	551-08-6	3333	N.A.	N.A.	1.04
129	Davana oil	8016-03-3	2359	172.510	69n	0.65
130	3,5-Dimethyl-1, 2-cyclopentanedione	13494-07-0	3269	N.A.	2235c	0.65
131	Ethyl cinnamate	103-36-6	2430	172.515	323c	0.65
132	Farnesol	4602-84-0	2478	172.515	78c	0.65
133	Geranyl phenylacetate	102-22-7	2516	172.515	231c	0.65
134	alpha-Irone	79-69-6	2597	172.515	145c	0.65
135	Jasmine absolute	8022-96-6	2598	182.20	245n	0.65
136	Kola nut tincture	68916-19-8	2607	182.20	149n	0.65
137	Linalool oxide	1365-19-1	3746	172.515	N.A.	0.65
138	Linalyl acetate	115-95-7	2636	182.60	203c	0.65
139	*para*-Methoxybenzaldehyde	123-11-5	2670	172.515	103c	0.65

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TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
140	2-Methylbutyric acid	116-53-0	2695	172.515	2002c	0.65
141	Myristic acid	544-63-8	2764	172.860	16c	0.65
142	gamma-Octalactone	104-50-7	2796	172.515	2274c	0.65
143	Opoponax oil	8021-36-1	N.A.	172.510	313n	0.65
144	Tagetes oil	8016-84-0	3040	172.510	443n	0.65
145	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	21835-01-8	3152	N.A.	759c	0.52
146	4-Methylacetophenone	122-00-9	2677	172.515	156c	0.26
147	Isobutyraldehyde	78-84-2	2220	172.515	92c	0.13
148	3-Methylbutyraldehyde	590-86-3	2692	172.515	94c	0.13
149	2,3-Dimethylpyrazine	5910-89-4	3271	N.A.	N.A.	0.13
150	2,5-Dimethylpyrazine	123-32-0	3272	N.A.	2210c	0.13
151	2,6-Dimethylpyrazine	108-50-9	3273	N.A.	2211c	0.13
152	Dimethyltetrahydrobenzofuranone	13341-72-5	3764	N.A.	N.A.	0.13
153	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	3658-77-3	3174	N.A.	536c	0.13
154	4-(*para*-Hydroxyphenyl)-2-butanone	5471-51-2	2588	172.515	755c	0.13
155	alpha-Ionone	127-41-3	2594	172.515	141c	0.13
156	beta-Ionone	8013-90-9	2595	172.515	142c	0.13
157	Isovaleric acid	503-74-2	3102	172.515	8c	0.13
158	Lime oil	8008-26-2	2631	182.20	141n	0.13
159	Mace absolute	8007-12-3	N.A.	182.20	296n	0.13
160	Nutmeg oil	8008-45-5	2793	182.20	296n	0.13
161	Caprylic acid	124-07-2	2799	184.1025	10c	0.13
162	Phenylacetaldehyde	122-78-1	2874	172.515	116c	0.13
163	5,6,7,8-Tetrahydroquinoxaline	34413-35-9	N.A.	N.A.	721c	0.13
164	Thyme oil	8007-46-3	3064	182.20	456n	0.13
165	Valeraldehyde	110-62-3	3098	172.515	93c	0.13

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturers Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

Inhalation Toxicity Study Design

Groups of 30 Sprague-Dawley rats of each sex were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 consecutive weeks to concentrations of 0.06, 0.2, or 0.8 mg/L WTPM of smoke from test cigarettes containing flavoring (study 1) or to flavoring or casing ingredients (study 2). Additional groups of 30 rats/sex were exposed to the same concentrations of smoke from reference cigarettes, similar to the test cigarettes but without the flavoring or casing ingredients (as described above), or to filtered air only (sham controls). This exposure regimen (1 h/day, 5 days/wk) reflects current laboratory practices for animal inhalation studies comparing the effects of smoke from test and reference cigarettes, and does not simulate human usage patterns. However, this difference should not influence the validity of the results.

Each group of 30 rats/sex was subdivided into 2 groups: 20 rats/sex scheduled for necropsy immediately after 13 wk of exposure (interim sacrifice) and up to 10 rats/sex scheduled for necropsy following 13 wk of recovery from smoke exposure (final sacrifice). Target smoke concentrations were 0.06, 0.2, or 0.8 mg WTPM/L for the test and reference cigarettes. An additional group of 30 rats/sex served as sham controls.

Biological endpoints for the 13-wk exposure and 13-wk recovery groups included clinical appearance, body weight, organ weights, and gross and microscopic lesions. Plasma nicotine, COHb, and respiratory parameters were measured periodically during the 13-wk exposure period and clinical pathology parameters were measured at the end of the 13-wk exposure period.

Smoke Generation and Exposure System

Animal exposures were conducted in AMESA exposure units (C. H. Technologies, Westwood, NJ). The smoke exposure machines were designed to contain 30 cigarettes on a smoking head that rotated 1 revolution per minute (Baumgartner and Coggins,

TABLE 3
Ingredients added to study 2 test cigarettes

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Invert sugar	8013-17-0	N.A.	184-1859	N.A.	20,000
2	Block chocolate	N.A.	N.A.	N.A.	N.A.	2,500
3	Plum extract	90082-87-4	N.A.	N.A.	371n	2,200
4	Fig extract	90028-74-3	N.A.	N.A.	198n	2,000
5	Molasse extract and tincture	68476-78-8	N.A.	N.A.	371n	2,000
6	Gentian root extract	97676-22-7	2506	172-510	214n	1,000
7	Lovage extract	8016-31-7	2650	172-510	261n	1,000
8	Peppermint oil	8006-90-4	2848	182-20	282n	250

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturer's Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

1980; Ayres et al., 1990). A vacuum port aligned with, and drew a puff from, one test or reference cigarette at a time as the head rotated. Air was drawn through the vacuum port by a peristaltic pump operating at a flow rate of ~1.05 L/min, creating a 2-s, 35-ml puff through each cigarette once each minute. The smoke vacuum flow rate was regulated by a concentration control unit consisting of a real-time aerosol monitor [(RAM)-1; MIE, Inc., Bedford, MA], a computer, and an electronic flow controller (Emerson Electric Co., Brooks Instrument Division, Hatfield, PA). The computer monitored analog voltage output of the RAM and adjusted the amount of smoke that was drawn from the glass mixing bowl by the flow controller until RAM voltage matched the calculated target voltage. The exposure units contained 3 tiers, each with 24 animal exposure ports. The exposure ports were connected to a delivery manifold, which transferred smoke to the animal breathing zone, and to an outer concentric manifold that drew the exhaled and excess smoke to an exhaust duct. Each cigarette was retained for seven puffs.

Exposure Atmosphere Characterization

The protocol-prescribed limits for the smoke concentration (WTPM/L) were target ±10% coefficient of variation (%CV). Smoke exposure concentrations were continuously monitored with a RAM at a representative exposure port. Mean exposure concentration was calculated from the mass collected on the filter and the total volume of air drawn through the filter, which was determined by the sample time and flow rate. RAM voltage readings were recorded during filter sample collection and were used to calculate a RAM response factor for subsequent exposures.

Two filters per exposure group per week were chemically analyzed for total nicotine. Nicotine standard reference material (98%) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The WTPM:nicotine and CO:nicotine ratios were calculated for the exposure atmospheres. The concentration of CO in the test and reference atmospheres was determined using Horiba PIR-2000 CO analyzers (Horiba Instruments, Inc., Irvine, CA), monitored by DOS-based computers.

Particle size distribution of the smoke was measured using Mercer-style cascade impactors designed specifically for the size range of particles found in cigarette smoke. The mass collected on each impactor stage was analyzed gravimetrically for WTPM and the resulting data were interpreted by probit analysis (NEWCAS; Hill et al., 1977) to obtain the particle size distribution, mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD). Temperature and RH of the exposure atmospheres were measured from a representative animal exposure port once every 2 wk for each exposure group.

Animals and Animal Care

Sprague-Dawley (Crl:CD) rats 4–5 wk of age were purchased from Charles River Laboratories (Raleigh, NC), held for 13 days in quarantine status prior to initial smoke exposure. Health screens were performed following group assignment and at 24 days after arrival. These health evaluations included necropsy, microscopic examination of selected tissues and examination for parasites. The 24 days after arrival screening included serological testing for antibodies to common viral pathogens. Viral antibody testing was also performed on sera collected from 10 sentinel rats at the end of the 13-wk exposure period and from another 10 at the end of the recovery period. All sera were tested for antibodies to Sendai virus, Kilham's rat virus (KRV)/Toolan's H-1 virus, pneumonia virus of mice (PVM), rat corona virus/sialodacryoadenitis virus, and *Mycoplasma pulmonis*. During the 13-wk exposure period, the animals were housed in individual stainless-steel cages on open racks. During the recovery period, the animals were housed in individual polycarbonate cages (Lab Products, Maywood, NJ) bedded with

ALPHA-dri alpha cellulose bedding (Sheperd Specialty Papers, Kalamazoo, MI). The cage space met the requirements stated in the current *Guide for Care and Use of Laboratory Animals* (National Academy of Sciences, 1996).

Body Weight and Clinical Observations

All rats were observed twice daily for mortality and moribundity. Each rat was examined every 4 wk for clinical signs. Individual body weights were measured during the randomization procedure, on exposure day 1, biweekly thereafter, and at necropsy.

Respiratory Function Measurements

Tidal volume (TV), respiratory rate (RR), and minute volume (MV), derived from flow signals from spontaneously breathing animals, were measured in 4 rats/sex/group during wk 2, 8, and 13 using whole-body phethysmography (Coggins et al., 1981). Each animal was monitored once during a single exposure period. MV and the actual WTPM were used to estimate the average total inhaled mass for the 1-h exposure period for each animal.

Carboxyhemoglobin and Plasma Nicotine Determinations

During wk 2 and 10, blood was collected from designated animals at the end of the 1-h smoke exposure. Animals were removed from the exposure unit and bleeding was initiated within ~5 min. The blood samples were obtained from the retro-orbital plexus of carbon dioxide (CO_2)-anesthetized animals into tubes containing potassium ethylenediaminete traacetic acid (K^+-EDTA). The sample tubes were immediately placed into an ice bath and maintained under these conditions until analyzed for blood carboxyhemoglobin (COHb). Plasma nicotine was quantitatively determined using gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring.

Clinical Pathology

On the day of the 13-wk interim sacrifice, the rats were anesthetized with ~70% CO_2 in room air and blood samples were obtained from the retro-orbital plexus. One sample was collected in a tube (Monoject, Sherwood Medical, St. Louis, MO) containing K^+-EDTA for hematologic determinations. Another sample was collected in a tube devoid of anticoagulant but containing a separator gel (Vacutainer, Franklin Lakes, NJ) for serum chemistry analysis. The following parameters were determined using an Abbott Cell-Dyn 3700 (Abbott Diagnostics Systems, Abbott Park, IL) multiparameter hematology instrument: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, volume of packed red cells (VPRC), the red cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), platelet count, and WBC differential counts. Results of the differential cell counts were reported as both relative and absolute values. Reticulocytes were stained supravitally with new methylene blue and enumerated as reticulocytes per 1000 enthrocytes using the Miller disc method (Brecher and Schneiderman, 1950).

A Roche Hitachi 912 system (Roche Diagnostic Corp., Indianapolis, IN) chemistry analyzer was used to determine the following serum analytes: urea nitrogen (BUN), creatinine, glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), sodium, potassium, chloride, calcium, phosphorus, total bilirubin, cholesterol, and triglycerides.

Necropsy and Tissue Collection

A complete necropsy was done on all 13-wk exposure groups and 13-wk recovery group animals. Rats designated for scheduled sacrifices or sacrificed due to moribund condition were weighed and anesthetized with 70% CO_2 in air, followed by exsanguination before cessation of heartbeat. All abnormalities were recorded on the individual animal necropsy forms. Lungs, liver, kidneys, testes, adrenals, spleen, brain, and heart from all scheduled sacrifice animals were weighed. These organ weights and the body weights at necropsy were used to calculate organ:body weight ratios. In addition, organ:brain weight ratios were calculated. The time from removal of the organ until weighing was minimized to keep tissues moist.

A complete set of over 40 tissues was removed from each animal at necropsy and examined. All tissues were fixed in 10% neutral buffered formalin (NBF) except for the eyes, which were fixed in Karnovsky's fixative. After the lungs were weighed, they were perfused with 10% NBF at 25 cm hydrostatic pressure.

Histopathology

All tissues were fixed in 10% NBF for a minimum of 48 h before being trimmed. Paraffin blocks were microtomed at 5 μm. All sections were stained with hematoxylin and eosin (H&E) stains for standard histopathologic evaluation of morphologic changes. Duplicate slides of nasal tissues, larynx, lung, and trachea were stained with periodic acid-Schiff/Alcian blue (PAS/AB) stains for evaluation of goblet cell populations. The lungs, nasal cavity (four sections), nasopharynx, larynx (three cross sections), trachea (three transverse sections), tracheobronchial lymph nodes, mediastinal (thymic) lymph nodes, heart, and all gross lesions were examined microscopically. The lungs were sectioned to present a maximal section of the mainstem bronchi. The nasal cavity was prepared in four sections using the landmarks described by Young (1981). Three transverse laryngeal sections were prepared from the base of the epiglottis, the ventral pouch, and through the caudal larynx at the level of the vocal folds (Renne et al., 1992). In addition, sections of brain, adrenals, spleen, liver, kidneys, and gonads from animals in the sham control and the groups exposed to 0.8 mg/L of smoke from the test or reference cigarettes were examined microscopically. Exposure-related microscopic lesions were observed in the tissues from the rats exposed to 0.8 mg/L; target organs were examined microscopically in the lower concentration groups to ascertain a no-effect concentration.

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

Cell proliferation rates were measured on respiratory tract tissues collected from 10 rats of each sex from each exposure group and the sham controls necropsied immediately after 13 wk of exposure, using a monoclonal antibody to 5-bromo-2′-deoxyuridine (BrdU). Tissues evaluated using the BrdU assay included the respiratory epithelium lining the median nasal septum and distal portions of maxillary and nasal turbinates, the transitional epithelium at the base of the epiglottis, the luminal epithelium dorsolateral to the ventral pouch, the luminal epithelium lining the cranial trachea, the luminal epithelium of the mainstem bronchi and adjacent bronchioles, and selected areas of alveolar epithelium. Data from both sides of bilaterally symmetrical tissues (nose, ventral pouch, mainstem bronchi) were combined for tabulation of results.

Statistical Methods

Body weight, body weight gain, organ:body weight, and organ:brain weight ratios were statistically analyzed for each sex by exposure concentration group using the Xybion PATH/TOX system. Data homogeneity was determined by Bartlett's test. Dunnett's t-test was performed on homogeneous data to identify differences between each concentration group and the sham control group, and between corresponding concentrations of test and reference cigarette smoke-exposed groups. Nonhomogeneous data were analyzed using a modified t-test. Respiratory physiology, clinical pathology, COHb, and plasma nicotine data parameters were statistically evaluated using SAS software (Statistical Analysis System, SAS, Inc., Cary, NC). One-way analysis of variance (ANOVA) between exposure groups was first conducted, followed by Bartlett's test for homogeneity of variance. A two-sided Dunnett's multiple comparison test was employed to determine which exposure groups were different from the controls. An unpaired two-sided t-test was used to compare equivalent exposure groups between cigarette types. Differences were considered significant at $p \leq .05$. The statistical evaluation of incidence and severity of lesions was made using the Kolmogorov–Smirnov two-sample test (Siegel, 1956). All treatment group means were compared to the sham control mean, and means of groups exposed to the test cigarette smoke were compared to the corresponding reference cigarette smoke-exposed group means. Cell proliferation data were compared statistically using Tukey's studentized range test with SAS software.

RESULTS

Cigarette Performance

The results of characterization of the test and reference cigarettes for study 1 and study 2 are presented in Tables 4 and 5. These results show that the filler weight and the number of puffs per cigarette, nicotine yield, and nicotine-free dry particulate matter (NFDPM) were comparable for test and reference cigarettes in both studies. The yields of nicotine and NFDPM and the puff count were also comparable. These results are consistent with the negligible differences in the configuration of both prototype cigarettes, which basically consist of the total relative amount of flavor ingredient contained in the test cigarettes (1% or 3% of the filler weight). A comparison of the burning rates in study 1 illustrates that the addition of the ingredients had little, if any effect on the burning characteristics of the test cigarettes.

TABLE 4
Key parameters for laboratory control of prototype study 1 cigarettes

Parameter	Target	Run average: Test cigarette	Run average: Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	0.963	0.965
Standard deviation	—	0.019	0.018
Non tobacco weight	0.212	0.212	0.215
Net tobacco	0.800	0.751	0.750
Air dilution (%)	32	35	34.1
Standard deviation	—	3.0	3.1
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	49	49
Expanded tobacco (%)	9.7	10.1	9.1
Nicotine (mg/cig)	0.9	0.92	0.97
Nicotine (mg/puff)	n.a.	0.118	0.123
NFDPM (mg/cig)	12.0	11.3	11.5
NFDPM (mg/puff)	n.a.	1.45	1.46
CO (mg/cig)	n.a.	12.4	13.1
CO (mg/puff)	n.a.	1.59	1.66
Puffs/cigarette	n.a.	7.8	7.9
Burning rate (mg tobacco/min)	n.a.	68.1	64.4

Note. Cig, cigarette.

In Vitro Mutagenicity Assays

Figures 1, 2, 3, and 4 summarize the results of Ames assays on test cigarettes from study 1 and 2 with and without metabolic activation. TA100, TA98, and TA1537 strains showed a positive response only with metabolic activation. No response was observed in TA 102 or TA1535. No sporadic responses in revertants were recorded. The highest sensitivity and specificity of the mutagenic response were observed using TA98 with metabolic activation. From the comparison of the data obtained for the test and reference cigarettes, it was concluded that the addition of ingredients did not result in a positive mutagenic response in any of the strains under the conditions already described. Hence, the use of the tested ingredients had no influence on the mutagenic activity of the cigarettes.

TABLE 5
Key parameters for laboratory control of prototype study 2 cigarettes

Parameter	Target	Run average	
		Test cigarette	Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	1.002	1.025
Standard deviation	—	0.0208	0.0173
Nontobacco weight	0.212	0.212	0.212
Net tobacco	0.800	0.790	0.813
Air dilution (%)	32	33.2	36.6
Standard deviation	—	1.6	1.4
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	50	47
Expanded tobacco (%)	9.5	9.6	9.3
Nicotine (mg/cig)	0.9	0.93	0.93
Nicotine (mg/puff)	n.a.	0.112	0.107
NFDPM (mg/cig)	12.0	11.4	11.0
NFDPM (mg/puff)	n.a.	1.37	1.26
CO (mg/cig)	n.a.	12.9	12.8
CO (mg/puff)	n.a.	1.55	1.47
Puffs/cigarette	n.a.	8.3	8.7

Note. Cig, cigarette.

Exposure Atmosphere Characterization

Tables 6 and 7 summarize the exposure data for the inhalation exposure periods for study 1 and study 2. The mean exposure concentrations (WTPM) were all within 3% of the target concentration, with CVs of 6.6%, or less. Nicotine and CO concentrations correlated well with WTPM in reference and test cigarette smoke atmospheres in both study 1 and study 2. Particle sizes were slightly larger in the study 1 test and reference cigarette smokes. All concentrations of the smoke from each cigarette were highly respirable for the rat model under investigation.

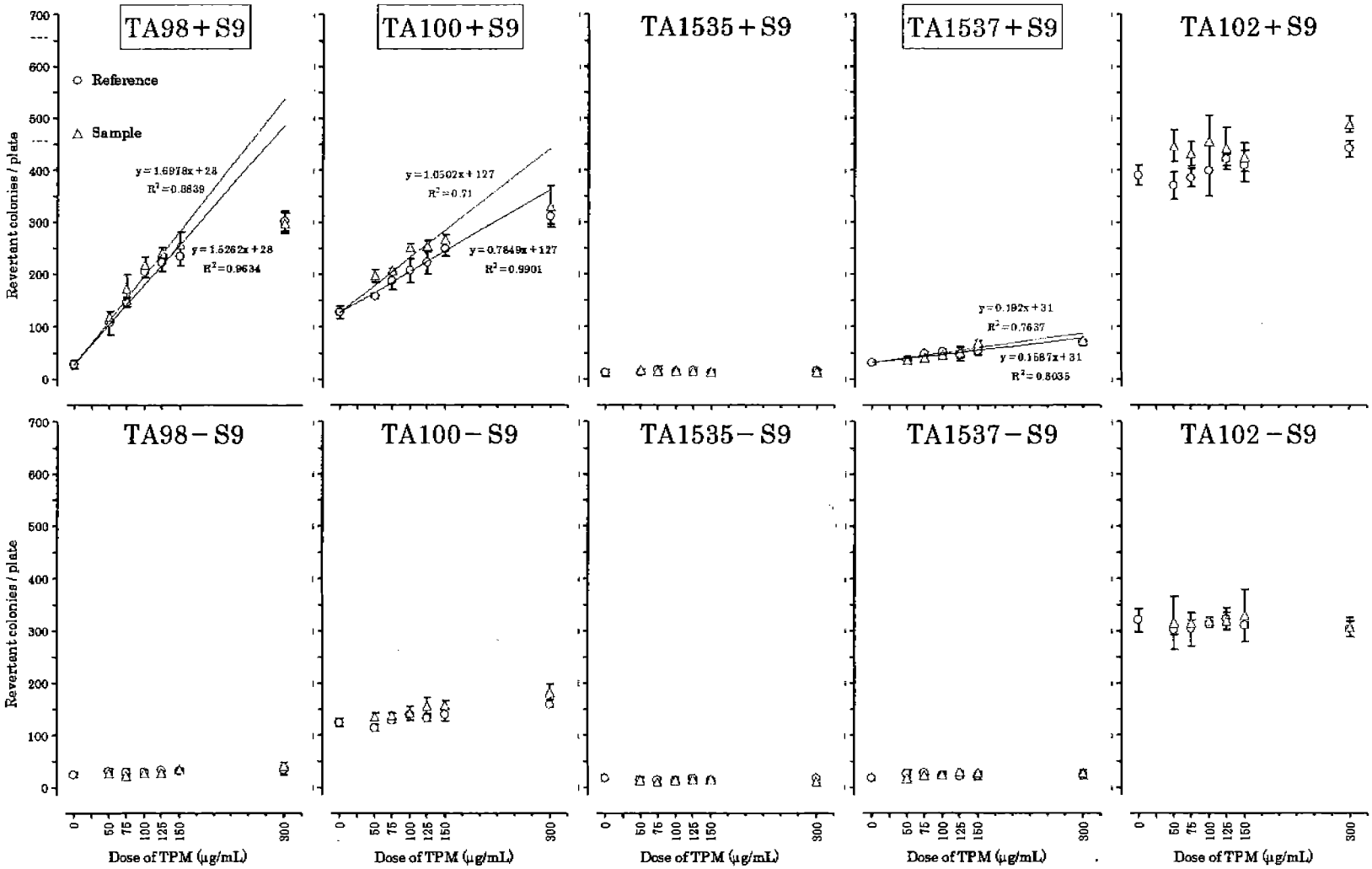
Body Weights and Clinical Observations

No significant mortality occurred in either study. Exposure-related adverse clinical signs were absent. Clinical observations noted were minor in consequence and low in incidence.

Mean body weight data for all groups on study throughout the exposure and recovery periods are illustrated in Figure 5. In study 1, mean body weights were consistently decreased compared to sham controls during the exposure period in male rats exposed to 0.8 mg/L of reference cigarette smoke and in males exposed to all 3 concentrations of test cigarette smoke. With the exception of day 71 (0.8 mg/L test), all female smoke-exposed groups in study 1 were comparable to sham control females throughout the study. In study 2, mean body weights were consistently decreased compared to sham controls in males exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.8 mg/L of reference cigarette smoke. Mean body weights of smoke-exposed groups were similar to sham control weights during the recovery period of both study 1 and study 2. The only consistent statistical difference in body weight changes between the test and reference cigarette smoke-exposed groups in either study was the decreased mean body weight in males exposed to 0.8 mg/L of reference cigarette smoke during the exposure period of study 1.

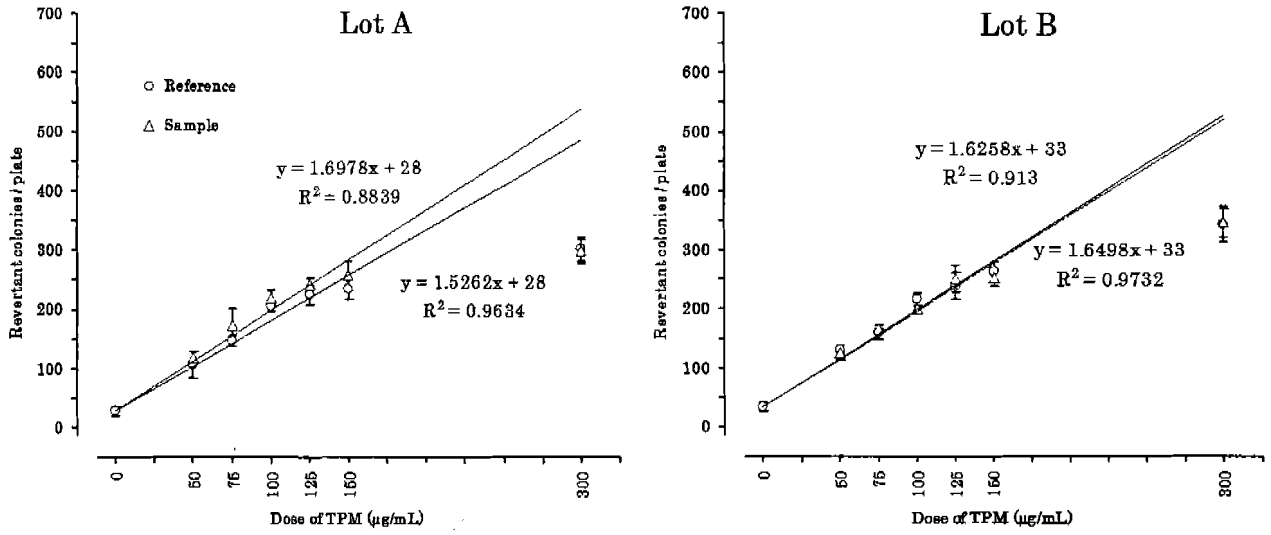
Organ Weights

Comparisons of selected group mean organ weights between smoke-exposed and sham controls in study 1 are presented in Table 8. Statistically significant differences in organ weights in groups of smoke-exposed rats were primarily low mean organ weights compared to their respective sham controls. There was no clear pattern of differences in any absolute or relative organ weight in smoke-exposed groups compared to sham controls, or in groups exposed to test versus reference cigarette smoke at either the interim sacrifice or the recovery sacrifices. Sham controls for the interim sacrifice of study 2 were inadvertently not fasted overnight prior to necropsy, which made comparison of absolute and relative organ weights of smoke-exposed and sham control groups from the interim sacrifice of questionable scientific value; thus these comparisons were not made for study 2. Statistical comparison of absolute and relative organ weights between groups exposed to test and reference cigarette smoke in study 2 showed very few statistically significant differences, none of which were considered toxicologically



N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot.

FIG. 1. Ames assay results, study 1 cigarettes.



MEAN ± SD of Specific Activity (50 to 150 µg/plate)

Reference	1576 ± 141.9	Reference.....	1734 ± 170.9
Sample.....	1783 ± 167.3	Sample.....	1703 ± 151.2

FIG. 2. Ames assay results, study 1 with TA98 metabolic activation.

significant. Comparison of organ weights in rats necropsied following the 13-wk recovery of study 2 indicated no consistent differences between sham control and smoke-exposed groups, or between groups exposed to similar concentrations of test and reference cigarette smoke.

Respiratory Physiology

Reductions in RR and/or TV resulted in consistently lower MV in rats exposed to test or reference cigarette smoke compared to sham controls in both study 1 and study 2. There was no consistent difference in MV between groups of rats exposed to test and reference cigarette smoke in either study. Because the overall MV in study 1 was similar among groups exposed to smoke, total inhaled mass was proportional to increasing smoke concentration in this study. In study 2, decreases in MV in groups exposed to 0.8 or 0.2 mg/L compared to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to the exposure concentration of inhaled smoke.

Clinical Pathology

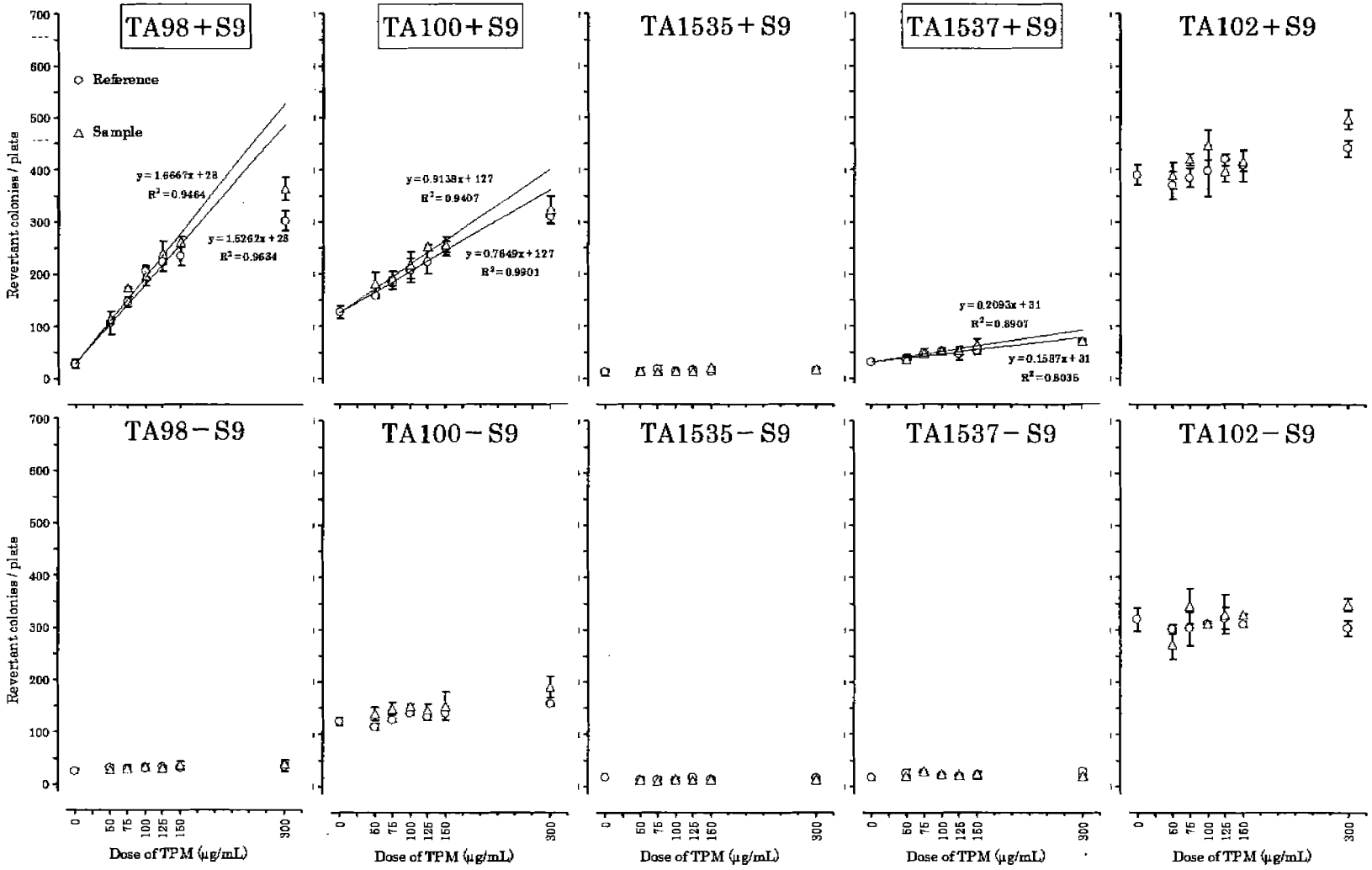
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 standard deviations of historic values for control Sprague-Dawley rats of comparable age. There were also statistically significant differences in several hematology and clinical chemistry parameters between groups exposed to similar concentrations of test and reference cigarette smoke. These differences are not considered to be of toxicologic significance, nor were they exposure related.

Whole-blood COHb levels were increased in a graded dose-response fashion as a function of exposure concentration for all test and reference cigarette smoke-exposed groups in both studies. In study 2 rats bled during exposure wk 2, there was a statistically significant decrease in COHb levels in both sexes exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.2 mg/L of test cigarette smoke, compared to groups exposed to reference cigarette smoke. There were no other clear differences in whole blood COHb levels between the test and reference cigarette groups at equivalent exposure levels in either study.

Plasma nicotine levels increased in a graded dose-response fashion for test and reference males and female groups in both studies. In study 2, test female groups exposed to 0.8 mg/L had significantly lower plasma nicotine levels than the 0.8 mg/L reference females at both 2- and 10-wk sampling. Comparing males to females at all exposure levels for test and reference cigarettes, the females consistently had higher plasma nicotine levels in both studies.

Pathology

Few gross lesions were observed in either study, with no evidence of changes attributable to exposure to smoke from the test



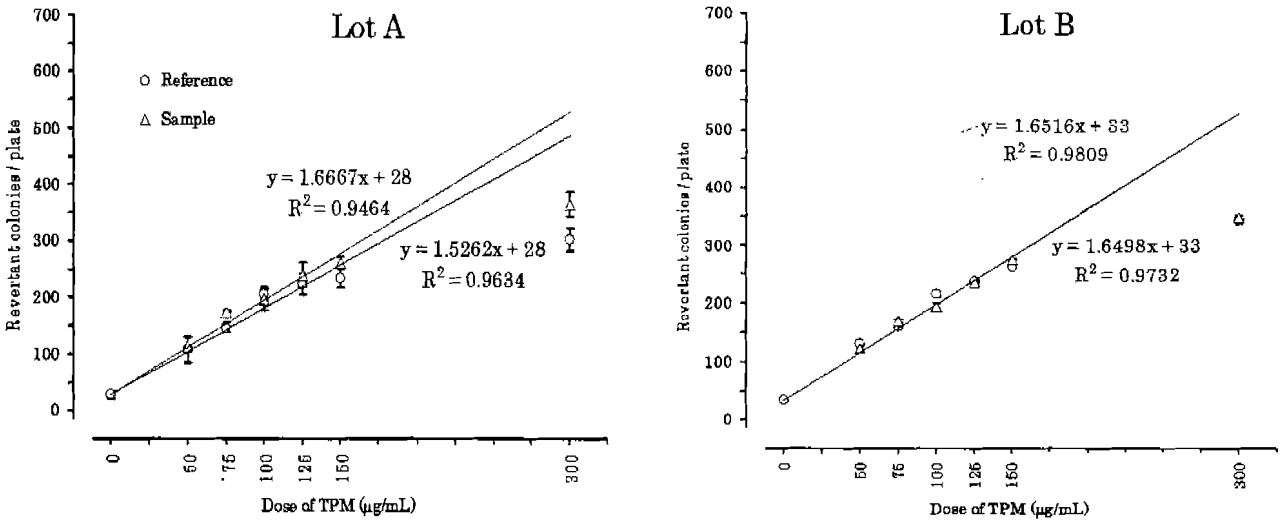
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

FIG. 3. Ames assay results, study 2 cigarettes.

TABLE 6
Study 1, exposure concentration data for rats exposed to mainstream smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 126$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 63$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.800	0.787 ± 0.035 (4.4)	68.2 ± 2.5 (3.7)	584 ± 27 (4.6)	98.4 ± 4.3	0.73 ± 0.08
0.200	0.199 ± 0.009 (4.5)	15.5 ± 1.0 (6.5)	144 ± 6 (4.2)	99.3 ± 4.3	0.74 ± 0.12
0.060	0.061 ± 0.004 (6.6)	4.4 ± 0.5 (11.4)	47 ± 3 (6.4)	101 ± 6	0.69 ± 0.09
Reference target exposure concentration (mg WTPM/L)					
0.800	0.795 ± 0.023 (2.9)	70.1 ± 2.1 (2.9)	608 ± 20 (3.3)	99.4 ± 2.7	0.74 ± 0.08
0.200	0.202 ± 0.004 (2.0)	15.8 ± 0.7 (4.5)	147 ± 4 (2.7)	101 ± 2	0.72 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.4 ± 0.4 (9.8)	50 ± 2 (4.8)	100 ± 4	0.74 ± 0.10

Note. CO, carbon monoxide; WTPM, wet total particulate matter.



MEAN±SD of Specific Activity (50 to 150 µg/plate)

Reference.....	1576±141.9	Reference.....	1734±170.9
Sample.....	1726±138.6	Sample-1.....	1701±107.9

FIG. 4. Ames assay results, study 2 cigarettes with TA98 metabolic activation.

TABLE 7
Study 2, exposure concentration data for rats exposed to smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 134$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 67$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.8	0.798 ± 0.040 (5.0)	56.8 ± 2.6 (4.6)	646 ± 34 (5.3)	100 ± 5	0.65 ± 0.01
0.2	0.194 ± 0.007 (3.6)	12.9 ± 0.6 (4.7)	158 ± 9 (5.7)	97 ± 4	0.62 ± 0.04
0.060	0.060 ± 0.002 (3.3)	4.0 ± 0.2 (5.0)	54 ± 3 (5.6)	100 ± 3	0.66 ± 0.03
Reference target exposure concentration (mg WTPM/L)					
0.8	0.784 ± 0.031 (4.0)	55.1 ± 2.3 (4.2)	676 ± 31 (4.6)	98 ± 4	0.57 ± 0.03
0.2	0.201 ± 0.004 (1.8)	13.0 ± 0.4 (3.4)	170 ± 15 (8.7)	100 ± 2	0.64 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.1 ± 0.2 (4.4)	57 ± 3 (5.8)	99 ± 3	0.66 ± 0.06

Note. CO, carbon monoxide; WTPM, wet total particulate matter.

or the reference cigarettes. Exposure to smoke from reference or test cigarettes in both studies induced concentration-related proliferative, metaplastic, and inflammatory microscopic lesions in the respiratory tract after 13 wk of exposure. The incidence of exposure-related respiratory-tract lesions observed at microscopic examination of tissues from rats necropsied at the interim sacrifice immediately following 13 wk of exposure is summarized in Table 9 for study 1 and Table 10 for study 2.

Hyperplasia of respiratory epithelium lining the anterior nasal cavity was present in all rats exposed to 0.8 mg/L in both studies, a few rats exposed to 0.2 mg/L in both studies, and in 3/40 rats exposed to 0.06 mg/L in study 1. Areas most severely and most frequently affected were the distal portions of the nasal and maxillary turbinates in sections of nose just caudal to the incisor teeth. In affected rats, the epithelium in the distal turbinates was up to six cells thick. There was also a clear dose response in the severity of nasal respiratory epithelial hyperplasia, with severity ranging from minimal to moderate. Comparison of incidence and severity data for nasal respiratory epithelial hyperplasia in rats exposed to similar concentrations of smoke from the test and reference cigarettes did not indicate any statistically significant differences in either study. Minimal goblet-cell hyperplasia was observed in the mucosal epithelium lining the median nasal septum in some smoke-exposed and sham control rats. Although not statistically significant compared to concurrent sham controls, the incidence of nasal goblet cell hyperplasia in male rats exposed to the 0.8-mg/L concentration of smoke from the reference cigarette or test cigarette in study 1 were considered to be toxicologically significant. There was no clear difference in the incidence of goblet cell hyperplasia between groups exposed to similar concentrations of reference and test cigarette smoke in either study.

Exposure to smoke from the reference or test cigarette in both study 1 and study 2 induced squamous metaplasia, hyperplasia, and hyperkeratosis of the transitional epithelium lining the base of the epiglottis and the epithelium lining the dorsal border of the ventral pouch and the adjacent laryngeal lumen. In control rats, the epithelium lining the base of the epiglottis was a mixture of ciliated columnar epithelium and slightly flattened, oval, rounded, or cuboidal cells one or two cells thick over a poorly defined basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, the base of the epiglottis was covered by a stratified squamous epithelium up to eight cells thick with a variably keratinized surface layer and a distinct basal cell layer. There was a concentration-related increase in severity of squamous metaplasia and hyperplasia of epiglottis epithelium in rats exposed to test or reference cigarette smoke. Statistical analysis did not indicate any significant differences in incidence or severity of these lesions between test and reference cigarette smoke-exposed groups in either study. Hyperkeratosis (accumulation of keratinized squamous cells on the surface) was observed in association with squamous metaplasia of the epithelium lining the base of the epiglottis in most rats exposed to smoke from reference or test cigarettes. Comparison of incidence/severity of hyperkeratosis in the epiglottis between test and reference cigarette smoke-exposed groups indicated a statistically

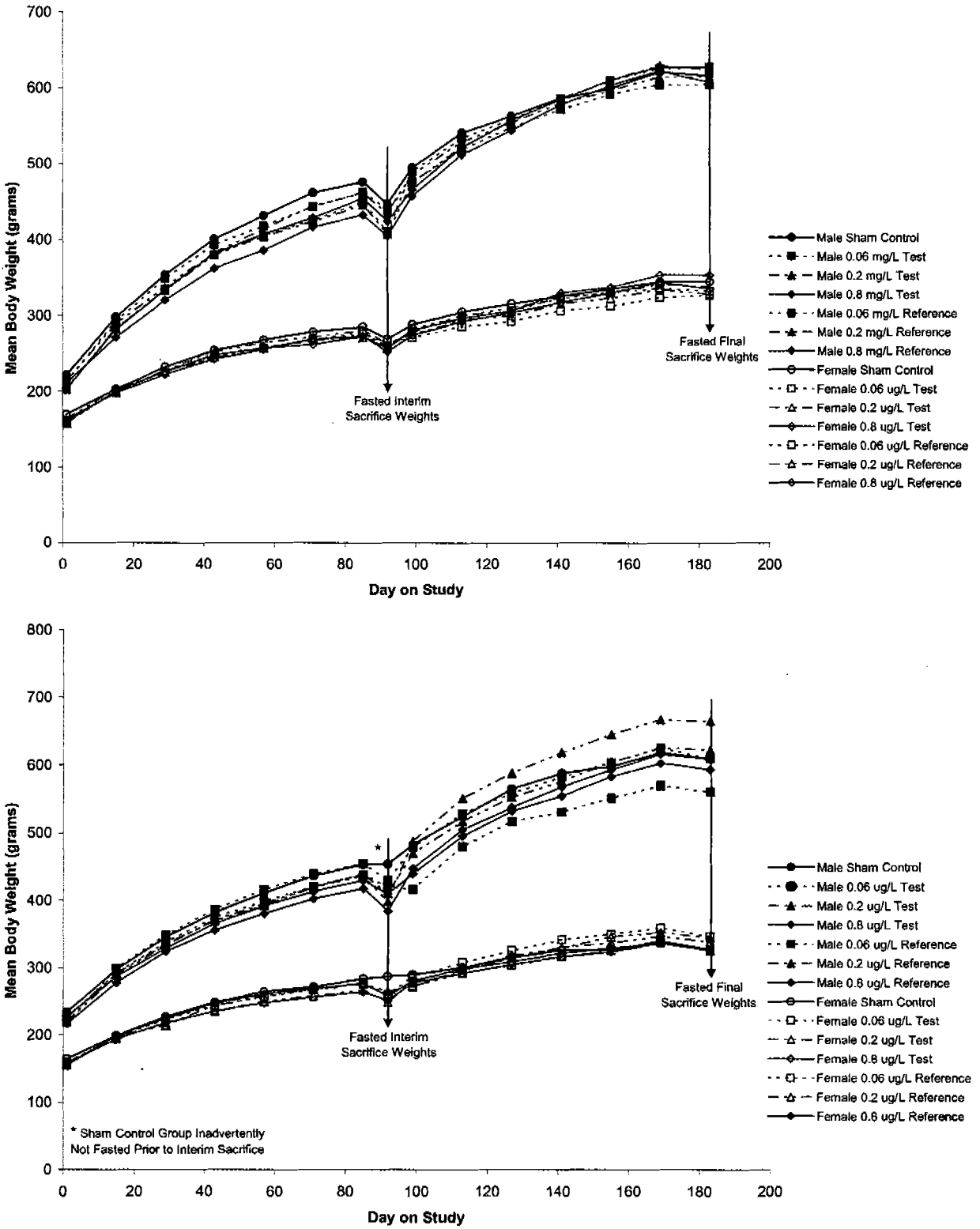


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

TABLE 8
Organ weights for rats exposed to smoke from study 1 cigarettes ($n = 20$, g ± SD)

		Test			Reference		
	Sham control	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L
Males							
Heart	1.60 ± 0.16	1.48 ± 0.15[a,b]	1.43 ± 0.16[a,c]	1.55 ± 0.15	1.60 ± 0.13	1.57 ± 0.16	1.52 ± 0.15
Kidneys	3.39 ± 0.33	3.17 ± 0.39	2.92 ± 0.30[a,c]	3.05 ± 0.33[a]	3.38 ± 0.33	3.20 ± 0.31	3.02 ± 0.27[a]
Lungs	1.95 ± 0.22	1.89 ± 0.17	1.82 ± 0.23[c]	1.93 ± 0.14	2.02 ± 0.28	1.98 ± 0.26	1.89 ± 0.15
Adrenals	0.066 ± 0.010	0.066 ± 0.012	0.059 ± 0.010	0.064 ± 0.012	0.062 ± 0.007	0.064 ± 0.008	0.063 ± 0.008
Females							
Heart	1.06 ± 0.09	1.02 ± 0.10	1.00 ± 0.10[c]	1.05 ± 0.12	1.03 ± 0.09	1.07 ± 0.09	1.09 ± 0.12
Kidneys	2.18 ± 0.21	2.02 ± 0.24	1.90 ± 0.19[a]	1.93 ± 0.18[a]	2.04 ± 0.21	1.99 ± 0.19[a]	1.95 ± 0.19[a]
Lungs	153 ± 0.13	1.50 ± 0.13	1.52 ± 0.17[c]	1.52 ± 0.15	1.55 ± 0.14	1.50 ± 0.17	1.60 ± 0.19
Adrenals	0.080 ± 0.010	0.081 ± 0.011	0.078 ± 0.008	0.082 ± 0.012	0.078 ± 0.008	0.080 ± 0.010	0.081 ± 0.013

[a] $p < .05$, Dunnett's t-test of significance, compared to sham control.
[b] $p < .05$, Dunnett's t-test of significance, compared to 0.06 reference group.
[c] $p < .05$, Dunnett's t-test of significance, compared to 0.2 reference group.

significant difference only in the 0.06-mg/L groups from study 1, in which females exposed to test cigarette smoke had a higher incidence/severity than females exposed to reference cigarette smoke. Chronic inflammation was present in the submucosa of the epiglottis in some rats exposed to reference or test cigarette smoke in study 1, most frequently in rats exposed to the 0.8 mg/L smoke concentration. Squamous metaplasia, hyperplasia, and hyperkeratosis were also present in the epithelium lining the opening of the ventral pouch and the adjacent laryngeal lumen in most rats exposed to smoke from the test or reference cigarette in both studies. In control rats, the epithelium lining the opening of the ventral pouch and adjacent laryngeal lumen was slightly flattened, oval, rounded, or cuboidal cells one or two cells thick with no discernible basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, this area was covered by a stratified squamous epithelium from three to six cells thick with a variably keratinized surface layer and a distinct basal cell layer. Comparison of incidence/severity of lesions at this site between test and reference cigarette smoke-exposed groups did not indicate any statistically significant differences in either study. Minimal or mild squamous metaplasia of the mucosal epithelium lining the caudal larynx was observed in 2/20 rats exposed to the 0.8 mg/L concentration of smoke from the test cigarette and 1/20 rats exposed to the 0.8 mg/L concentration of smoke from the reference cigarette in study 1.

Exposure to smoke from reference or test cigarettes induced a dose-related increase in minimal hyperplasia of the mucosal epithelium lining the tracheal lumen in both sexes of rats in study 1 and in males in study 2. Comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences in either study.

There were increased numbers of macrophages diffusely scattered through the pulmonary alveoli of rats exposed to smoke from reference or test cigarettes in both studies, compared to concurrent controls. There was some evidence of a dose response in the incidence and severity of macrophage accumulation in alveoli of smoke-exposed rats. This increase was graded as minimal in the vast majority of affected rats. Comparison of incidence and severity data for macrophages in alveoli of rats exposed to smoke from the test and reference cigarettes did not indicate any statistically significant differences. Minimal goblet-cell hyperplasia was observed in AB/PAS-stained sections of the mainstem bronchi of some rats exposed to smoke from reference or test cigarettes in both studies. There was some evidence of a dose response in the incidence of this lesion. Analysis of data indicated a statistically significant increase compared to controls in rats of both sexes exposed to the 0.8 mg/L concentration of smoke from reference cigarettes and in female rats exposed to the 0.8-mg/L concentration of smoke from the test cigarette in study 1, and in both sexes exposed to 0.8 mg/L of reference cigarette smoke in study 2. The incidence (7/20) of goblet-cell hyperplasia in males exposed to the 0.8-mg/L concentration of smoke from the test cigarette in both studies, although not statistically significant, was considered to be toxicologically significant. The incidence of bronchial goblet-cell hyperplasia was slightly higher in male rats exposed to smoke from reference cigarettes compared to similar concentrations of smoke from test cigarettes, but comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences. There was a very low incidence of a variety of microscopic lesions in other tissues examined in both studies, with no evidence of an effect of exposure to smoke from the reference or test cigarette on these tissues.

TABLE 9
Study 1, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	2 (0.2)	4 (0.3)	20 (2.2)	1 (0.1)	8 (0.4)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	6 (0.3)	3 (0.2)	9 (0.5)	5 (0.3)	5 (0.3)	10 (0.5)
Suppurative inflammation	2 (0.2)	2 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	9 (0.5)	20 (1.4)	19 (1.9)	16 (0.9)	20 (1.8)	20 (1.9)
Ventral pouch, squamous metaplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	9 (0.6)	19 (1.4)	1 (0.2)	17 (1.4)	18 (1.5)
Chronic inflammation	0 (0.0)	2 (0.1)	8 (0.4)	16 (0.9)	0 (0.0)	4 (0.2)	13 (0.7)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	6 (0.3)	6 (0.3)	18 (0.9)	5 (0.3)	12 (0.6)	16 (0.8)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	15 (0.8)	14 (0.7)	20 (1.4)	8 (0.4)	11 (0.6)	20 (1.1)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	7 (0.4)	3 (0.2)	4 (0.2)	11 (0.6)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	7 (0.4)	20 (2.0)	0 (0.0)	3 (0.2)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	2 (0.1)	2 (0.1)	7 (0.4)	2 (0.1)	2 (0.1)	4 (0.2)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	19 (1.4)[c]	20 (2.2)	20 (2.2)	13 (0.7)	20 (2.0)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	15 (1.3)	20 (1.8)	1 (0.1)	18 (1.5)	18 (1.5)
Chronic inflammation	0 (0.0)	3 (0.2)	2 (0.2)	10 (0.6)	0 (0.0)	4 (0.2)	17 (1.0)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	2 (0.1)	8 (0.4)	12 (0.6)	3 (0.2)	7 (0.4)	18 (0.9)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	10 (0.5)	13 (0.7)	20 (1.2)	12 (0.6)	17 (0.9)	20 (1.3)
Bronchi, goblet-cell hyperplasia	0 (0.0)	2 (0.1)	3 (0.2)	10 (0.5)	1 (0.1)	4 (0.2)	13 (0.7)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.
[c]$p < .05$, Kolmogorov–Smirnov test, compared to 0.06-mg/L reference group.

TABLE 10
Study 2, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	2 (0.1)	20 (2.0)	0 (0.0)	4 (0.2)	20 (1.9)
Goblet-cell hyperplasia	2 (0.1)	3 (0.2)	3 (0.2)	3 (0.2)	3 (0.2)	4 (0.2)	3 (0.2)
Suppurative inflammation	0 (0.0)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	6 (0.4)	15 (1.2)	20 (2.0)	13 (1.0)	20 (1.8)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.4)	16 (1.2)	0 (0.0)	5 (0.4)	16 (1.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	2 (0.1)	8 (0.4)	9 (0.5)	11 (0.6)	6 (0.3)	8 (0.4)	10 (0.5)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	4 (0.2)	11 (0.6)	16 (0.9)	20 (1.4)	11 (0.6)	14 (0.7)	20 (1.4)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	4 (0.2)	0 (0.0)	1 (0.1)	9 (0.5)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	4 (0.2)	20 (1.5)	0 (0.0)	4 (0.2)	20 (1.6)
Goblet-cell hyperplasia	3 (0.2)	3 (0.2)	5 (0.3)	5 (0.3)	5 (0.3)	2 (0.1)	8 (0.4)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, hyperkeratosis	0 (0.0)	16 (1.0)	20 (2.0)	20 (2.2)	15 (0.9)	20 (1.6)	20 (2.4)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	15 (1.2)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	14 (1.1)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.5)	18 (1.4)	0 (0.0)	9 (0.6)	20 (1.7)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	0 (0.0)	1 (0.1)	2 (0.1)	2 (0.1)	1 (0.1)	2 (0.1)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	9 (0.5)	10 (0.5)	19 (1.1)	10 (0.5)	10 (0.5)	17 (1.0)
Perivascular lymphoid infiltrate	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	0 (0.0)	7 (0.4)	3 (0.2)	4 (0.2)	10 (0.5)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.

Examination of tissue sections from rats necropsied at the end of the recovery period demonstrated nearly complete regression of nasal and tracheal lesions and a substantial decrease in the incidence and severity of smoke-induced lesions in the larynx and lungs in rats exposed to smoke from test or reference cigarettes in both studies. Macrophages observed in alveoli of smoke-exposed and control recovery group rats were in small focal aggregates, as opposed to the diffuse distribution of macrophages in lungs of rats necropsied at the interim sacrifice. There was no statistically significant difference in the incidence or severity of respiratory-tract lesions between recovery group rats previously exposed to similar concentrations of test and reference cigarette smoke in either study.

Evaluation of Cell Proliferation Rates

There was a dose-related trend toward higher mean nuclear labeling rates in the epithelium lining the median nasal septum in groups exposed to progressively higher concentrations of test or reference cigarette smoke compared to sham controls, but the increases were statistically significant only in females exposed to 0.8 mg/L of test cigarette smoke in study 1 and males exposed to 0.8 mg/L of reference cigarette smoke in study 2. Mean nuclear labeling rates of nasal epithelium lining the distal portions of the nasal and maxillary turbinates were statistically increased compared to control rates in both sexes of rats exposed to 0.8 mg/L of smoke from the test or reference cigarettes in both studies. Mean labeling rates in nasal and maxillary turbinates of study 1 males exposed to 0.8 mg/L of test cigarette smoke were statistically increased compared to labeling rates at these sites in males exposed to the same concentration of reference cigarette smoke.

Mean nuclear labeling rates in laryngeal epithelium were increased compared to sham control groups at all dose levels in both studies. Labeling rates in laryngeal epithelium were statistically different between several test and reference cigarette smoke-exposed groups in both studies, with no clear trend. The histopathology findings of laryngeal epithelial hyperplasia in smoke-exposed rats confirmed the relative sensitivity of these laryngeal sites to smoke-induced hyperplastic changes.

Mean nuclear labeling rates in the tracheal epithelium of rats exposed to smoke from test or reference cigarettes were not clearly different from those of sham controls of the same sex in either study. Labeling rates of bronchial, bronchiolar, and alveolar epithelium in both studies were difficult to evaluate due to wide standard deviations, low labeling rates, and variable sample sizes, and therefore labeling data from these sites were not used in evaluating effects of smoke exposure.

DISCUSSION

The studies described here were designed to evaluate the potential influence of ingredients on the chemical composition and the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against reference cigarettes identical except produced without flavors or casings. The configuration and ISO-condition tar, nicotine, and CO yields of all cigarettes investigated are representative of American blend cigarettes. Both test and reference cigarettes had the same tobacco blend and humectant composition (glycerine plus water) and were prepared by the same manufacturing process. Similarly, identical nontobacco materials (NTM) were used throughout. The weight of the filler remained constant between test and reference cigarettes. These studies illustrate that the application of 165 low-use flavoring or 8 high-use flavoring or casing ingredients had little, if any, observable effect on the deliveries or physical parameters of the cigarettes.

From comparison of the mutagenicity data obtained in Ames assays of studies 1 and 2 test and reference cigarettes, it was concluded that the addition of these ingredients did not increase the mutagenic response of any of the strains of *Salmonella typhimurium* under the conditions described, and the results did not suggest any mutagenic activity of the added ingredients.

The objectives of the two inhalation toxicity studies were to compare the biologic activity of mainstream smoke from the two test cigarettes with reference cigarettes in a series of two 13-wk inhalation exposures, each followed by a 13-wk recovery period. Data collected during the 13-wk exposures confirmed that both the particulate (WTPM, nicotine) and vapor (CO) phases of the inhalation atmospheres presented to the rats were well controlled and provided appropriate data for comparison of the responses of the study animals to smoke from the two cigarettes under investigation in each of the two studies. WTPM was used as the basis for exposure concentration in these studies, since the predominant known toxicologic effects of cigarette smoke are associated with the mainstream particulate phase (Coggins et al., 1980).

Blood COHb concentrations demonstrated that exposure of rats to smoke from either the test or reference cigarette resulted in reproducible biomarkers of exposure consistent with the concentration of CO in the smoke. Samples taken for plasma nicotine analysis confirmed exposure to nicotine in test or reference smoke, which resulted in exposure-related increases in plasma nicotine concentrations.

The only occurrence during either study that affected the utility of the data was the failure to fast the sham control rats prior to necropsy at the interim sacrifice immediately following the exposure period in study 2. This error did not allow direct comparison of the body and organ weights of controls with smoke-exposed groups sacrificed at that time point.

Other investigations have noted effects similar to those we observed of cigarette smoke exposure on body weight, including the relative resistance of females to this change (Coggins et al., 1989; Baker et al., 2004). We concluded that the decreased body weights in smoke-exposed groups in both studies compared to sham controls were the result of smoke exposure. However, we do not consider these effects on body weight to be toxicologically significant due to their recovery after smoke exposure was terminated, and due to the lack of any concurrent clinical observations that would indicate any significant dysfunction.

In study 1 there were a number of statistically significant differences in absolute or relative organ weights between test or reference cigarette smoke-exposed groups and sham controls necropsied immediately following 13 wk of smoke exposure. However, these statistical differences showed no clear dose-response pattern, and no exposure-related histopathologic effects were observed in any weighed organ except the lungs. It is possible that the increased lung/body weight ratios in study 1 rats exposed to 0.8-mg/L of smoke from test or reference cigarettes were related to the minimal increase in numbers of macrophages in alveoli of these rats. These increases in lung/body weight ratio more likely reflect the decreased body weight in these groups at the interim sacrifice. In any case, these and the other statistical differences in absolute or relative organ weights in smoke-exposed rats compared to sham controls are not considered toxicologically significant. There was no consistent difference in organ weights between groups of rats exposed to similar concentrations of test and reference cigarette smoke in either study. Increases in total inhaled mass were proportional to increasing exposure concentration in study 1, but in study 2 decreases in MV in groups exposed to 0.8- or 0.2-mg/L relative to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to exposure concentration of smoke.

Inhalation exposure to smoke from test or reference cigarettes in both studies clearly induced microscopic changes in the nasal cavity, larynx, trachea, and lungs of exposed rats. Results of histopathologic examination of the recovery groups illustrated that these respiratory-tract lesions were either completely resolved or in the process of resolving by 13 wk after cessation of smoke exposure, and thus represent an adaptive response to the inhaled smoke. The nasal cavity and larynx were much more affected by inhaled smoke than the lungs in our studies, and the mucosal epithelium lining the base of the epiglottis and adjacent ventral pouch was the most affected site. The extreme susceptibility of the rodent laryngeal mucosa to inhaled smoke and other xenobiotics has been described in detail (Lewis, 1980, 1991; Gopinath et al., 1987; Burger et al., 1989). Since the most notable cellular changes observed in the respiratory tract of rodents in response to inhaled smoke involve cellular proliferation and metaplasia, a quantitative measure of cell turnover in affected tissue is a useful tool to measure the effect of exposure. Cell proliferation rate measurements in nasal turbinates and laryngeal epithelium using nuclear labeling with BrdU correlated well with histopathology data, reinforcing the conclusion that exposure to smoke from test or reference cigarette smoke for 13 wk clearly induced epithelial hyperplasia at these sites. Results of BrdU labeling in the trachea and lungs were less clear, and probably reflect the more subtle effects of inhaled smoke on the epithelium at these sites.

The effects of inhaled cigarette smoke on the respiratory tract of rats in both the studies described herein are similar to those described in a number of previously reported cigarette smoke inhalation studies in rats (Dalbey et al., 1980; Gaworski et al., 1997; Coggins et al., 1989; Ayres et al., 2001; Vanscheeuwijck et al., 2002) and hamsters (Lewis, 1980; Wehner et al., 1990). Four recently published papers have described studies similar to those presented here, in which smokes from cigarettes with and without flavoring or casing ingredients were compared on the basis of chemical composition and biologic effects on rodents (Gaworski et al., 1998; Paschke et al., 2002; Carmines, 2002; Baker et al., 2004). Results of the studies presented here are consistent with the conclusions of these authors that the presence of flavoring and casing ingredients studied to date did not significantly change the type or extent of toxicologic effects observed in rodents inhaling cigarette smoke.

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Flavouring Group Evaluation 52 (FGE.52): Consideration of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (57th meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated by EFSA in FGE.20 (2005) (Commission Regulation (EC) No 1565/2000 of 18 July 2000)

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission

(Question No EFSA-Q-2008-032C)

(Adopted on 3 July 2007)

PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise 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 Scientific Panel is requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The present consideration concerns 44 hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (57th meeting) and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 35 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated in the Flavouring Group Evaluation 20 (FGE.20).

The Panel concluded that the 44 substances in the JECFA flavouring group of hydroxy- and alkoxy-substituted benzyl derivatives are structurally related to the group of benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated by EFSA in the FGE.20.

Further two substances were evaluated by the JECFA in this group but are not in the Register (2-methoxybenzoic acid and ethyl vanillin propylene glycol acetal) and therefore not dealt with in this consideration.

The Panel agrees with the application of the Procedure as performed by the JECFA for 43 of the 44 substances considered in this FGE. For butyl 4-hydroxybenzoate [FL-no: 09.754] additional data would be required before it can be evaluated as a flavouring substance, using the Procedure.

For eight substances [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075] the JECFA evaluation is only based on Maximised Survey-derived Daily Intake MSDI values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For all 44 substances use levels are needed to calculate the modified Theorical Added Maximum Daily Intake (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 44 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications are available for 40 of the 44 JECFA evaluated substances. For four substances [FL-no: 06.132, 09.087, 09.751 and 09.763] further information on specifications are requested.

Thus, for 12 substances [FL-no: 04.093, 06.132, 08.071, 08.076, 08.092, 09.087, 09.145, 09.751, 09.754, 09.763, 09.807 and 16.075] the Panel has reservations (only USA production volumes available and/or missing data on specifications and/or isomerism/composition). For one of these 12 substances, butyl 4-hydroxybenzoate [FL-no: 09.754], the Panel concluded that additional data would be required before it can be evaluated as a flavouring substance using the Procedure. For the remaining 32 JECFA evaluated hydroxy- and alkoxy-substituted benzyl derivatives [FL-no: 02.128, 02.165, 02.213, 04.094, 05.015, 05.016, 05.017, 05.018, 05.019, 05.047, 05.055, 05.056, 05.091, 08.040, 08.043, 08.112, 09.019, 09.035, 09.058, 09.220, 09.430, 09.706, 09.713, 09.714, 09.748, 09.749, 09.750, 09.752, 09.753, 09.796, 09.811 and 09.933] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substance” based on the MSDI approach.

KEYWORDS

Hydroxy- and alkoxy-substituted benzyl derivatives, JECFA 57th meeting, FGE.20, butyl 4-hydroxybenzoate, butyl paraben.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) 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 2006/252/EC (EC, 2006). 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, 2000), which is broadly based on the opinion of the Scientific Committee on Food (SCF, 1999).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2006, during its 55th, 57th, 59th, 61st, 63rd and 65th meetings, the JECFA evaluated about 900 substances which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000). These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC (EC, 1999a) and its consecutive amendments.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), hereafter named the “EFSA Procedure”. This Procedure is based on the opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b) hereafter named the “JECFA Procedure”. The Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

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. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate 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.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?") (JECFA, 1999b).

In line with the opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of the JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 46 flavouring substances consisting of hydroxy- and alkoxy-substituted benzyl derivatives (JECFA, 2002b). Two of these are not in the Register (2-methoxybenzoic acid and ethyl vanillin propylene glycol acetal). This consideration will therefore only deal with 44 JECFA evaluated substances. Butyl 4-hydroxybenzoate [FL-no: 09.754] has been evaluated as a flavouring substance by the JECFA at its 59th meeting where it was concluded that butyl 4-hydroxybenzoate was of no safety concern at the current intakes as a flavouring substance (JECFA, 2003a). In 2006 the JECFA has also considered butyl 4-hydroxybenzoate as a food additive and concluded that: "*The reproductive toxicity of the parabens appears to increase with increasing length of the alkyl chain, and there are specific data showing adverse reproductive effects in male rats of butyl paraben. In view of this and the fact that butyl paraben was not included in the group ADI for parabens, the Committee decided to withdraw the specifications for this substance"* (JECFA, 2007b).

1.1.2. EFSA Considerations

The Panel concluded that all the 44 substances in the JECFA flavouring group of hydroxy- and alkoxy-substituted benzyl derivatives are structurally related to the group of benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated by EFSA in the Flavouring Group Evaluation 20 (FGE.20).

1.2. Isomers

1.2.1. JECFA Status

The substance [FL-no: 06.132] in the group of JECFA evaluated hydroxy- and alkoxy- substituted benzyl derivatives has two chiral centres.

1.2.2. EFSA Considerations

Information is lacking about the stereoisomerism for [FL-no: 06.132].

1.3. Specifications

1.3.1. JECFA Status

JECFA specifications are available for all 44 substances (JECFA, 2001c; JECFA, 2002d). See Table 1. For one substance, p-Anisyl formate [FL-no: 09.087], the JECFA has reservations. Although a JECFA specification is available for butyl 4-hydroxybenzoate as a flavouring substance (JECFA, 2002d), the JECFA has withdrawn the specification for butyl 4-hydroxybenzoate as a food additive at its 67th meeting in 2006 (see Section 1.1.1).

1.3.2. EFSA Considerations

The available specifications are considered adequate except that information on stereoisomerism is missing for [FL-no: 06.132], see Section 1.2. For [FL-no: 09.087 and 09.751] further information on the composition is requested and for [FL-no: 09.763] an ID test is missing.

2. Intake Estimations

2.1. JECFA Status

For 36 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1. For the eight remaining substances [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075] production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for eight substances, MSDI values for the EU cannot be calculated for these [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075].

3. Genotoxicity Data

3.1. Genotoxicity Studies - Text Taken from the JECFA (JECFA, 2002a)

In vitro

The hydroxy- and alkoxy-substituted benzyl derivatives were not mutagenic in standard assays for reverse mutation with plate incorporation and/or preincubation in *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA2637, at concentrations ranging up to those that are cytotoxic or at maximum test concentrations recommended by ICH/OECD, in the absence and presence of metabolic activation (S9) (White et al., 1977; Sasaki & Endo, 1978; Douglas et al., 1980; Florin et al., 1980; Kawachi et al., 1980a; Kawachi et al., 1980b; Nestmann et al., 1980; Rapson et al., 1980); and (Kasamaki et al., 1982; Pool & Lin, 1982; Sekizawa & Shibamoto, 1982; Haworth et al., 1983; Wild et al., 1983; Ball et al., 1984; Ishidate et al., 1984; Haresaku et al., 1985; Nagabhushan & Bhide, 1985); and (Mortelmans et al., 1986; Fujita & Sasaki, 1987; Heck et al., 1989; Watanabe & Morimoto, 1989c; Dillon et al., 1992; Müller et al., 1993; King & Harnasch, 1997; Dillon et al., 1998)). An assay for mutation in *S.*

typhimurium strain TA1535/pSK1002, in which *umu* gene expression was the end-point, gave negative results with salicylaldehyde [FL-no: 05.055] (Nakamura et al., 1987). Assays for mutation or DNA repair in *Escherichia coli* strains WP2 *uvrA*, WP2s, CSH26/pYM3, CSH26/pSK1002, PQ37, and Sd-4-73 with methyl anisate [FL-no: 09.713], vanillyl alcohol [FL-no: 02.213], vanillin [FL-no: 05.018], vanillyl butyl ether [FL-no: 04.093], and piperonal [FL-no: 05.016] (Szybalski, 1958; Sekizawa & Shibamoto, 1982; Ohshima et al., 1989; Watanabe & Morimoto, 1989c; Takahashi et al., 1990), and *Saccharomyces cerevisiae* strains D3, D4, D7, and XV185-14C with veratraldehyde [FL-no: 05.017] (Nestmann & Lee, 1983) also gave negative results.

Mixed results were obtained with the hydroxy- and alkoxy-substituted benzyl derivatives in the assay for DNA repair in *Bacillus subtilis* strains H17 and M45 for *rec* mutation, both positive and negative results being reported for piperonal [FL-no: 05.016] and negative results for *para*-methoxybenzaldehyde [FL-no: 05.015], vanillin [FL-no: 05.018], ethyl vanillin [FL-no: 05.019], and methyl salicylate [FL-no: 09.749] (Oda et al., 1979; Kawachi et al., 1980a; Kawachi et al., 1980b; Sekizawa & Shibamoto, 1982). Some of the differences in the results were apparently laboratory-specific. Oda et al. (Oda et al., 1979) reported only negative results with some of the same compounds; however, the studies were reported in Japanese with English abstracts and could not be fully evaluated for methodological or other differences. It was not clear whether cytotoxicity was a factor in the results. No mutations were observed in silkworms treated with methylsalicylate [FL-no: 09.749] (Kawachi et al., 1980a; Kawachi et al., 1980b).

Both negative and positive results were obtained in assays in isolated mammalian cells with some of the hydroxy- and alkoxy-substituted benzyl derivatives. Mixed results were reported with *para*-methoxybenzaldehyde and vanillin in assays for sister chromatid exchange in several Chinese hamster cell lines and in human lymphocytes (Jansson et al., 1986; Jansson & Zech, 1987; Sasaki et al., 1987; Jansson et al., 1988). Negative results were obtained in this assay with ethyl vanillin [FL-no: 05.019], salicylaldehyde [FL-no: 05.055], and methyl salicylate [FL-no: 09.749] (Kawachi et al., 1980a; Kawachi et al., 1980b; Sasaki et al., 1987; Jansson et al., 1988). Similarly, mixed results were obtained in assays for chromosomal aberration in Chinese hamster and human cell lines with *para*-methoxybenzaldehyde [FL-no: 05.015], vanillin [FL-no: 05.018], ethyl vanillin [FL-no: 05.019], piperonal [FL-no: 05.016], and methyl salicylate [FL-no: 09.749] (Kawachi et al., 1980a; Kawachi et al., 1980b; Kasamaki et al., 1982; Ishidate et al., 1984; Kasamaki & Urasawa, 1985; Jansson & Zech, 1987). The results in the assays for sister chromatid exchange and chromosomal aberrations were generally obtained independently of the presence or absence of metabolic activation. Mixed, but mostly positive, results were obtained with veratraldehyde [FL-no: 05.017], *para*-methoxybenzaldehyde [FL-no: 05.015], and ethyl vanillin [FL-no: 05.019] in the assay for forward mutation in L5178Y mouse lymphoma cells, both with and without metabolic activation (Garberg et al., 1988; Wangenheim & Bolcsfoldi, 1988; Heck et al., 1989). Vanillin [FL-no: 05.018] and piperonal [FL-no: 05.016] were inactive in this assay (Heck et al., 1989). Vanillin weakly induced micronuclei in human Hep-G2 cells, with only a moderate response at the highest concentration tested (Sanyal et al., 1997). No unscheduled DNA synthesis was observed in rat hepatocytes exposed to veratraldehyde [FL-no: 05.017], vanillin [FL-no: 05.018], or ethyl vanillin [FL-no: 05.019] (Heck et al., 1989). Piperonal [FL-no: 05.016] caused unscheduled DNA synthesis in one test, but the finding could not be confirmed in subsequent tests (Heck et al., 1989), and the result was considered to be questionable.

para-Methoxybenzaldehyde [FL-no: 05.015] or benzaldehyde alone did not induce strand breaks in supercoiled DNA from the phage PM2, although positive results were reported with both substances

in the presence of $CuCl_2$. The finding that the effect depended on the concentration of copper suggests that DNA-damaging species are produced during redox reactions of aromatic (and aliphatic) aldehydes with $CuCl_2$ (Becker et al., 1996).

Numerous assays for anti-mutagenicity have been conducted *in vitro* with some of the hydroxy- and alkoxy-substituted benzyl derivatives, including evaluations in several sub-mammalian and mammalian cell lines. Anti-mutagenic activity was reported with *para*-methoxybenzaldehyde [FL-no: 05.015] and ethyl vanillin [FL-no: 05.019] (Ohta et al., 1986b; Imanishi et al., 1990; Ohta, 1995). Mixed results were reported with vanillin [FL-no: 05.018] (Takahashi et al., 1990; Tamai et al., 1992; Sanyal et al., 1997). Analysis of the concentrations, test organisms, and study methods did not provide an explanation for the discrepant results in these studies. No anti-mutagenic effect was observed with piperonal [FL-no: 05.016] or methyl salicylate [FL-no: 09.749] (Ohta et al., 1983; Ohta et al., 1986a; Ohta et al., 1986b).

In vivo

The hydroxy- and alkoxy-substituted benzyl derivatives were inactive in all assays *in vivo* in mammals given the compounds orally or by intraperitoneal injection at doses that were significant fractions of the reported lethal doses. Micronuclei were not induced by *para*-ethoxybenzaldehyde [FL-no: 05.056] at a dose of 1005 mg/kg bw, ethyl vanillin [FL-no: 05.019] at 1000 mg/kg bw, vanillin [FL-no: 05.018] at 500 mg/kg bw, or piperonyl acetate [FL-no: 09.220] at 620 mg/kg bw (Wild et al., 1983; Furukawa et al., 1989). Piperonal [FL-no: 05.016] administered by intraperitoneal injection at 1000 mg/kg bw caused a slight increase in the number of early fetal deaths as compared with the incidence in control mice; however, the authors reported that the result was not statistically significant, and no similar finding was reported after administration by oral gavage (Epstein et al., 1972).

In assays for sex-linked recessive lethal mutation in fruit flies (*Drosophila melanogaster*), negative results were obtained with *para*-ethoxybenzaldehyde [FL-no: 05.056], ethyl vanillin [FL-no: 05.019], and piperonyl acetate [FL-no: 09.220] after feeding at concentrations of 751, 8309, and 4855 µg/ml, respectively (Wild et al., 1983). Vanillin [FL-no: 05.018] induced an anti-mutagenic response in fruit flies, and both vanillin and *para*-methoxybenzaldehyde [FL-no: 05.015] were anti-mutagenic in mice (Imanishi et al., 1990; Sasaki et al., 1990b; de Andrade et al., 1992). The data on vanillin, including the results *in vitro*, suggest some anti-mutagenic activity, although the relevance of this finding is questionable and impossible to extrapolate to the low concentrations to which persons are likely to be exposed from its use as a flavour in food.

Conclusion on genotoxicity

The hydroxy- and alkoxy-substituted benzyl derivatives did not have mutagenic activity in bacterial or other submammalian cellular systems. Mixed results were obtained in an assay for DNA repair in bacteria and in assays for clastogenicity in isolated mammalian cells. These findings probably reflect the known activity of alcohols or aldehydes in biological systems, as they were seen both with and without metabolic activation, and cytotoxicity was often a limitation at high concentrations. Negative results were obtained in tests for genotoxicity in mice and *Drosophila in vivo*. In a 2-year study in mice, no difference in tumour incidence from that in controls was found in groups fed doses up to 900 mg/kg bw per day of butyl-*para*-hydroxybenzoate [FL-no: 09.754] (Inai et al., 1985). The Committee therefore concluded that the hydroxy- and alkoxy-substituted benzyl derivatives do not have genotoxic potential *in vivo.*

For a summary of *in vitro / in vivo* genotoxicity data considered by the JECFA see Table 2.1.

3.2. Genotoxicity Studies - Text Taken from EFSA (EFSA, 2006e)

In vitro

Data from *in vitro* tests are available for eight candidate substances [FL-no: 09.631, 09.367, 05.129, 05.158, 08.080, 05.153, 08.087 and 02.205] and 29 supporting substances. Data from *in vivo* tests are available for two candidate substances [FL-no: 09.367 and 08.080] and for ten supporting substances.

All the seven candidate substances [FL-no: 09.631, 09.367, 05.129, 05.142, 08.080, 05.153, and 08.087] tested for bacterial gene mutations gave negative results. For five candidate substances [FL-no: 09.367, 05.129, 05.158, 08.080, and 08.087] both positive and/or negative results were reported in various other *in vitro* test systems (Rec assay, chromosomal aberration test, SCE and mammalian cell gene mutation assay (mouse lymphoma tests)) for most of which the validity cannot be evaluated or are known to be of very limited relevance.

The same situation was observed for the supporting substances. All the available bacterial gene mutation assays on supporting substances gave negative results. For fourteen of these substances, both positive and negative results were reported in other *in vitro* test systems (Rec assay, chromosomal aberration test, SCE and mammalian cell gene mutation assay) for most of which, however, the validity cannot be evaluated.

In vivo

The available *in vivo* studies on candidate substances reported negative results for ethyl 4-hydroxybenzoate [FL-no: 09.367] in a chromosome aberration assay in rat bone marrow cells and for gallic acid [FL-no: 08.080] in a bioassay in the rat liver. However, due to very limited details on method and results the validity of these studies cannot be evaluated.

The Panel noted that benzyl acetate was positive in an *in vivo* Comet assay, which may indicate a genotoxic activity at high dose levels. The study was considered of limited validity. However, all other *in vivo* studies with benzyl acetate are negative and several of these studies, among which an UDS-test in the liver and a mouse bone marrow micronucleus test were considered to be of good quality (NTP, 1993d). Additionally, in the long term carcinogenicity studies with benzyl acetate, no carcinogenic effects were observed in mice and rats after administration via the diet (NTP, 1993d). In a previous study by NTP (NTP, 1986c) in which this substance was administered by gavage in corn oil, concern was raised in particular about pancreatic tumours in rats, but for these tumours a confounding influence of the vehicle was suspected. In two other genotoxicity studies, specifically aiming at the determination of benzyl acetate-induced DNA damage (UDS test and alkaline elution assay) in rat pancreas, no indications of a genotoxic effect were obtained although these studies were of limited or inassessible validity. Taking all this information into account, the Panel considered the positive result from the *in vivo* Comet assay as insufficient ground to preclude the evaluation of benzyl acetate via the Procedure.

Furthermore, all the studies carried out with ten different supporting substances among which were benzyl alcohol, benzyl acetate and benzaldehyde, give no indication of a genotoxic potential *in vivo* in several studies for different genetic endpoints and by different routes of administration.

Conclusion on genotoxicity:

While some of the *in vitro* studies indicated equivocal weak positive or positive results, considering the weight of evidence from candidate and supporting substances and the *in vivo* studies the Panel concluded no safety concern with respect to genotoxicity of the substances in the present flavouring group.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA see Table 2.2 and 2.3.

3.3. EFSA Considerations

The Panel considered that while some of the *in vitro* studies indicated equivocal weak positive or positive results, the weight of evidence from candidate and supporting substances and the *in vivo* studies do not preclude evaluation of the 44 JECFA evaluated hydroxy- and alkoxy- substituted benzyl derivatives through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 44 Hydroxy- and Alkoxy-substituted Benzyl Derivatives Evaluated by JECFA (JECFA, 2002a):

According to the JECFA 35 of the substances belong to structural class I and nine to structural class II using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded 40 of the 44 flavouring substances at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for the substances are below the thresholds for structural classes I and II (step A3).

The four remaining substances [FL-no: 05.016, 05.018, 05.019 and 09.749] were concluded at step A5 – i.e. the intakes are above the threshold for the structural class, the substances are not endogenous, but a NOAEL is available that can provide an adequate margin of safety to the estimated intake of the substances.

In conclusion, the JECFA evaluated all 44 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 44 substances are summarised in Table 3.1: Summary of Safety Evaluation of 44 Hydroxy- and Alkoxy-Substituted Benzyl Derivatives (JECFA, 2002b).

4.2. Application of the Procedure to 35 Benzyl Alcohols, Benzaldehydes, a Related Acetal, Benzoic Acids, and Related Esters Evaluated by EFSA (EFSA, 2006e):

Thirty-three of the flavouring substances are classified into structural class I, one is classified into structural class II and one is classified into structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The Panel concluded all of the 35 flavouring substances at step A3 in the EFSA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for structural classes I, II and III respectively (step A3).

In conclusion the Panel considered that the 35 substances evaluated through the Procedure were of no safety concern at the estimated levels of intake based on the MSDI approach.

The stepwise evaluations of the 35 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA, 2006e).

4.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA at its 57th meeting (JECFA, 2002a) for 43 of the 44 substances in the group of hydroxy- and alkoxy-substituted benzyl derivatives.

More recent studies on butyl 4-hydroxybenzoate [FL-no: 09.754] considered in the EFSA opinion on methyl, ethyl and propyl 4-hydroxybenzoates, evaluated as food additives, have demonstrated that in juvenile rats given dietary doses of approximately 10, 100 or 1000 mg/kg body weight (bw) per day for eight weeks, effects were observed on male reproductive organs, sperm parameters or sex hormones at all doses (EFSA, 2004b; JECFA, 2007b). In juvenile mice given dietary doses of butyl 4-hydroxybenzoate of 15-1500 mg/kg bw per day for ten weeks, effects on sperm counts and serum concentrations of testosterone were observed (JECFA, 2007b). As no NOAEL could be demonstrated for these effects on male reproductive parameters in rodents the Panel concluded that additional data would be required before butyl 4-hydroxybenzoate [FL-no: 09.754] can be evaluated as a flavouring substance using the Procedure.

5. Conclusion

The Panel concluded that the 44 substances in the JECFA flavouring group of hydroxy- and alkoxy-substituted benzyl derivatives are structurally related to the group of benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated by EFSA in the Flavouring Group Evaluation 20 (FGE.20).

Further two substances were evaluated by the JECFA in this group but are not in the Register (2-methoxybenzoic acid and ethyl vanillin propylene glycol acetal) and therefore not dealt with in this consideration.

The Panel agrees with the application of the Procedure as performed by the JECFA for 43 of the 44 substances considered in this FGE. For butyl 4-hydroxybenzoate [FL-no: 09.754] additional data would be required before it can be evaluated as a flavouring substance, using the Procedure.

For eight substances [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075] the JECFA evaluation is only based on MSDI values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For all 44 substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 44 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications are available for 40 of the 44 JECFA evaluated substances. For three substances [FL-no: 06.132, 09.087 and 09.751] further information on the composition is requested for and for one substance [FL-no: 09.763] an ID test is missing.

Thus, for 12 substances [FL-no: 04.093, 06.132, 08.071, 08.076, 08.092, 09.087, 09.145, 09.751, 09.754, 09.763, 09.807 and 16.075] the Panel has reservations (only USA production volumes

available and/or missing data on specifications and/or isomerism/composition). For one of these 12 substances, butyl 4-hydroxybenzoate [FL-no: 09.754], the Panel concluded that additional data would be required before it can be evaluated as a flavouring substance using the Procedure. For the remaining 32 JECFA evaluated hydroxy- and alkoxy-substituted benzyl derivatives [FL-no: 02.128, 02.165, 02.213, 04.094, 05.015, 05.016, 05.017, 05.018, 05.019, 05.047, 05.055, 05.056, 05.091, 08.040, 08.043, 08.112, 09.019, 09.035, 09.058, 09.220, 09.430, 09.706, 09.713, 09.714, 09.748, 09.749, 09.750, 09.752, 09.753, 09.796, 09.811 and 09.933] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substance” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

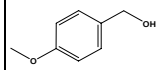
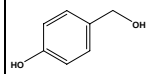
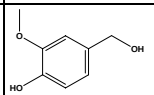
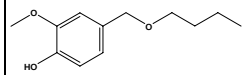
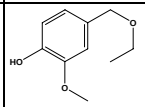
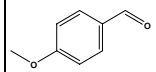
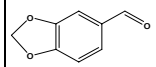
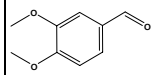
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
02.128 871	p-Anisyl alcohol		2099 66 105-13-5	Liquid $C_8H_{10}O_2$ 138.17	Insoluble Miscible	259 24-25 IR 97 %	1.540-1.547 1.107-1.115	
02.165 955	4-Hydroxybenzyl alcohol		3987 623-05-2	Solid $C_7H_8O_2$ 124.14	Slightly soluble Soluble	n.a. 110-112 IR 99 %	n.a. n.a.	
02.213 886	Vanillyl alcohol		3737 690 498-00-0	Solid $C_8H_{10}O_3$ 154.17	Soluble Soluble	n.a. 115 IR 98 %	n.a. n.a.	According to JECFA: Boiling point is "n/a (decomposes at the melting point)".
04.093 888	Butyl vanillyl ether		3796 82654-98-6	Liquid $C_{12}H_{18}O_3$ 210.27	Insoluble Miscible	241 IR 95 %	1.511-1.521 1.048-1.068	
04.094 887	Ethyl 4-hydroxy-3-methoxybenzyl ether		3815 13184-86-6	Liquid $C_{10}H_{14}O_3$ 182.22	Insoluble Miscible	212 NMR 98 %	1.528-1.532 1.106-1.113	
05.015 878	4-Methoxybenzaldehyde		2670 103 123-11-5	Liquid $C_8H_8O_2$ 136.15	Poorly soluble Miscible	248 IR 97 %	1.568-1.574 1.115-1.123	
05.016 896	Piperonal		2911 104 120-57-0	Solid $C_8H_6O_3$ 150.13	Slightly soluble Freely soluble	263 37 IR 98 %	n.a. n.a.	
05.017 877	Veratraldehyde		3109 106 120-14-9	Solid $C_9H_{10}O_3$ 166.18	Insoluble Soluble	281 42-45 IR 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

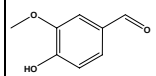
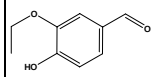
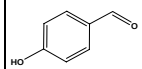
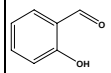
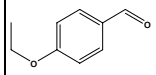
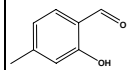
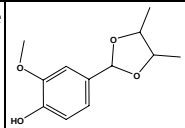
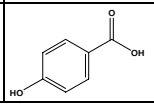
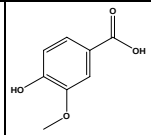
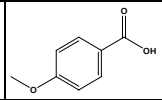
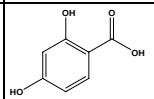
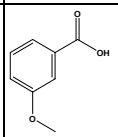
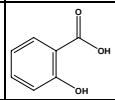
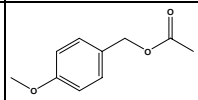
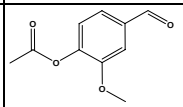
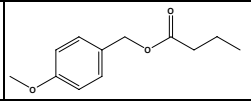
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
05.018 889	Vanillin		3107 107 121-33-5	Solid $C_8H_8O_3$ 152.15	Slightly soluble Freely soluble	285 80-81 IR 97 %	n.a. n.a.	
05.019 893	Ethyl vanillin		2464 108 121-32-4	Solid $C_9H_{10}O_3$ 166.18	Insoluble Very soluble	285 78 IR 98 %	n.a. n.a.	
05.047 956	4-Hydroxybenzaldehyde		3984 558 123-08-0	Solid $C_7H_6O_2$ 122.12	Slightly soluble Freely soluble	n.a. 116 IR 99 %	n.a. n.a.	According to JECFA: Melting point is "116° [sublimes at atmospheric pressure]".
05.055 897	Salicylaldehyde		3004 605 90-02-8	Liquid $C_7H_6O_2$ 122.12	Slightly soluble Miscible	196-197 IR 95 %	1.570-1.576 1.159-1.170	
05.056 879	4-Ethoxybenzaldehyde		2413 626 10031-82-0	Liquid $C_9H_{10}O_2$ 150.18	Poorly soluble Miscible	250 IR 97 %	1.556-1.564 1.078-1.084	According to JECFA: Boiling point is "250 (minimum)".
05.091 898	2-Hydroxy-4-methylbenzaldehyde		3697 2130 698-27-1	Solid $C_8H_8O_2$ 136.15	Insoluble Freely soluble	207 57 IR 98 %	n.a. n.a.	
06.132 960	Vanillin butan-2,3-diol acetal (mixture of stereo isomers) 6)		4023 63253-24-7	Solid $C_{12}H_{16}O_4$ 224.26	Insoluble Soluble	n.a. 48-52 IR NMR MS 95 %	n.a. n.a.	CASrn does not specify stereoisomers.
08.040 957	4-Hydroxybenzoic acid		3986 693 99-96-7	Solid $C_7H_6O_3$ 138.12	Slightly soluble Freely soluble	n.a. 213-214 IR 99 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

FL-no JECFA-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)	EFSA comments
08.043 959	Vanillic acid		3988 697 121-34-6	Solid $C_8H_8O_4$ 168.15	Slightly soluble Soluble	n.a. 210-212 IR 99 %	n.a. n.a.	
08.071 883	p-Anisic acid		3945 10077 100-09-4	Solid $C_8H_8O_3$ 152.15	Soluble Freely soluble	275-280 184 IR 98 %	n.a. n.a.	
08.076 908	2,4-Dihydroxybenzoic acid		3798 89-86-1	Solid $C_7H_6O_4$ 154.12	Soluble Soluble	n.a. 225 IR 97 %	n.a. n.a.	According to JECFA: Melting point is "225° (decomposes, rapid heating required)".
08.092 882	3-Methoxybenzoic acid		3944 586-38-9	Solid $C_8H_8O_3$ 152.15	Soluble Freely soluble	170-172 107-109 IR 98 %	n.a. n.a.	
08.112 958	Salicylic acid		3985 10165 69-72-7	Solid $C_7H_6O_3$ 138.12	Very slightly soluble Very soluble	211 (26 hPA) 158-160 IR 99 %	n.a. n.a.	
09.019 873	p-Anisyl acetate		2098 209 104-21-2	Liquid $C_{10}H_{12}O_3$ 180.20	Insoluble Miscible	235 IR 97 %	1.511-1.517 1.104-1.111	
09.035 890	Vanillyl acetate		3108 225 881-68-5	Solid $C_{10}H_{10}O_4$ 194.19	Slightly soluble Soluble	148 (13 hPa) 77-79 IR 97 %	n.a. n.a.	
09.058 875	p-Anisyl butyrate		2100 286 6963-56-0	Liquid $C_{12}H_{16}O_3$ 208.26	Insoluble Miscible	270 IR 97 %	1.500-1.505 1.047-1.067	

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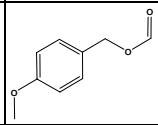
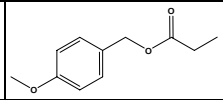
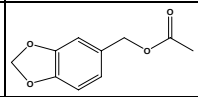
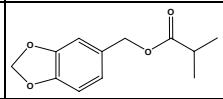
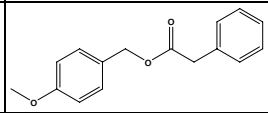
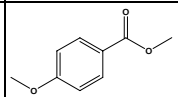
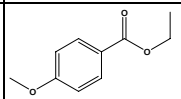
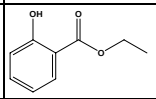
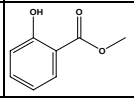
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
09.087 872	p-Anisyl formate 9)		2101 354 122-91-8	Liquid $C_9H_{10}O_3$ 166.18	Insoluble Miscible	220 IR 90 %	1.519-1.525 1.136-1.145	According to JECFA: "Minimum assay value is 90%".
09.145 874	p-Anisyl propionate		2102 426 7549-33-9	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	100-103(0.7hPa) IR 97 %	1.505-1.510 1.070-1.086	
09.220 894	Piperonyl acetate		2912 2068 326-61-4	Liquid $C_{10}H_{10}O_4$ 194.19	Insoluble Miscible	150-151 (13hPa) IR 97 %	1.523-1.529 1.227-1.239	
09.430 895	Piperonyl isobutyrate		2913 305 5461-08-5	Liquid $C_{12}H_{14}O_4$ 222.24	Insoluble Miscible	91-92(0.007hPa) IR 97 %	1.506-1.513 1.154-1.160	
09.706 876	Anisyl phenylacetate		3740 233 102-17-0	Liquid $C_{16}H_{16}O_3$ 256.30		370 IR 97 %	1.553-1.563 1.125-1.133	
09.713 884	Methyl 4-methoxybenzoate		2679 248 121-98-2	Solid $C_9H_{10}O_3$ 166.18	Very slightly soluble Soluble	255 48 IR 97 %	n.a. n.a.	
09.714 885	Ethyl 4-methoxybenzoate		2420 249 94-30-4	Liquid $C_{10}H_{12}O_3$ 180.20	Insoluble Miscible	270 IR 97 %	1.522-1.528 1.101-1.105	
09.748 900	Ethyl salicylate		2458 432 118-61-6	Liquid $C_9H_{10}O_3$ 166.18	Slightly soluble Miscible	234 IR 98 %	1.518-1.525 1.125-1.131	
09.749 899	Methyl salicylate		2745 433 119-36-8	Liquid $C_8H_8O_3$ 152.15	Slightly soluble Miscible	222 IR 98 %	1.534-1.538 1.176-1.185	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

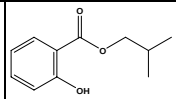
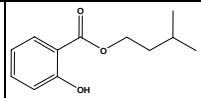
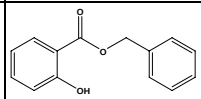
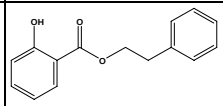
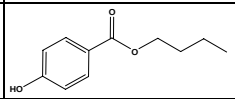
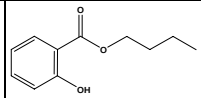
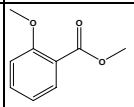
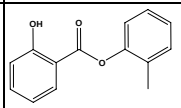
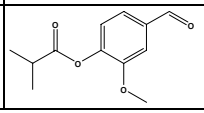
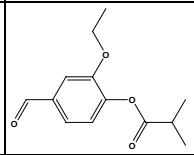
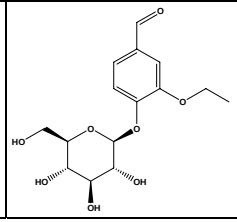
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
09.750 902	Isobutyl salicylate		2213 434 87-19-4	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	260 IR 98 %	1.506-1.570 1.062-1.069	
09.751 903	Isopentyl salicylate 9)		2084 435 87-20-7	Liquid $C_{12}H_{16}O_3$ 208.26	Insoluble Miscible	277 IR 98 %	1.504-1.509 1.046-1.055	According to JECFA: Min. assay value is "98 (sum of isoamyl and amyl salicylate)".
09.752 904	Benzyl salicylate		2151 436 118-58-1	Liquid $C_{14}H_{12}O_3$ 228.25	Insoluble Miscible	300 24-26 IR 98 %	1.573-1.584 1.173-1.183	
09.753 905	Phenethyl salicylate		2868 437 87-22-9	Solid $C_{15}H_{14}O_3$ 242.28	Insoluble Soluble	190 (7 hPa) 44 IR 98 %	n.a. n.a.	
09.754 870	Butyl 4-hydroxybenzoate		2203 525 94-26-8	Solid $C_{11}H_{14}O_3$ 194.23	Insoluble Soluble	156-157 (5 hPa) 67-70 IR 98 %	n.a. n.a.	
09.763 901	Butyl salicylate		3650 614 2052-14-4	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	268 98 %	1.508-1.520 1.070-1.080	ID 7).
09.796 880	Methyl 2-methoxybenzoate		2717 2192 606-45-1	Liquid $C_9H_{10}O_3$ 166.18	Very slightly soluble Miscible	246 IR 97 %	1.529-1.537 1.144-1.160	
09.807 907	o-Tolyl salicylate		3734 617-01-6	Solid $C_{14}H_{12}O_3$ 228.25	Insoluble Soluble	180 (3 hPa) NMR 99 %	1.576-1.584 1.164-1.174	
09.811 891	Vanillin isobutyrate		3754 20665-85-4	Liquid $C_{12}H_{14}O_4$ 222.24	Insoluble Miscible	130-132 (3 hPa) IR 98 %	1.522-1.526 1.110-1.136	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

FL-no JECFA-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)	EFSA comments
09.933 953	Ethyl vanillin isobutyrate		3837 188417-26-7	Solid $C_{13}H_{16}O_4$ 236.27	Insoluble Freely soluble	 57 IR 97 %	n.a. n.a.	
16.075 892	Ethyl vanillin beta-D-glucopyranoside		3801	Solid $C_{15}H_{20}O_8$ 328.32	Slightly soluble Slightly soluble	n.a. 199-200 NMR 99 %	n.a. n.a.	CASrn to be included in the Register: 122397-96-0. According to JECFA: Boiling point is "n/a (decomposes on heating)".

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.

6) Stereoisomeric composition not specified.

7) ID: Missing identification test.

8) MP: Missing melting point.

9) Composition of mixture not specified.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro / in vivo*) for 44 Hydroxy- and Alkoxy-Substituted Benzyl Derivatives (JECFA, 2002a)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

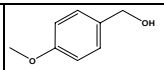
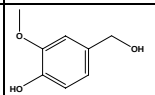
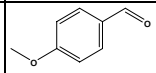
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
02.128 871	p-Anisyl alcohol Anisyl alcohol		Reverse mutation (plate incorporation)	*S. typhimurium* TA100	≥ 500 mg/plate	Negative[c]	(Ball et al., 1984)
02.213 886	Vanillyl alcohol		SOS DNA repair	*Escherichia coli* PQ37	Not reported	Positive[c]	(Ohshima et al., 1989)
05.015 878	4-Methoxybenzaldehyde p-Methoxybenzaldehyde		Reverse mutation (preincubation)	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	5000 mg/plate[a]	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA98, TA100	≥ 500 mg/plate	Negative[b]	(Kasamaki et al., 1982)
			Chromosomal aberration	Chinese hamster fibroblasts	500 mg/ml[a]	Negative[c]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	*S. typhimurium* TA102, TA97	≥ 1000 mg/plate	Negative[b]	(Fujita & Sasaki, 1987)
			Mutation	*B. subtilis* H17, M45	22 mg/disc	Negative[c]	(Oda et al., 1979)
			Reverse mutation	*S. typhimurium* TA102	5000 mg/plate	Negative[b]	(Müller et al., 1993)
			Reverse mutation	*S. typhimurium* TA100	≥ 1000 mg/plate	Negative	(Rapson et al., 1980)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 470 mg/ml 540–780 mg/ml	Negative Positive[c]	(Wangenheim & Bolcsfoldi, 1988)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	410 mg/plate	Negative[b]	(Florin et al., 1980)
			Chromosomal aberration	Chinese hamster B241 cell line	0.0068 mg/ml	Positive[b]	(Kasamaki et al., 1982)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

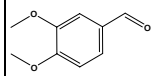
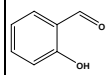
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Mutation	Phage PM2	1400 mg/ml	Negative	(Becker et al., 1996)
			Sister chromatid exchange	Human lymphocytes	≥ 270		
			DNA alkaline unwinding	Mouse lymphoma L5178Y/TK$^{+/-}$ cells	≥ 820 mg/ml 960–1100 mg/ml	Negative[b] Positive[b]	(Garberg et al., 1988)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	≥ 14 mg/ml	Negative	(Sasaki et al., 1987)
05.017 877	Veratraldehyde		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	8000 mg/plate	Negative[b]	(Nestmann et al., 1980)
			Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	8000 mg/plate	Negative[b]	(Douglas et al., 1980)
			Mutation	*Saccharomyces cerevisiae* D7, XV185-14C	Not reported	Negative[c]	(Nestmann & Lee, 1983)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 6666 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	1000 mg/plate[a]	Negative[b]	(Heck et al., 1989)
			Forward mutation	Mouse lymphoma L5178Y cells	1400 mg/ml[a]	Positive[b]	(Heck et al., 1989)
			Reverse mutation (preincubation)	*S. typhimurium* TA100, TA102, TA104, TA1538, TA982	Not reported	Negative[b]	(Dillon et al., 1992)
			Reverse mutation (preincubation)	*S. typhimurium* TA100, TA102, TA104	33–3300 mg/plate	Negative[b]	(Dillon et al., 1998)
			Unscheduled DNA synthesis	Rat hepatocytes	100 mg/ml[a]	Negative	(Heck et al., 1989)
05.055 897	Salicylaldehyde		Mutation	*S. typhimurium* TA1535/pSK1002	110 mg/ml	Negative[b]	(Nakamura et al., 1987)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	370 mg/plate	Negative[b]	(Florin et al., 1980)
			Reverse mutation (preincubation)	*S. typhimurium* TA98, TA100	Not reported	Negative[b]	(Sasaki & Endo, 1978)
			Sister chromatid exchange	Human lymphocytes	≥ 61 mg/ml	Negative[d]	(Jansson et al., 1988)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

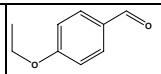
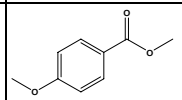
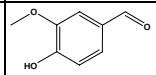
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
05.056 879	4-Ethoxybenzaldehyde p-Ethoxybenzaldehyde		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3600 mg/plate	Negative[b]	(Wild et al., 1983)
09.713 884	Methyl 4-methoxybenzoate Methyl anisate		Mutation	*Escherichia coli* Sd-4-73	Not reported	Negative[c]	(Szybalski, 1958)
05.018 889	Vanillin		Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	10 000 mg/plate[a]	Negative[b]	(Heck et al., 1989)
			Mutation	*B. subtilis* H17, M45	21 mg/disc	Negative[c]	(Oda et al., 1979)
			Chromosomal aberration	Chinese hamster fibroblasts	1000 mg/ml	Negative[c]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	5000 mg/plate	Negative[b]	(Pool & Lin, 1982)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 10 000 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Mutation	*Escherichia coli* Sd-4-73	Not reported	Negative[c]	(Szybalski, 1958)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Not reported	Negative[b]	(Nagabhushan & Bhide, 1985)
			Reverse mutation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	10 000 mg/plate[a]	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA100	≥ 1000 mg/plate	Negative	(Rapson et al., 1980)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 1500 mg/ml[a]	Negative[b]	(Heck et al., 1989)
			Mutation	*Escherichia coli* CSH26/pYM3, CSH26/pSK1002	≥ 15 000 mg/ml	Negative	(Takahashi et al., 1990)
			Reverse mutation	*S. typhimurium* TA98, TA100	≥ 1000 mg/plate	Negative[b]	(Kasamaki et al., 1982)
			Chromosomal aberration	Chinese hamster B241 cells	≥ 0.006 mg/ml	Negative	(Kasamaki & Urasawa, 1985)
			Sister chromatid exchange	Human lymphocytes	0–150 mg/ml	Positive	(Jansson et al., 1986)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

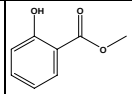
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Mitotic gene conversion	*S. cerevisiae*	10 000 mg/ml	Negative	(Rosin, 1984)
			Chromosomal aberration	Chinese hamster V79 lung cells	15 000–150 000 mg 300 000 mg	Negative[c] Positive[c]	(Tamai et al., 1992)
			Chromosomal aberration	Human lymphocytes	$\geq$ 610 mg/ml	Negative	(Jansson & Zech, 1987)
			Chromosomal aberration	Chinese hamster B241 cell line	0.003 mg/ml	Negative	(Kasamaki et al., 1982)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	$\geq$ 15 mg/ml	Negative	(Sasaki et al., 1987)
			Sister chromatid exchange	Human lymphocytes	150–300 mg/ml	Positive	(Jansson & Zech, 1987)
			Unscheduled DNA synthesis	Rat hepatocytes	500 mg/ml[a]	Negative	(Heck et al., 1989)
			SOS DNA repair	*Escherichia coli* PQ37	Not reported	Positive[c]	(Ohshima et al., 1989)
			Micronucleus formation	Human hepatoma (Hep-G2) cells	50 mg/ml 500 mg/ml	Negative Positive	(Sanyal et al., 1997)
09.749 899	Methyl salicylate		Chromosomal aberration	Hamster lung fibroblasts	Not reported	Positive[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Mutation	*B. subtilis* H17, M45	23 mg/disc	Negative[c]	(Oda et al., 1979)
			Chromosomal aberration	Chinese hamster fibroblasts	250 mg/ml[a]	Negative[c]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	10 000 mg/plate	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	$\geq$ 330 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA100, TA98	Not reported	Negative[b]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Mutation	*B. subtilis* H17, M45	Not reported	Negative[b]	(Kawachi et al., 1980a; Kawachi et al., 1980b)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

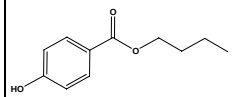
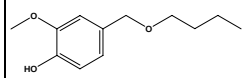
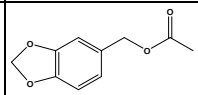
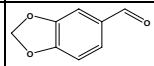
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Chromosomal aberration	Human embryo fibroblasts	Not reported	Negative[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Sister chromatid exchange	Human embryo fibroblasts	Not reported	Negative[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Mutation	Silkworm	Not reported	Negative[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
09.754 870	Butyl 4-hydroxybenzoate		Chromosomal aberration	Chinese hamster fibroblasts	60 mg/ml[a]	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	S. typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	1000 mg/platec	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation	S. typhimurium TA98, TA100	< 1000 mg/plate	Negative[b]	(Haresaku et al., 1985)
04.093 888	Butyl vanillyl ether		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98	5000 mg/plate	Negative[b]	(Watanabe & Morimoto, 1989c)
			Mutation	*Escherichia coli* WP2 *uvr*A	5000 mg/plate	Negative[b]	(Watanabe & Morimoto, 1989c)
09.220 894	Piperonyl acetate		Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 3300 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3600 mg/plate	Negative[b]	(Wild et al., 1983)
05.016 896	Piperonal		Reverse mutation (histidine substitution)	*Escherichia coli* WP2*uvr*A*trp*$^{-}$	2400 mg	Negative[b]	(Sekizawa & Shibamoto, 1982)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	10 000 mg/plate[a]	Negative[b]	(Heck et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100	0.05–5000 mg/plate	Negative[b]	(Kasamaki et al., 1982)
			Reverse mutation	*S. typhimurium* TA1537, TA1538, TA98, TA100	≥ 5000 mg/plate	Negative[b]	(White et al., 1977)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

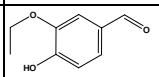
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Mutation	*B. subtilis* H17, M45	20 mg/disc	Negative[c]	(Oda et al., 1979)
			Reverse mutation	*S. typhimurium* TA100, TA1535, TA98, TA1537, TA1538	2400 mg	Negative[b]	(Sekizawa & Shibamoto, 1982)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA1537, TA98, TA100	≥ 10 000 mg/plate	Negative[b]	(Haworth et al., 1983)
			Unscheduled DNA synthesis	Rat hepatocytes	500 mg/ml	Positive	(Heck et al., 1989)
			Chromosomal aberration	Chinese hamster B241 cell line	0.075 mg/ml	Positive	(Kasamaki et al., 1982)
			Chromosomal aberration	Chinese hamster B241 cell line	≥ 0.15 mg/ml	Negative	(Kasamaki & Urasawa, 1985)
			Mutation	*B. subtilis* H17/M45	5000 mg/disc	Positive[c]	(Sekizawa & Shibamoto, 1982)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 1000 mg/ml	Negative[b]	(Heck et al., 1989)
05.019 893	Ethyl vanillin		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	≥ 3600 mg/plate	Negative[b]	(Wild et al., 1983)
			Mutation	*B. subtilis* H17, M45	21 mg/disc	Negative[c]	(Oda et al., 1979)
			Chromosomal aberration	Chinese hamster fibroblasts	250 mg/ml[a]	Positive[c]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 8000 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	10 000 mg/plate[a]	Negative[b]	(Ishidate et al., 1984)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 1000 mg/ml 800 mg/ml	Negative[d] Weakly positive[c]	(Heck et al., 1989)
			Reverse mutation (preincubation)	*S. typhimurium* TA97, TA102	≥ 1000 mg/plate	Negative[b]	(Fujita & Sasaki, 1987)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	10 000 mg/plate	Negative[b]	(Heck et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	200 mg/ml	Negative	(Heck et al., 1989)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

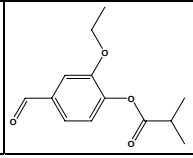
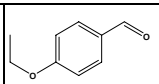
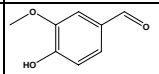
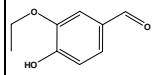
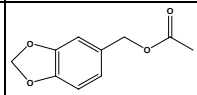
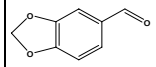
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Sister chromatid exchange	Human lymphocytes	≥ 330 mg/ml	Negative[c]	(Jansson et al., 1988)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	≥ 17 mg/ml	Negative	(Sasaki et al., 1987)
09.933 953	Ethyl vanillin isobutyrate		Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	≥ 5000 mg/plate	Negative[b]	(King & Harnasch, 1997)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vivo							
05.056 879	4-Ethoxybenzaldehyde p-Ethoxybenzaldehyde		Sex-linked recessive lethal mutation	*Drosophila melanogaster*	750 mg/ml	Negative	(Wild et al., 1983)
			Micronucleus formation	NMRI mice	≥ 1000 mg/kg bw	Negative	(Wild et al., 1983)
05.018 889	Vanillin		Micronucleus formation	Male BDF1 mice	500 mg/kg bw	Negative	(Inouye et al., 1988)
05.019 893	Ethyl vanillin		Sex-linked recessive lethal mutation	*D. melanogaster*	8300 mg/ml	Negative	(Wild et al., 1983)
			Micronucleus formation	Male BDF1 mice	Not reported	Negative	(Furukawa et al., 1989)
			Micronucleus formation	NMRI mice	1000 mg/kg bw	Negative	(Wild et al., 1983)
09.220 894	Piperonyl acetate		Sex-linked recessive lethal mutation	*D. melanogaster*	4900 mg/ml	Negative	(Wild et al., 1983)
			Micronucleus formation	NMRI mice	≥ 970 mg/kg bw	Negative	(Wild et al., 1983)
05.016 896	Piperonal		Dominant lethal mutation	ICR/Ha Swiss mice	≥ 620 mg/kg bw[e]	Negative	(Epstein et al., 1972)
			Dominant lethal mutation	ICR/Ha Swiss mice	1000 mg/kg bw[f]	Negative	(Epstein et al., 1972)

a Highest dose if result was negative; lowest active dose if result was positive.

b Without metabolic activation.

c With and without metabolic activation.

d With metabolic activation.

e Administered by intraperitoneal injection.

f Administered by oral gavage.

Table 2.2: Genotoxicity (*in vitro*) EFSA / FGE.20

Substances listed in brackets are JECFA-evaluated substances

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Benzyl alcohol [02.010])	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (plate incorporation method)	*S. typhimurium* TA100	1000 µg/plate	Negative[2]	(Ball et al., 1984)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[2]	(Rogan et al., 1986)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	6666 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmole/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	50,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate	Negative[2]	(Milvy & Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate	Negative[1]	(NTP, 1989)	Valid study in accordance with OECD guideline 471 (except that only four strains were used). Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Mutation assay	*Escherichia coli* WP2 *uvr*A	1000 to 8000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	10 µg/disc	Weakly positive	(Kuroda et al., 1984b)	Study published in Japanese with English abstract. Data extracted from figure. Validity of the study cannot be evaluated. Inhibition of growth was reported.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Weakly positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						evaluated. A weak positive result (i.e. 4 mm≤ D<8 mm). was reported (D=5 mm). No information on the use of metabolic activation.
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg /ml[4] (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Equivocal[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 473. Lowest effective dose was 4000 µg/ml with and without S9. No dose-response observed. Positive results were not reproducible in all trials. Absence of cytotoxicity reported up to the highest dose.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Negative[2] Weakly positive[3]	(NTP, 1989)	Valid study in accordance with OECD guideline 473. A positive result was reported only in the presence of S9 at relatively high concentrations of 4000 µg/ml in 3 of 4 tests carried out with harvest times between 12 and 18 hours. No data on cytotoxicity reported.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 5000 µg/ml	Weakly positive	(NTP, 1989)	Valid study in accordance with OECD guideline 479. Dose-related increase in frequency of SCE at concentrations from 500 - 1250 µg/ml (without metabolic activation) and 500 - 4000 µg/ml (with metabolic activation). No data on cytotoxicity reported. Number of chromosomes per cell reduced at 4000 µg/ml with S9.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 1250 µg/ml[2] 16 to 4000 µg/ml[3]	Weakly positive[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 479. Significant increase (20%) in SCE only at the highest doses. No dose-response observed. No second trial using high concentrations to reproduce the positive effects performed. Absence of cytotoxicity reported up to the highest dose.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Up to 5000 µg/ml	Questionable	(McGregor et al., 1988a; Myhr et al., 1990)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing performed). Positive responses observed in some experiments at concentrations of 3500 and higher. No dose-response was observed. The highest concentration was letal in some experiments. Positive and negative responses could not be reproduced in all experiments.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	150 to 5000 µg/ml	Negative[3] Positive[2]	(NTP, 1989)	Valid study in accordance with OECD guideline 476. In one of three trials without S9 a positive result (relative mutant fraction ≥1.6) was reported at 4500 µg/ml with relative total growth of 20%. The concentration of 5000 µg/ml was letal in

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						this trial, whereas in another one of three trials without S9 3500 µg/ml was letal.
	Mutation assay	*E. coli* WP2 *uvr*A	Not reported	Negative	(Kuroda et al., 1984a)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Cytotoxicity assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Rat hepatocytes	10 mM	Negative	(Storer et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	DNA damage assay	*E. coli* P3478	50 µl/disc	Negative[1]	(Fluck et al., 1976)	
(Benzyl formate [09.077])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm≤ D<8 mm).was reported (D=4 mm). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	500 to 4000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
(Benzyl acetate [09.014])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4≤ D<8).was reported (D could not clearly be determined). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	250 to 2000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells; Human lymphoblast TK6 cells	Mouse cells 0, 250, 500, 1000 µg/ml; Human cells 0, 500, 1000, 1250, 1500 µg/ml	Negative[2] Positive[3]	(Caspary et al., 1988)	Published non-GLP study in accordance with OECD guideline 476 (except that no colony sizing was performed). Thus, the study is considered not fully valid. The lowest significantly effective doses in the presence of S9 were 500 µg/ml in mouse cells and 1500 µg/ml in human cells. Cytotoxicity was reported above 500 µg/ml with and without S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0-1600 µl/ml (6 concentrations)	Positive[2]	(McGregor et al., 1988a)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP. The study was not in accordance with OECD guideline 476 (no colony sizing performed, only in the absence of metabolic activation) and

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						thus not considered valid. The lowest significantly effective doses was 900 µg/ml at which the relative total growth was 50%. The highest dose was lethal. A positive response was observed in two of three experiments. No dose-response was observed.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Not reported	Negative[2] Positive[3,]	(Rudd et al., 1983)	Study carried out within a larger NTP project. Only abstract available. Validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	Not reported	Negative[2] Inconclusive[3]	(Honma et al., 1999a)	Published collaborative study on 40 chemicals. Protocol was in accordance with OECD guideline 476, except that no colony sizing was performed. As the results are insufficiently reported, their validity cannot be evaluated. In the presence of S9 metabolic activation one laboratory achieved a statistically significant dose-dependant result, but did not induce mutations greater than three times the spontaneous response. The second laboratory did not obtain a positive response.
	Chromosomal aberration test	Chinese hamster ovary cells	160-1600 µg/ml[2]; 500-5000 µg/ml[3]	Negative[1]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster lung fibroblast cells	2400 µg/ml	Negative[1]	(Matsuoka et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Chinese hamster ovary cells	50-500 µg/ml[2]; 500-5000 µg/ml[3]	Negative[1,]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Unscheduled DNA synthesis test	Rat hepatocytes	Not reported	Negative	(Mirsalis et al., 1983)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Micronucleus test	Human lymphocytes and hepatoma cell line *Hep G2*	500 µM	Negative[1]	(Kevekordes et al., 2001)	
(Benzyl propionate [09.132])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Benzyl benzoate [09.727])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
(Benzaldehyde [05.013])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	37,500 nl/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	50 to 300 µl/plate	Negative[1]	(Rockwell & Raw, 1979)	Assay of urine samples from rats given benzaldehyde by oral gavage.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & Raw, 1979)	Samples assayed prior to administration to rats.
	Ames test	*S. typhimurium* TA98; TA100; TA2637	2000 mg/plate	Negative[1]	(Nohmi et al., 1985)	Article published in Japanese. Data reported from English

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Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						summary.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Ames test	*S. typhimurium* TA100; TA102; TA104	3333 µg/plate	Negative[1]	(NTP, 1990c)	
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki & Endo, 1978)	
	Ames test (preincubation method)	*S. typhimurium* TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (preincubation method)	*S. typhimurium* TA100	2000 nM/	Negative[1]	(Vamvakas et al., 1989)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Ames test (preincubation method)	*S. typhimurium* TA97a; TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1998)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1537; TA7001; TA7002; TA7003; TA7004; TA7006; Mix of TA7001–7006 TA7005	1000 µg/ml	Negative[1] Negative[2]; Positive[3]	(Gee et al., 1998)	
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	Not reported	Negative[2] Positive[3]	(Matsui et al., 1989)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	251 nl/ml	Negative	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	12.5 to 800 nl/ml	Negative[2] Weakly positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (12.5-800, 25-600, 400-600 nl/ml) were used in three independent experiments within which positive responses were observed. A 2.8 to 5.2-fold increase in mutant frequency was observed in the presence of S9.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0 to 800 µg/ml (6 concentrations)	Positive[2]	(McGregor et al., 1991)	Published summary report including detailed method and results from study on 27 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing performed). Statistically significant increase in mutant fraction at the highest non-lethal concentration (400 µg/ml) in two experiments. Concentration of 640 and 800 µg/ml were lethal. Thus, significant increases in mutant fraction were close to toxic doses. No dose-response was observed. Since a positive response was observed without S9, no experiment was carried out with S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y +/- cells	600 µg/ml	Negative[2]	(Bigger & Clarke, 1991)	
	Chromosomal aberration test	Chinese hamster cells	0, 800, 1000, 1200 µg/ml	Positive[2] Weak positive[3]	(Sofuni et al., 1985)	Article published in Japanese. Data extracted from English summary and tables. Validity of the study cannot be evaluated. Cytotoxicity was observed at the two maximum concentrations tested. In the presence and in the absence of S9 a positive response was only observed at cytotoxic concentrations. Polyploidization (11%) was reported at non-cytotoxic concentrations.
	Chromosomal aberration test	Chinese hamster ovary cells	50-500 µg/ml[2]; 160-1600 µg/ml[3]	Negative[1]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0053 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Information is only given for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Sister chromatid exchange assay	Chinese hamster ovary cells	5-160 µg/ml[2]; 160-1600 µg/ml[3]	Positive[2] Weakly positive[3]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on a preliminary assay. Although some deatails of results are not reported the study is considered valid. Weakly positive results with metabolic activation were observed at the highest concentration which was cytotoxic and resulted in 50% growth reduction.
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 1000 µM (up to 106 µg/ml)	Negative[3]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-212 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Benzoic acid [08.021])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative[1]	(Anderson & Styles, 1978)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1536	3.6 µg/plate	Negative[1]	(Cotruvo et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[3]	(McCann et al., 1975)	
	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	100 µg/plate	Negative[2]	(Milvy & Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA1535; TA1537; TA1538	0.5% (5 mg/ml)	Negative[1]	(FDA, 1975b)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama & Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Umu mutation assay	*S. typhimurium* TA1535/ pSK1002	1607 µg/ml	Negative[1]	(Nakamura et al., 1987)	
	Rec assay (liquid method)	*B. subtilis* M45 (rec^-), H17 (rec^+)	Not reported	Positive	(Nonaka, 1989)	Only abstract available. Details on method and results not reported. Use of metabolic activation not reported. The validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Positive	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) of sufficient quality to be taken into account in the evaluation. A weak positive result (D>2 mm) was observed at concentrations of 4 mg/disc or more. At 5 mg/disc D=2.9 mm.
	Mutation assay	*S. cerevisiae* D3	0.18%	Negative[1]	(Cotruvo et al., 1977)	
	Mutation assay	*S. cerevisiae* D4	0.15%	Negative[1]	(FDA, 1975b)	
	Indirect DNA repair test	*E. coli* PQ37	400 µg/ml	Negative	(Glosnicka & Dziadziuszko, 1986)	Genotoxicity measured as ability to induce ß-galactosidase.
	SOS Chromotest	*E. coli* PQ37	50 µg	Negative[1]	(Kevekordes et al., 1999)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Equivocal[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Total incidence of cells with aberrations was 8%.. Negative response for polyploidization.
	Sister chromatid exchange	Human lymphocytes	0-2 mM (0-244 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay					guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	*In vitro*Micronucleus assay	Mouse lymphoma L5178Y cells	1000 µg/ml	Negative[1]	(Nesslany & Marzin, 1999)	
(Methyl benzoate [09.725])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	0 to 666 µg/plate (-S9); 0 to 6666 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD guideline 471.
	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Methyl 4-methylbenzoate [09.631]	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537;	0 to 333 µg/plate (-S9); 0 to 3333 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD guideline 471.
(Isopentyl benzoate [09.755])	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
(4-Isopropylbenzyl alcohol [02.039])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & Raw, 1979)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell & Raw, 1979)	Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
(Tolualdehydes (mixed *o, m, p*) [05.027])	Ames test (preincubation method)	*S. typhimurium* TA104	0.8 µM/plate	Negative[1]	(Marnett et al., 1985a)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	18,750 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102	0.8 mM/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA100; TA1535; TA1537	666 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Unscheduled DNA synthesis test	Rat hepatocytes	1000 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	300 µg/ml (+S9), 600 µg /ml (-S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
(4-Isopropylbenzaldehyde [05.022])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & Raw, 1979)	
	Ames test (plate method)incorporation	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell & Raw, 1979)	Assay of urine samples from rats given 4-isopropyl benzaldehyde (cuminaldehyde) by gavage.
	Umu test	*S. typhimurium* TA1535/ pSK1002	1 µmole/ml	Negative	(Miyazawa et al., 2000)	Results indicated that 4-isopropyl benzaldehyde (cuminaldehyde) was positive for antimutagenicty, but not genotoxic.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 333 µM (up to 50 µg/ml)	Negative[2]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
(4-Hydroxybenzoic acid [08.040])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
(Salicylic acid [08.112])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama & Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	Not reported	Negative[2]	(McCann et al., 1975)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Weakly positive	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) of sufficient quality to be taken into account in the evaluation. A weak positive result (D>2 mm) was observed at concentrations of 2 mg/disc or more. At 5 mg/disc D=4.7 mm.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Mutation assay	*S. cerevisiae* rad18	Up to 0.1 mM (up to 13.8 µg/ml; 8 concentrations)	Weakly positive	(Zetterberg, 1979)	Published non-GLP study with limited report of experimental details and result. Use of metabolic activation not reported. The validity of the study cannot be evaluated. The dose level tested was clearly cytotoxic. An increase in mutant frequency was not evident until 95-99% of cells were killed.
Ethyl 4-hydroxybenzoate [09.367]	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980a)	dito.
	Chromosomal aberration assay	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980a)	dito.
	Chromosomal aberration assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	dito.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	Up to 250 µg/ml	Positive	(Ishidate et al., 1978)	Published non-GLP study in Japanese with English summary and tabulated results. Some important details of method and results are not available. There is no information on the use of metabolic activation. The substance was tested up to the maximum dose tolerated. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive

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Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay					screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human fibroblastic cells HE2144	0, 83, 166 µg/ml	Negative[2]	(Sasaki et al., 1980)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mutation assay	Silk worms	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl 4-hydroxybenzoate [09.754])	Ames test	*S. typhimurium* TA98; TA100	1000 µg/plate	Negative[1]	(Haresaku et al., 1985)	
	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 1000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster fibroblast cells	60 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (plate incorporation assay)	*S. typhimurium* TA100	500 µg/plate	Negative[2]	(Ball et al., 1984)	
(Veratraldehyde [05.017])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA15378	8000 µg/plate	Negative[1]	(Nestmann et al., 1980)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	8000 µg/plate	Negative[1]	(Douglas et al., 1979)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TTA1537	6666 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA100; TA102; TA104; TA982; TA1538	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (preincubation protocol)	*S. typhimurium* TA100; TA102; TA104	33 - 3333 µg/plate	Negative[1]	(Dillon et al., 1998)	
	Mutation assay	*S. cerevisiae* D7; XV185-14C	Not reported	Negative[2]	(Nestmann & Lee, 1983)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	250 to 1800 µg/ml	Positive[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (250, 1400-1600, 1400-1800 µg/ml) were used in three independent experiments within which positive responses were observed. A 2.3 to 6.2fold increase in the mutation frequency was observed both with and without S9.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
	Unscheduled DNA synthesis test	Rat hepatocytes	100 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(4-Methoxybenzaldehyde [05.015])	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA1537	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Engelhardt, 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	408 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita & Sasaki, 1987)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	22 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Müller et al., 1993)	
	Ames test	*S. typhimurium* TA 100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Mutation assay	Phage PM2	1362 µg/ml	Negative	(Becker et al., 1996)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0068 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, ring and dicentric aberrations, chromatic exchanges).
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0-3.0 mM (0-408 µg/ml) 3.6-5.1 mM (484-691 µg/ml)	Negative[2] Positive[2]	(Wangenheim & Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Jung et al., 1992)	Results confirmed at three separate contract laboratories

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	14 µg/ml	Negative	(Sasaki et al., 1987)	
	DNA alkaline unwinding assay	Mouse lymphoma L5178Y TK+/- cells	0, 4, 5, 6 mole/l (0, 544, 680, 816 µg/ml) 7, 8 mole/l (953, 1089 µg/ml)	Negative[2] Positive[2]	(Garberg et al., 1988)	Published study on 78 compounds not in accordance with standard guidelines. Test suitable for rapid screening only. Strand breaks or mutations observed only at cytotoxic concentrations.
2-Methoxybenzaldehyde [05.129]	Mutation assay	*E. coli* WP2*uvr*A, *trpE*	5000 µg/plate	Negative[2]	(Watanabe et al., 1989)	Published non-GLP study with limited report of experimental details and results. Study evaluating the enhancing effect on *N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced mutagenesis in pretreated cells and not on the mutagenicity of the substance itself. Absence of an enhancing effect reported.
	Sister chromatid exchange assay	Human lymphocytes	0-0.25 mM (0-34 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
3-Methoxybenzaldehyde [05.158]	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	dito.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0- 2.5 mM (0- 340 µg/ml) 3 mM (408 µg/ml)	Negative[2] Positive[2]	(Wangenheim & Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
(4-Ethoxybenzaldehyde [05.056])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[2]	(Wild et al., 1983)	
(Methyl 4-methoxybenzoate [09.713])	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Gallic acid [08.080]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	3000 µg/plate	Negative[1]	(Chen & Chung, 2000)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate (solvent DMSO) 0, 100, 333, 1000, 3333, 10,000 µg/plate (solvent acetone)	Negative[1] Equivocal[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471. Results on gallic acid from two different laboratories using different solvent. A negative response was observed in both laboratories with TA98, TA1535, TA1537. A negative result was also reported with TA100 in the laboratory using DMSO as solvent. With acetone, a low-level response with a dose-related trend was found with TA100 both in the absence and in the presence of metabolic activation. The effect was reproducible in a second, not reproducible in a third experiment.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535	5000 µg/plate	Negative[1]	(Rashid et al., 1985)	Inhibition was noted at the 5000-µg/plate dose-level; however this may have been due to toxicity. No mutagenicity was observed at the 1000-µg/plat dose-level.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	15 µM/plate	Negative[1]	(Wang & Klemencic, 1979)	

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA100	100 µg/plate	Weakly positive[2] Positive[3]	(Yamaguchi, 1981)	Published non-GLP. Insufficient report of important details of method and results, thus the validity of the result cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sugimura et al., 1976)	
	Chromosomal aberration test	Chinese hamster ovary cells	50 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Results are reported for one concentration only which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be reduced by the addition of S9.
	Chromosomal aberration test	Chinese hamster ovary K1 cells	up to 2 mM (up to 340 µg/ml)	Negative[1]	(Tayama & Nakagawa, 2001)	Published non-GLP study. Part of the study with insufficient report of important details of method and results. The validity of the results cannot be evaluated.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	0, 0.25, 0.5, 1.0, 1.5, 2.0 mM (0, 42.5, 85, 170, 255, 340 µg/ml)	Positive[2]	(Tayama & Nakagawa, 2001)	Published non-GLP study. Well conducted part of the study, however with insufficient report of some important details of method and results (results with metabolic activation not reported)..
	Mitotic gene conversion assay	*S. cerevisiae* D7	0, 100, 1000 µg/ml	Negative[2] Positive[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Gallic acid did not induce a significant extent of gene conversions under acidic conditions. At neutral pH no convertogenic activity was reported at 100 µg/ml, however, gallic acid was considerably convertogenic at 1000 µg/ml. The presence of catalase completely inhibited the convertogenic activity.gene conversions. Under alkaline conditions (pH 10), the concentration of 100 µg/ml was reported to induce a significant ($p < 0.01$) increase of Trp^+ convertants.
(Vanillin [05.018])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA 1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(Pool & Lin, 1982)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Ames test (preincubation assay)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 1000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Not reported	Negative[1]	(Nagabhushan & Bhide, 1985)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	2500 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
	Mutation assay	*E. coli* CSH26/pYM3; CSH26/pSK 1002	15,215 µg/ml	Negative	(Takahashi et al., 1990)	
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Chromosomal aberration test	Chinese hamster cell line B241	5, 20, 40 nM (0.0008, 0.003, 0.006 µg/ml)	Negative	(Kasamaki & Urasawa, 1985)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster V79 lung cells	15,215 -152,150 µg	Negative[2]	(Tamai et al., 1992)	
	Chromosomal aberration test	Human lymphocytes	0, 1, 2, 4 mM (0, 152, 304, 608 µg/ml)	Negative	(Jansson & Zech, 1987)	Published non-GLP study not in accordance with OECD guideline 473 (no metabolic activation). Insufficient report of important details of method and results. No information on cytotoxicity. This study is not considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	20 nM (0.003 µg/ml)	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are only reported for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. No significant increase increase of single types of aberrations and of total aberrations.
	Sister chromatid exchange assay	Human lymphocyte cells	0 – 1.0 mM (0 - 152 µg/ml)	Positive[2]	(Jansson et al., 1986)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). This study is not considered valid. Dose-dependent effect reported. Insufficient report of important details of method and results.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	15 µg/ml	Negative	(Sasaki et al., 1987)	
	Sister chromatid exchange assay	Human lymphocytes	0, 1, 2 mM (0, 152, 304 µg/ml)	Positive[2]	(Jansson & Zech, 1987)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. Dose-dependent

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						effect reported This study is not considered valid.
	Mutation assay	Mouse lymphoma L5178Y cells	1000 µg/ml (-S9), 1500 µg/ml (+S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	500 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Micronucleus assay	Human hepatoma (Hep-G2) cells	5, 50 µg/ml 500 µg/ml	Negative [2] Positive[2]	(Sanyal et al., 1997)	Published non-GLP study carried out only in the absence of metabolic activation. Thus, the study is not considered valid. A statistically significant increase of spontaneus micronucleus frequency was reported at the highest concentration. Low concentrations of vanillin (0.25 – 5 µg/ml) but not higher (50, 500 µg/ml) showed an inhibitory effect on micronuclei induced by heterocyclic amines.
(Vanillic acid [08.043])	Chromosomal aberration test	Chinese hamster ovary cells	25,000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be increased by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
4-Hydroxy-3,5-dimethoxybenzaldehyde [05.153]	Ames test	*S. typhimurium* TA100	10,000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
4-Hydroxy-3,5- dimethoxybenzoic acid [08.087]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Chromosomal aberration test	Chinese hamster ovary cells	3000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be reduced by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid.
(Salicylaldehyde [05.055])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki & Endo, 1978)	
	Ames test	*S. typhimurium* TA98; TA100	16 µg/ml	Negative[1]	(Kono et al., 1995)	
	Mutation assay	*S. typhimurium* TA1535/ pSK1002	111 µg/ml	Negative[1]	(Nakamura et al., 1987)	

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration test	CHL/IU cells	Not reported (max. 5 mg/ml)	Positive[1]	(Kusakabe et al., 2002)	Published study in accordance to OECD guideline 473. However, some details on method and results are insufficiently reported. Thus the validity of the study cannot be evaluated. Positive result with minimum effective dose manifesting over 50% cytotoxicity at short-term treatment (6 h, less than 50% cells with chromosomal aberrations without S9, less than 20% cells with chromosomal aberrations with S9). Reduced effect at continuous treatment without S9 (24 h less than 10% cells with chromosomal aberrations). No chromosomal aberrations after 48 h treatment without S9. After 48 h treatment without S9 18% polyploid cells..
	Sister chromatid exchange assay	Human lymphocyte cells	0-0.5 mM (0-61 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Methyl salicylate [09.749])	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	333.3 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	dito.
	Chromosomal aberration test	Chinese hamster fibroblasts	250 µg/ml[4] (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Positive[1]	(Kuboyama & Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation. At 100 µg/plate a positive response was observed in strain TA98 in the presence of S9 mix obtained from hamsters a negative response was observed in TA98 in the presence of S9 mix obtained from rat, mouse and guinea pig.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	23 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	0 to 5000 µg/disc	Negative	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation.
	Mutation assay	Silkworm	Not reported	Negative	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	dito.
	Sister chromatid exchange assay	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	dito.
(Butyl vanillyl ether [04.093])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative[1]	(Watanabe & Morimoto, 1989c)	
	Mutation assay	*E. coli* WP2 *uvr*A	5000 µg/plate	Negative[1]	(Watanabe & Morimoto, 1989c)	
(Ethyl vanillin [05.019])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	8000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (six concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita & Sasaki, 1987)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblast cells	250 µg/ml (three concentrations, maximal concentration inducing 50% cell-growth inhibition)[4]	Positive[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Polyploidization in 48% of cells reported at 48 hours. Negative response for chromosomal aberrations.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	125-800 µg/ml	Negative[2] Weak positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (125-500 µg/ml, 600 µg/ml, 800 µg/ml) were used in three independent experiments within which positive responses were observed. In the presence of S9 a 2.1 to 3-fold increase in the mutant frequency was reported.
	Unscheduled DNA synthesis test	Rat hepatocytes	199 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-332 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	17 µg/ml	Negative	(Sasaki et al., 1987)	
(Ethyl vanillin isobutyrate)	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(King & Harnasch, 1997)	
(Piperonyl acetate [09.220])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
(Piperonal [05.016])	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 *E. coli* WP2*uvr*Atrp$^-$	0, 300, 600, 1200, 2400 µg/plate	Negative[1]	(Sekizawa & Shibamoto, 1982)	Valid study in accordance with OECD guideline 471. The plate incorporation method was used -S9; the preincubation method +S9.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 5000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1537; TA1538	5000 µg/plate	Negative[1]	(White et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	20 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	5000 µg/disc	Positive[2]	(Sekizawa & Shibamoto, 1982)	Well designed and reported study, however with some limitations with respect to results. DNA-repair tests in the

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						presence of S9 were not successful (no data reported).
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0075 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Data are only reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Chromosomal aberration test	Chinese hamster cell line B241	0.15 µg/ml	Negative	(Kasamaki & Urasawa, 1985)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	1000 µg/ml[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	10 to 502 µg/ml	Positive	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.

NR = not reported

[1] *With and without S9 metabolic activation.*

[2] *Without S9 metabolic activation.*

[3] *With S9 metabolic activation.*

[4] *Concentration listed is either the highest tested if the result was negative or the concentration at which the maximum effect was observed for positive results.*

Table 2.3: Genotoxicity (*in vivo*) EFSA / FGE.20

Substances listed in brackets are JECFA-evaluated substances

Table 2.3: GENOTOXICITY (*in vivo*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Benzyl alcohol [02.010])	*In vivo* Sex-linked recessive lethal mutations(SLRL)	*D. melanogaster*	Diet	5000 ppm	Negative	(Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	8000 ppm	Negative	(Foureman et al., 1994)	
	In vivo Micronucleus test	Mouse bone marrow cells	IP injection	200 mg/kg bw	Negative	(Hayashi et al., 1988)	
	In vivo Replicative DNA synthesis test	Mouse and rat hepatocytes	Not reported	Not reported	Negative	(Yoshikawa, 1996)	Screening test for the detection of non-genotoxic hepatocarcinogens. The substance was administered once at the maximum tolerated dose or at half the maximum tolerated dose to male mice and rats. Hepatocytes were prepared after 24, 39 and 48 hours.
	In vivo Replicative DNA synthesis test	Mouse hepatocytes	Oral gavage	800 mg/kg	Negative	(Miyagawa et al., 1995)	
	In vivo Replicative DNA synthesis test	Rat hepatocytes	Oral or SC injection	600 mg/kg	Negative	(Uno et al., 1994)	
(Benzyl acetate [09.014])	*In vivo* SLRL	*D. melanogaster*	Diet	300 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	20,000 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection	1700 mg/kg bw	Negative	(NTP, 1993d)	
	In vivo Chromosomal aberration test	Mouse bone marrow cells	IP injection	0 to 1700 mg/kg bw	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. The highest dose caused toxicity and cell cycle delay. Test not fully in compliance with the OECD guideline (insufficient cells per animal studied). GLP status not stated. The study is considered of limited validity.
	In vivo Micronucleus test	Mouse bone marrow cells	3 IP injection with 24 h intervals	0, 312, 625 and 1250 mg/kg bw	Negative	(NTP, 1993d; Shelby et al., 1993)	Test substance same batch as NTP chronic bioassays. Study in compliance with OECD guideline. GLP not stated. Micronuclei were determiend at 24 h after the last dose. A dose-related decrease in PCE/NCE ratio was observed. The study is considered valid.
	In vivo Micronucleus test	Mouse erythrocytes	Dietary exposure for 13 weeks.	0 to 50,000 ppm (equal to 0 to 7900 mg/kg bw/day for males and 0 to 9400 mg/kg bw/day for females)	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. In life phase under GLP; for determination of genotoxic effects. GLP not specified. Test in compliance with OECD guideline. The test is considered valid, but of limited relevance because no chance in PCE/NCE ratio was observed.
	In vivo Unscheduled DNA	Rat hepatocytes	Oral gavage	0, 50, 200 and 1000	Negative	(Mirsalis et al., 1989)	Test substance same batch as NTP chronic bioassays.

Table 2.3: GENOTOXICITY (*in vivo*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
	synthesis test			mg/kg bw			Test in compliance with OECD guidelines. GLP not stated. The test is considered valid.
	In vivo Unscheduled DNA synthesis test	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz & Mirsalis, 1984)	Only abstract available. Non guideline test. Validity cannot be assesed.
	In vivo DNA damage	Rat pancreatic cells	IP injection	0, 150, 500 and 1500 mg/kg bw	Negative	(Longnecker et al., 1990)	Alkaline elution assay. GLP status not specified. Limited number of animals/group; DNA damage monitored at 1 hr post dosing. The study is of limited validity.
	In vivo Comet assay	Mouse/ Rat	Oral	1600 mg/kg (mouse); 1200 mg/kg (rat)	Positive	(Sekihashi et al., 2002)	Non-GLP and non-guideline test; but in compliance with recommended protocols. Some important details of method and results insufficiently reported. No toxicity data reported. The administered dose was 0.5 x LD50. Sampling time was 3, 8 and 24 hours after dosing. Positive result reported in mice for stomach, colon, kidney, urinary bladder and brain, in rats for stomach, colon, liver, kidney, urinary bladder, lung. After 24 h no significant effect in mice, significant effects in rat only in lung and kidney. The study is of limited validity.
(Benzaldehyde [05.013])	*In vivo* SLRL	*D. melanogaster*	Diet	1150 ppm	Negative	(Woodruff et al., 1985)	
	In vivo SLRL	*D. melanogaster*	Injection	2500 ppm	Negative	(Woodruff et al., 1985)	
(Salicylic acid [08.112])	*In vivo* Chromosomal aberration assay	Mouse bone marrow cells	IP injection gavage	0, 50, 100, 200 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Published study widely in accordance with OECD guideline 475 and well reported (except that only males were tested, only one sampling time was chosen and signs of toxicity were not reported). Oral and i.p. dose were selected to be 1/3 and 1/5 of the reported oral LD50.
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection gavage	0, 25, 50, 100 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Well described published study of good quality. Oral and i.p. dose were selected to be 1/3 and 1/10 of the reported oral LD50.
Ethyl 4-hydroxybenzoate [09.367]	*In vivo* Chromosomal aberration assay	Rat bone marrow cells	Not reported	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
(4-Ethoxybenzaldehyde [05.056])	*In vivo* Basc test Micronucleus test	*D. melanogaster*	NR	751 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	1005 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
Gallic acid [08.080]	*In vivo* Medium-term rat liver bioassay	Male rats initiated with IP injection of diethylnitrosamine	Not reported.	Not reported	Negative	(Shirai, 1997)	Published non-GLP study. Unusual study protocol not following OECD guidelines. Some important details of method missing and only summarized results of a large screening study reported. Thus, the validity of the study cannot be evaluated.

Table 2.3: GENOTOXICITY (*in vivo*) EFSA / FGE.20

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Vanillin [05.018])	*In vivo* Micronucleus test	Male BDF1 mice	Oral gavage	500 mg/kg bw	Negative	(Inouye et al., 1988)	Published non-GLP study not in accordance with OECD guideline 474 (smaller group size, only males tested, no toxicity data reported, single dose level used, no negative control, effect on PCE/NCE ratio not reported.) Induction of micronuclei in mitomycin-treated mice was suppressed by post-treatment with vanillin due to an anticlastogenic effect. Vanillin itself did not induce micronucleated PCEs (vanillin control group without mitomycin-treatment, six sampling times from 5 to 65 h).
(Salicylaldehyde [05.055])	*In vivo* Spot test	*D. melanogaster* BINSC *D. melanogaster* Oregon-R	NR	1.05 to 1.40 ppm 0.09 to 0.35 ppm	Negative Negative	(Kono et al., 1995)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated
(Ethyl vanillin [05.019])	*In vivo* Basc test	*D. melanogaster*	NR	8309 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	Male BDF1 mice	IP injection	Not reported	Negative	(Furukawa et al., 1989)	*Only abstract available. Insufficient report of experimental details and result to evaluate the validity of the study*.
	In vivo Micronucleus test	NMRI mice	NR	1000 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonyl acetate [09.220])	*In vivo* Basc test	*D. melanogaster*	NR	4855 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	970 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonal [05.016])	*In vivo* Dominant lethal assay	ICR/Ha Swiss mice	IP injection	0, 124, 620 mg/kg bw	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.
	In vivo Dominant lethal assay	ICR/Ha Swiss mice	Oral gavage	0, 1000 mg/kg bw (repeated doses on 5 successive days)	Negative	(Epstein et al., 1972)	Dito.

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of 44 Hydroxy- and Alkoxy-Substituted Benzyl Derivatives Evaluated by JECFA (JECFA, 2002b)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

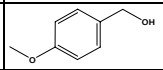
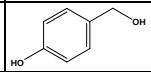
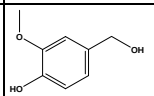
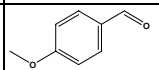
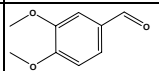
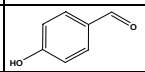
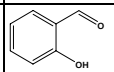
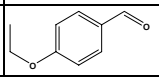
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.128 871	p-Anisyl alcohol		130 58	Class I A3: Intake below threshold	4)	6)	6)
02.165 955	4-Hydroxybenzyl alcohol		5.2 0.06	Class I A3: Intake below threshold	4)	6)	6)
02.213 886	Vanillyl alcohol		5.4 6	Class I A3: Intake below threshold	4)	6)	6)
05.015 878	4-Methoxybenzaldehyde		370 580	Class I A3: Intake below threshold	4)	6)	6)
05.017 877	Veratraldehyde		120 55	Class I A3: Intake below threshold	4)	6)	6)
05.047 956	4-Hydroxybenzaldehyde		55 56	Class I A3: Intake below threshold	4)	6)	6)
05.055 897	Salicylaldehyde		84 16	Class I A3: Intake below threshold	4)	6)	6)
05.056 879	4-Ethoxybenzaldehyde		0.073 0.01	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

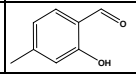
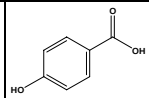
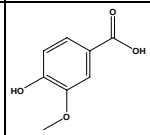
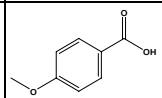
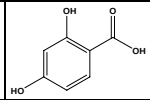
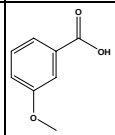
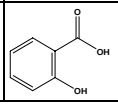
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
05.091 898	2-Hydroxy-4-methylbenzaldehyde		0.61 0.3	Class I A3: Intake below threshold	4)	6)	6)
08.040 957	4-Hydroxybenzoic acid		16 17	Class I A3: Intake below threshold	4)	6)	6)
08.043 959	Vanillic acid		24 26	Class I A3: Intake below threshold	4)	6)	6)
08.071 883	p-Anisic acid		ND 0.1	Class I A3: Intake below threshold	4)	7)	7)
08.076 908	2,4-Dihydroxybenzoic acid		ND 6	Class I A3: Intake below threshold	4)	7)	7)
08.092 882	3-Methoxybenzoic acid		ND 0.01	Class I A3: Intake below threshold	4)	7)	7)
08.112 958	Salicylic acid		0.024 0.03	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

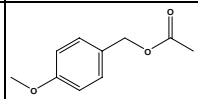
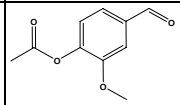
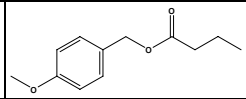
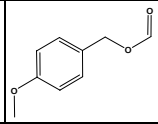
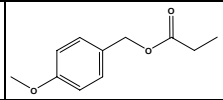
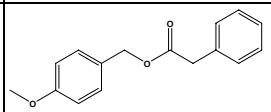
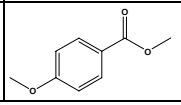
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.019 873	p-Anisyl acetate		50 300	Class I A3: Intake below threshold	4)	6)	6)
09.035 890	Vanillyl acetate		1.8 1	Class I A3: Intake below threshold	4)	6)	6)
09.058 875	p-Anisyl butyrate		29 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.087 872	p-Anisyl formate		39 24	Class I A3: Intake below threshold	4)	6)	According to JECFA: "Minimum assay value is 90%", composition of mixture to be specified.
09.145 874	p-Anisyl propionate		ND 5	Class I A3: Intake below threshold	4)	7)	7)
09.706 876	Anisyl phenylacetate		0.0024 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.713 884	Methyl 4-methoxybenzoate		0.97 0.01	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

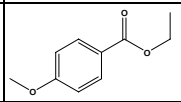
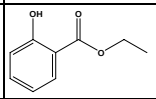
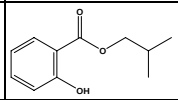
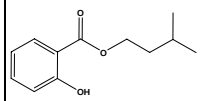
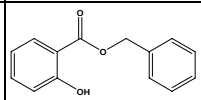
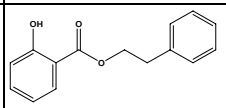
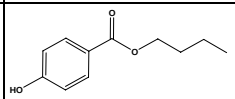
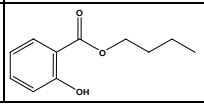
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.714 885	Ethyl 4-methoxybenzoate		9.1 2	Class I A3: Intake below threshold	4)	6)	6)
09.748 900	Ethyl salicylate		27 1700	Class I A3: Intake below threshold	4)	6)	6)
09.750 902	Isobutyl salicylate		0.97 6	Class I A3: Intake below threshold	4)	6)	6)
09.751 903	Isopentyl salicylate		41 7	Class I A3: Intake below threshold	4)	6)	According to JECFA: Min. assay value is "98 (sum of isoamyl and amyl salicylate)", composition of mixture to be specified.
09.752 904	Benzyl salicylate		26 29	Class I A3: Intake below threshold	4)	6)	6)
09.753 905	Phenethyl salicylate		0.12 4	Class I A3: Intake below threshold	4)	6)	6)
09.754 870	Butyl 4-hydroxybenzoate		ND 0.03	Class I A3: Intake below threshold	4)	7) Additional data required	7) Additional data required.
09.763 901	Butyl salicylate		0.012 0.0007	Class I A3: Intake below threshold	4)	6)	Id test is requested.

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

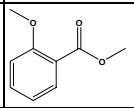
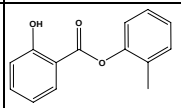
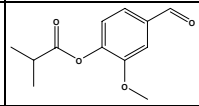
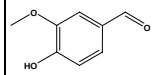
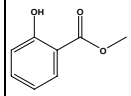
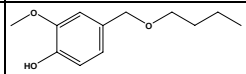
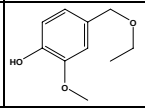
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.796 880	Methyl 2-methoxybenzoate		49 8	Class I A3: Intake below threshold	4)	6)	6)
09.807 907	o-Tolyl salicylate		ND 30	Class I A3: Intake below threshold	4)	7)	7)
09.811 891	Vanillin isobutyrate		55 0.04	Class I A3: Intake below threshold	4)	6)	6)
05.018 889	Vanillin		47000 150000	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 1000 mg/kg bw/day in a 2-year study in rats is > 100 times the estimated daily intake of vanillin when used as a flavouring substance.	6)
09.749 899	Methyl salicylate		410 44000	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 50 mg/kg bw/day in a 2-year study in dogs is > 100 times the estimated daily intake of methyl salicylate when used as a flavouring substance.	6)
04.093 888	Butyl vanillyl ether		ND 0.1	Class II A3: Intake below threshold	4)	7)	7)
04.094 887	Ethyl 4-hydroxy-3-methoxybenzyl ether		20 22	Class II A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

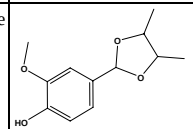
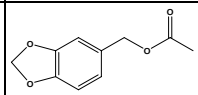
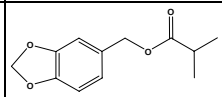
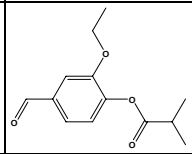
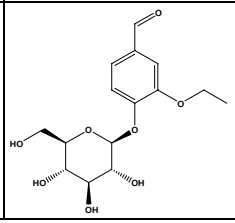
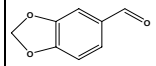
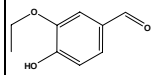
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
06.132 960	Vanillin butan-2,3-diol acetal (mixture of stereo isomers)		3.4 3	Class II A3: Intake below threshold	4)	6)	CASrn does not specify stereoisomers, stereoisomeric composition to be specified.
09.220 894	Piperonyl acetate		34 11	Class II A3: Intake below threshold	4)	6)	6)
09.430 895	Piperonyl isobutyrate		0.085 3	Class II A3: Intake below threshold	4)	6)	6)
09.933 953	Ethyl vanillin isobutyrate		0.61 ND	Class II A3: Intake below threshold	4)	6)	6)
16.075 892	Ethyl vanillin beta-D-glucopyranoside		ND 30	Class II A3: Intake below threshold	4)	7)	CASrn to be included in the Register: 122397-96-0. 7)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
05.016 896	Piperonal		1500 3200	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 250 mg/kg bw/day in a 2-year study in rats is > 100 times the estimated daily intake of piperonal when used as a flavouring substance	6)
05.019 893	Ethyl vanillin		5400 43000	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 500 mg/kg bw/day in a 14-week study in rats is > 100 times the estimated daily intake of ethyl vanillin when used as a flavouring substance	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, Class II = 540, 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 as flavouring substance based on the MSDI approach.

7) MSDI based on USA production figure.

ND: not determined

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.20)

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

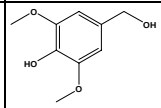
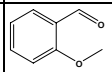
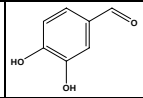
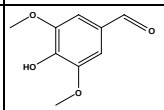
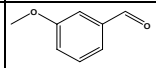
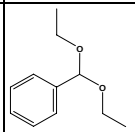
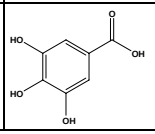
FL-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		0.037	Class I A3: Intake below threshold	4)	6)	
05.129	2-Methoxybenzaldehyde		0.16	Class I A3: Intake below threshold	4)	6)	
05.142	3,4-Dihydroxybenzaldehyde		8.5	Class I A3: Intake below threshold	4)	6)	
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde		0.74	Class I A3: Intake below threshold	4)	6)	
05.158	3-Methoxybenzaldehyde		0.011	Class I A3: Intake below threshold	4)	6)	
06.017	(Diethoxymethyl)benzene		1.7	Class I A3: Intake below threshold	4)	6)	
08.080	Gallic acid		0.011	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

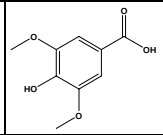
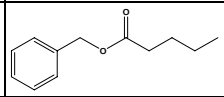
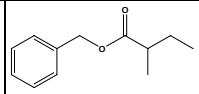
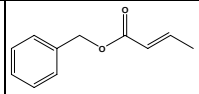
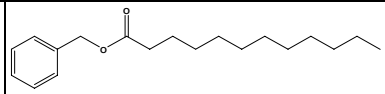
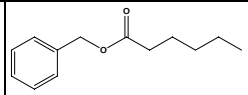
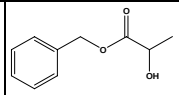
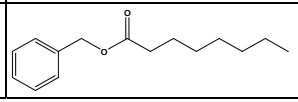
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		1.2	Class I A3: Intake below threshold	4)	6)	
09.152	Benzyl valerate		1.7	Class I A3: Intake below threshold	4)	6)	
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	7)	
09.314	Benzyl crotonate		0.37	Class I A3: Intake below threshold	4)	6)	
09.315	Benzyl dodecanoate		0.13	Class I A3: Intake below threshold	4)	6)	
09.316	Benzyl hexanoate		0.75	Class I A3: Intake below threshold	4)	6)	
09.317	Benzyl lactate		0.91	Class I A3: Intake below threshold	4)	7)	
09.318	Benzyl octanoate		0.12	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

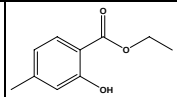
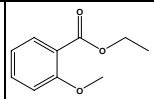
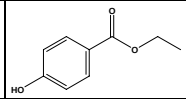
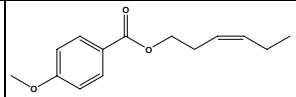
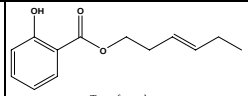
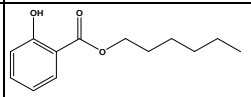
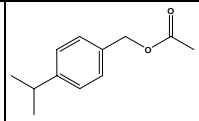
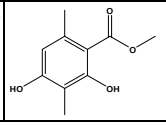
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.362	Ethyl 2-hydroxy-4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.363	Ethyl 2-methoxybenzoate		5.5	Class I A3: Intake below threshold	4)	6)	
09.367	Ethyl 4-hydroxybenzoate		10	Class I A3: Intake below threshold	4)	6)	
09.560	Hex-3(cis)-enyl anisate		0.12	Class I A3: Intake below threshold	4)	6)	
09.570	Hex-3-enyl salicylate	 Trans form shown	0.13	Class I A3: Intake below threshold	4)	7)	
09.581	Hexyl salicylate		0.018	Class I A3: Intake below threshold	4)	6)	
09.611	4-Isopropylbenzyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		0.012	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

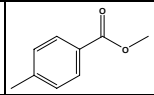
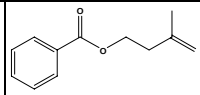
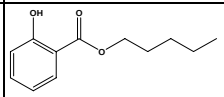
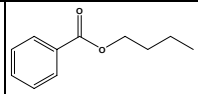
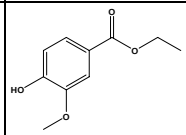
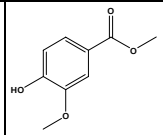
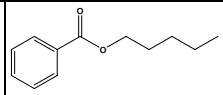
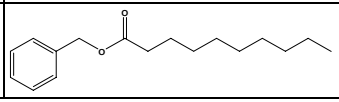
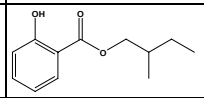
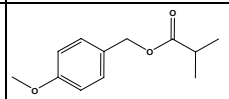
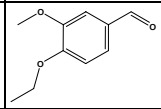
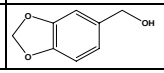
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.631	Methyl 4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.656	3-Methylbut-3-enyl benzoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.762	Pentyl salicylate		0.24	Class I A3: Intake below threshold	4)	6)	
09.779	Butyl benzoate		3.7	Class I A3: Intake below threshold	4)	6)	
09.798	Ethyl vanillate		0.024	Class I A3: Intake below threshold	4)	6)	
09.799	Methyl vanillate		0.011	Class I A3: Intake below threshold	4)	6)	
09.825	Pentyl benzoate		1.1	Class I A3: Intake below threshold	4)	6)	
09.835	Benzyl decanoate		0.35	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (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
09.852	2-Methylbutyl 2-hydroxybenzoate		0.011	Class I A3: Intake below threshold	4)	7)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	Class I A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
02.205	Piperonyl alcohol		0.011	Class III A3: Intake below threshold	4)	6)	

1) 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, Class II = 540, 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.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30[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 45 flavouring substances in the Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4), using the Procedure in Commission Regulation (EC) No 1565/2000. This revision 4 is made due to inclusion of four additional substances, *o*-, *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenoate [FL no: 09.858]. None of the substances were 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 Panel concluded that all the substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for all 45 candidate substances.

KEYWORDS

Flavourings, safety, benzyl alcohols, benzaldehydes, benzoic acids, esters, acetals, FGE.20, revision.

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1 On request from the European Commission, Question No (EFSA-Q-2012-00621, EFSA-Q-2012-00620, EFSA-Q-2012-00619, EFSA-Q-2012-00618), adopted on 20 November 2012.

2 Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Leon Brimer, Laurence Castle, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Maria de Fatima Tavares Pocas, Fidel Toldra, Detlef Wölfle. Correspondence: cef@efsa.europa.eu.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Scientific 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 requested to evaluate 45 flavouring substances in the Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These flavouring substances belong to chemical groups 23 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.20, FGE.20Rev4, includes the evaluation of four additional substances, *o*- *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenoate [09.858].

Four flavouring substances can exist as optical isomers [FL-no: 06.104, 09.313, 09.317 and 09.852] and four substances can exist as geometrical isomers [FL-no: 09.314, 09.560, 09.570 and 09.858].

Forty-one candidate substances are classified into structural class I and four [FL-no: 02.205, 05.066, 05.221 and 06.104] are classified into structural class II according to the decision tree approach presented by Cramer et al., 1978.

Twenty-five 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 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 41 flavouring substances allocated to structural class I have intakes in Europe from 0.001 to 610 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day). The four substances in structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104] have estimated intakes of 0.011, 1.2, 0.61 and 100 microgram/*capita*/day, respectively. These intakes are below the threshold values of 540 microgram/person/day for structural class II.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 41 of the candidate substances belonging to structural class I is approximately 1400 microgram/*capita*/day and the combined intake of the four candidate substances belonging to structural class II is approximately 100 microgram/*capita*/day. These values are lower than the threshold of concern for structural class I and II substances. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100

microgram/*capita*/day for structural class I and II, respectively, which exceed the thresholds of concern. However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

For the substances in this group the available genotoxicity data do not preclude the evaluation of the candidate substances using the Procedure.

It is anticipated that the candidate substances in FGE.20Rev4 would be metabolised to innocuous products.

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 on the basis of the default MSDI approach the 45 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 770 to 120000 microgram/person/day for 41 flavouring substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for six flavouring substances [FL-no: 05.129, 05.142, 05.153, 05.158, 08.080 and 09.858]. The estimated intakes, based on the mTAMDI, of the four flavouring substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II were 3900, 1600, 7000 and 3900 microgram/person/day, respectively, which are all above the threshold of concern for the structural class (540 microgram/person/day for structural class II). The six substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products. Thus, on the basis of the mTAMDI, the estimated intakes for 39 flavouring substances considered in this Opinion, exceed the relevant threshold for their structural class to which the flavouring substance has been assigned. Therefore, for these 39 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 45 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 each of the 45 flavouring substances.

For these 45 flavouring substances the Panel concluded that they would present no safety concern at their estimated levels of intake based of 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 Union list of flavourings and source materials is established in Commission Regulation (EC) No 872/2012 (EC, 2012a).

HISTORY OF THE EVALUATION

The Flavouring Group Evaluation 20 (FGE.20) dealt with 35 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters.

The Revision 1 of FGE.20, FGE.20Rev1, included the assessment of one additional candidate substance, vanillin propylene glycol acetal [FL-no: 06.104]. For this substance there are hydrolysis data and for a related substance [FL-no: 02.248] *in vitro* genotoxicity data. Additional information for three substances [FL-no: 09.313, 09.317 and 09.852] was made available since FGE.20 was published. Toxicity and metabolism data on a substance, vanillin 3-l-menthoxypropane-1,2-diol acetal [FL-no: 02.248], related to the candidate substance vanillin propylene glycol acetal [FL-no:06.104], are included.

The Revision 2 of FGE.20, FGE.20Rev2, included the assessment of five additional candidate substances [FL-no: 05.221, 08.132, 08.133, 09.693 and 09.696]. Toxicity data are available for four of the five substances. *In vitro* genotoxicity data are available for [FL-no: 05.221 and 08.133], long-term toxicity data are available for [FL-no: 08.133] and acute toxicity data are available for [FL-no: 08.133, 09.693 and 09.696]. Two of the substances [FL-no: 09.693 and 09.696] were considered with respect to genotoxicity in FGE.202 in which the Panel concluded that the genotoxicity data available do not preclude their evaluation through the Procedure.

The Revision 3 of FGE.20, FGE.20Rev3, included the consideration of the SCF Opinion on benzoic acid (SCF, 2002c). Furthermore, the Industry has for two substances [FL-no: 06.104 and 09.570] submitted information on the stereoisomeric composition (EFFA, 2010a), which was missing in the previous version of the FGE. Finally, the Industry has submitted new information to support the re-allocation of structural class to the candidate substance piperonyl alcohol [FL-no: 02.205]. For piperonyl alcohol [FL-no: 02.205], the Flavouring Industry has submitted new information, since the publication of FGE.20Rev2, that suggests natural occurrence in several food sources of closely structurally related substances, which are most likely metabolised to piperonyl alcohol. Therefore Flavouring Industry considered it correct to answer the question whether the substance occur naturally

with a yes, and therefore the substance should be allocated to structural class II, instead of class III. The Panel agreed in this consideration and allocated [FL-no: 02.205] to structural class II.

FGE	Opinion adopted by EFSA	Link	No. Of candidate substances
FGE.20	7 December 2005	http://www.efsa.europa.eu/en/efsajournal/doc/296.pdf	35
FGE.20Rev1	29 November 2007	http://www.efsa.europa.eu/en/efsajournal/doc/976.pdf	36
FGE.20Rev2	26 November 2009	http://www.efsa.europa.eu/en/scdocs/doc/1405.pdf	41
FGE.20Rev3	17 May 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2176.htm	41
FGE.20Rev4	20 November 2012		45

The present Revision of FGE.20, FGE.20Rev4, includes the assessment of four additional substances *o*-, *m*- and *p*-tolualdehyde [FL-no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenoate [FL no: 09.858].

No toxicity or metabolism data were submitted for the new substances. A search in the open literature for these substances provided four additional studies on the biotransformation of tolualdehydes. Additional data on toxicity were not found. Some genotoxicity studies on the tolualdehyde isomers were already included in the previous versions of this FGE for a supporting substance (tolualdehyde, mixture of isomers; [FL no: 05.027]). These studies have been relocated in the tables with genotoxicity data, but no new information was included. No additional data were found for substance [FL no: 09.858].

Furthermore, additional information on composition has been submitted for [FL-no: 05.221] (EFFA, 2012k) and [FL-no: 06.104] (EFFA, 2012u).

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.

In addition, the Commission has asked EFSA to reflect newly submitted information on specifications in the revisions of FGEs.

ASSESSMENT

1. PRESENTATION OF THE SUBSTANCES IN FLAVOURING GROUP EVALUATION 20, REVISION 4

1.1. Description

The present Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 45 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters. These flavouring substances belong to chemical groups 23 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 45 candidate substances under consideration with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

This group of candidate substances includes 19 benzyl derivatives (subgroup 1), 25 hydroxy- and alkoxy-ringsubstituted benzyl derivatives (subgroup 2) and one hydroxy- and alkoxy-substituted biphenyl derivative (subgroup 3).

- Subgroup 1: Benzyl derivatives

  This subgroup comprises three tolualdehyde isomers (*o*-, *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and 15 alkyl esters, ten of which are benzyl esters [FL-no: 09.152, 09.313, 09.314, 09.315, 09.316, 09.317, 09.318, 09.611, 09.835 and 09.858] and five are benzoic acid esters [FL-no: 09.631, 09.656, 09.693, 09.779 and 09.825]. Four of these candidate esters contain a double-bond in the alkyl side chain [FL-no: 09.314, 09.656, 09.693 and 09.858] and two contain an alkyl substituent at the aromatic ring [FL-no: 09.631 and 09.611]. The remaining substance [FL-no: 06.017] is an acetal of benzaldehyde.

- Subgroup 2: Hydroxy- and alkoxy-ringsubstituted benzyl derivatives

  This subgroup includes two benzyl alcohols [FL-no: 02.164, and the derivative piperonyl alcohol FL-no: 02.205], six benzaldehyde derivatives [FL-no: 05.066, 05.129, 05.142, 05.153, 05.158 and 06.104], four benzoic acids [FL-no: 08.080, 08.087, 08.132 and 08.133] and 13 related esters [FL-no: 09.362, 09.363, 09.367, 09.560, 09.570, 09.581, 09.623, 09.696, 09.762, 09.798, 09.799, 09.852 and 09.895]. One of the esters is a benzyl ester [FL-no: 09.895], all the others are benzoic acid esters. Three of the esters contain a double-bond in the alkyl side chain [FL-no: 09.560, 09.570 and 09.696].

- Subgroup 3: Hydroxy- and alkoxy-substituted biphenyl derivative

  This subgroup contains one candidate substance, a hydroxy- and alkoxy-substituted biphenyl derivative [FL-no: 05.221].

The 45 flavouring substances (candidate substances) are closely related structurally to 77 flavouring substances (supporting substances) evaluated at the 46th and 57th JECFA meeting (JECFA, 1997a; JECFA, 2002b). The names and structures of the 77 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; JECFA, 1997a; JECFA, 2002b; SCF, 1995).

The 77 supporting substances include 34 benzyl derivatives (subgroup 1) and 43 hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2).

The hydrolysis products of the candidate esters are listed in Table 2b.

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.).

Three flavouring substances possess one chiral centre [FL-no: 09.313, 09.317 and 09.852] and one flavouring substance possesses two chiral centres [FL-no: 06.104]. Due to the presence and the position of double bonds, four candidate substances can exist as geometrical isomers [FL-no: 09.314, 09.560, 09.570 and 09.858]. For all eight substances, the stereoisomeric composition has been specified (see Table 1).

1.3. Natural Occurrence in Food

Twenty-five candidate substances have been reported to occur in fruit (cherry, mango, papaya, bilberry, black currants, mulberry, sapodilla, cloudberry, pineapple, grape), cocoa, potato, coffee, tea, beer, rum, sherry, whisky, wine, honey, spices, soybean, peanut, wort and pork. Quantitative data on the natural occurrence of these substances have been reported for the occurrence of 16 of these substances in food.

Table 1.3.1 Candidate Substances Reported to Occur in Food (TNO, 2000; EFFA, 2010a; TNO, 2012)

FL-no:	Name:	Quantitative data reported:
05.026	o-Tolualdehyde	0.6 mg/kg in allium species.
05.029	p-Tolualdehyde	Up to 0.3 mg/kg in tea, up to 0.004 mg/kg in honey.
05.129	2-Methoxybenzaldehyde	7000 mg/kg in cassia leaf (oil), up to 1500 mg/kg in cinnamon bark (oil).
05.142	3,4-Dihydroxybenzaldehyde	Up to 20 mg/kg in coffee, 313 mg/kg in bourbon vanilla.
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	Up to 0.7 mg/kg in beer, up to 9.2 mg/kg in grape, up to 0.014 mg/kg in mango, 0.08 mg/kg in pineapple, 8.3 mg/kg in pork, up to 19.9 mg/kg in rum, 0.035 mg/kg in sherry, 1.9 mg/kg in bourbon vanilla, up to 8.7 mg/kg in whisky, up to 0.86 mg/kg in red wine, up to 0.04 mg/kg in wort.
05.158	3-Methoxybenzaldehyde	3900 mg/kg in clove bud (oil).
08.080	Gallic acid	Up to 0.6 mg/kg in beer, up to 7 mg/kg in cherry, up to 11 mg/kg in grape, up to 6.1 mg/kg in whisky, up to 35 mg/kg in wine.
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	Up to 1.1 mg/kg in beer, 1.3 mg/kg in grape, up to 0.096 mg/kg in mango, up to 18 mg/kg in rum, up to 34 mg/kg in soybean, up to 1.4 mg/kg in whisky, up to 10 mg/kg in wine.
08.132	3-Hydroxybenzoic acid	Up to 2.7 mg/kg in honey.

Table 1.3.1 Candidate Substances Reported to Occur in Food (TNO, 2000; EFFA, 2010a; TNO, 2012)

FL-no:	Name:	Quantitative data reported:
08.133	3,4-Dihydroxybenzoic acid	Up to 1.4 mg/kg in brandy, up to 0.4 mg/kg in beer, up to 52 mg/kg in black currants, up to 6.8 mg/kg in honey, 4.3 mg/kg in mulberry, 0.15 mg/kg in rum, 10 mg/kg in soybean, up to 0.3 mg/kg in whisky, up to 10 mg/kg in wine.
09.152	Benzyl valerate	0.11 mg/kg in sea buckthorn.
09.314	Benzyl crotonate	0.0001 mg/kg in papaya.
09.779	Butyl benzoate	200 mg/kg in galanga (oil), 2 mg/kg in hog plum, up to 0.05 mg/kg in papaya.
09.798	Ethyl vanillate	0.3 mg/kg in rum, up to 113 mg/kg in red wine.
09.799	Methyl vanillate	0.05 mg/kg in cloudberry, up to 214 mg/kg in red wine.
09.825	Pentyl benzoate	0.001 mg/kg in bilberry, trace amounts in sapodilla fruit.

According to TNO the remaining 20 substances have not been reported to occur naturally in any food items.

Table 1.3.2 Candidate Substances Not Reported to Occur in Food (TNO, 2000; TNO, 2012)

FL-no:	Name:
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol
09.858	Phenylmethyl 2-methyl-2-butenoate
09.315	Benzyl dodecanoate
09.317	Benzyl lactate
09.318	Benzyl octanoate
09.362	Ethyl 2-hydroxy-4-methylbenzoate
09.560	Hex-3(cis)-enyl anisate
09.581	Hexyl salicylate
09.611	4-Isopropylbenzyl acetate
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate
09.656	3-Methylbut-3-enyl benzoate
09.693	Prenyl benzoate
09.696	Prenyl salicylate
09.762	Pentyl salicylate
09.835	Benzyl decanoate
09.852	2-Methylbutyl 2-hydroxybenzoate
09.895	4-Methoxybenzyl-2-methylpropionate
05.066	4-Ethoxy-3-methoxybenzaldehyde
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde
06.104	Vanillin propylene glycol acetal

2. SPECIFICATIONS

Purity criteria for the 45 candidate substances have been provided by the Flavour Industry (EFFA, 2003u; EFFA, 2004c; EFFA, 2007d; EFFA, 2011e; Flavour Industry, 2008c).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for all the 45 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 population[4] (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).

[4] 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.

The total annual volume of production of the candidate substances in the present Flavouring Group Evaluation (FGE.20Rev4) from use as flavouring substances in Europe has been reported to be approximately 12500 kg (EFFA, 2003u; EFFA, 2004d; EFFA, 2007d; EFFA, 2011e; EFFA, 2012m; Flavour Industry, 2008c). For 76 of the 77 supporting substances the total annual volume of production is approximately 660000 kg in Europe (vanillin [FL-no: 05.018] accounts for 390000 kg) (JECFA, 2002a). The annual volume of production in Europe for one of the supporting substances [FL-no: 09.754] has not been reported.

On the basis of the annual volumes of production reported for the 45 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated. Approximately 97 % of the annual volume of production for the candidate substances is accounted for by four substances [FL-no: 05.029; 06.104, 08.132 and 08.133]. The estimated daily *per capita* intake of these four candidate substances from use as flavouring substances is 160, 100, 610 and 610 microgram, respectively. For each of the remaining substances the estimated daily *per capita* intake is 10 microgram or less (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 all candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c). The candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. 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] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] 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).
[7] 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

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except [FL-no: 08.132, 08.133, 09.858]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.858]
03.0	Edible ices, including sherbet and sorbet	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.858]
04.1	Processed fruits	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
07..0	Bakery wares	All except [FL-no 05.129, 08.132, 08.133]
08.0	Meat and meat products, including poultry and game	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.825]
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: 05.026, 05.028, 05.029, 08.132, 08.133]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 09.858]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 09.858]
15.0	Ready-to-eat savouries	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.693, 09.858]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.858]

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 1 - 500 mg/kg food, and the maximum use levels are in the range of 5 - 2000 mg/kg (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c).

The mTAMDI value is 770 - 120000 microgram/person/day for the 41 candidate substances from structural class I (see Section 5). For the candidate substances [FL-no: 02.205, 05.066, 05.221, and 06.104] from structural class II (see Section 5) the mTAMDIs are 3900, 1600, 7000 and 3900 microgram/person/day, respectively.

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 45 candidate substances are subdivided into three subgroups. Subgroup 1 includes three tolualdehyde isomers and 16 benzyl derivatives of which 15 are benzyl esters or benzoic acid esters and one is an acetal, [FL-no: 06.017] (diethoxymethyl)benzene. Subgroup 2 includes 25 hydroxy- and alkoxy-substituted benzyl derivatives of which 12 are benzyl alcohols, benzaldehydes or benzoic acids and 13 are related esters. Subgroup 3 contains one derivative of biphenyl [FL-no: 05.221] (6,6'-dihydroxy-5,5'dimethoxy-biphenyl-3,3'-dicarbaldehyde).

Subgroup 1

Nine of the 15 esters from subgroup 1, benzyl valerate [FL-no: 09.152], benzyl 2-methylbutyrate [FL-no: 09.313], benzyl crotonate [FL-no: 09.314], benzyl dodecanoate [FL-no: 09.315], benzyl hexanoate [FL-no: 09.316], benzyl lactate [FL-no: 09.317], benzyl octanoate [FL-no: 09.318], benzyl decanoate [FL-no: 09.835] and phenylmethyl 2-methyl-2-butenoate [FL-no: 09.858], will yield benzyl alcohol, which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester, 4-isopropylbenzyl acetate [FL-no: 09.611], will yield 4-isopropylbenzyl alcohol, previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidised to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids. Of the remaining five candidate esters in subgroup 1, four are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825] and prenyl benzoate [FL-no: 09.693]. One ester, methyl 4-methylbenzoate [FL-no: 09.631], will yield 4-methylbenzoic acid upon hydrolysis. Benzoic acid will mainly be conjugated with glycine and excreted as hippuric acid. Conjugation with glycine may be a saturable process with increasing levels of exposure and glucuronide conjugation may become relatively more important.

One of the substances in subgroup 1 is an acetal, (diethoxymethyl)benzene [FL-no: 06.017]. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by the JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid and subsequently conjugated with glycine or glucuronic acid and eliminated via the urine. The same biotransformations will occur with the three tolualdehyde isomers ([FL no: 05.026, 05.028 and 05.029]). Additionally, for the tolualdehyde isomers, reduction of the aldehyde function to yield the corresponding alcohol has also been demonstrated. These alcohols can be converted into the corresponding sulphate esters, which in their turn can further react with glutathione to give benzylmercapturic acids. This metabolic pathway is more important for *o*-toluadehyde than for the other two isomers, but at any rate, only a limited fraction of the dose (< 10 %) will be eliminated via this route.

Subgroup 2

Subgroup 2 includes 13 esters of which one, 4-methoxybenzyl-2-methylpropionate [FL-no: 09.895], will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxybenzyl alcohol is expected to be excreted in the urine either unchanged or as glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative, 4-hydroxy-3,5-dimethoxybenzyl alcohol [FL-no: 02.164].

The remaining 12 esters in subgroup 2, ethyl 2-hydroxy-4-methylbenzoate [FL-no: 09.362], ethyl 2-methoxybenzoate [FL-no: 09.363], ethyl 4-hydroxybenzoate [FL-no: 09.367], hex-3(cis)-enyl anisate [FL-no: 09.560] (hex-3(cis)-enyl 4-methoxybenzoate), hex-3-enyl salicylate [FL-no: 09.570] (hex-3-enyl 2-hydroxybenzoate), hexyl salicylate [FL-no: 09.581] (hexyl 2-hydroxybenzoate), methyl 2,4-dihydroxy-3,6-dimethylbenzoate [FL-no: 09.623], prenyl salicylate [FL-no: 09.696] (3-methylbut-2-enyl 2-hydroxybenzoate), pentyl salicylate [FL-no: 09.762] (pentyl 2-hydroxybenzoate), ethyl vanillate [FL-no: 09.798] (ethyl 3-methoxy-4-hydroxybenzoate), methyl vanillate [FL-no: 09.799]

(methyl 3-methoxy-4-hydroxybenzoate), 2-methylbutyl 2-hydroxybenzoate [FL-no: 09.852] (2-methylbutyl salicylate) will yield alkoxy- and/or hydroxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine unchanged or as the glucuronic acid, glycine or sulphate conjugates. The same metabolic route is proposed for the candidate acids, 4-hydroxy-3,5-dimethoxybenzoic acid [FL-no: 08.087], 3-hydroxybenzoic acid [FL-no: 08.132] and 3,4-dihydroxybenzoic acid [FL-no: 08.133].

The main metabolic pathway for the acetal, vanillin propylene glycol acetal [FL-no: 06.104], after hydrolysis to the aldehyde, and for the five candidate aldehydes in subgroup 2, 4-ethoxy-3-methoxybenzaldehyde [FL-no: 05.066], 2-methoxybenzaldehyde [FL-no: 05.129], 3,4-dihydroxybenzaldehyde [FL-no: 05.142], 4-hydroxy-3,5-dimethoxybenzaldehyde [FL-no: 05.153], 3-methoxybenzaldehyde [FL-no: 05.158], is presumed to be oxidation of the aldehyde to the corresponding acids, followed by conjugation and excretion. The reduction to alcohols is a minor metabolic route and the oxidative pathway dominates clearly. For 2-methoxybenzaldehyde it has been shown that this reductive metabolic pathway leads to the formation of sulphate conjugates, which are converted into glutathione conjugates. The latter are eliminated via the urine as mercapturic acids. To a minor extent O-demethylation followed by conjugation may occur.

The candidate substance piperonyl alcohol [FL-no: 02.205] (3,4-methylenedioxybenzylalcohol) is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as glycine conjugate. Demethylenation of the methylenedioxy moiety is a very minor metabolic path for this compound.

The main metabolite of gallic acid [FL-no: 08.080] (3,4,5-trihydroxybenzoic acid) is expected to be 4-O-methyl gallic acid (3,5-dihydroxy-4-methoxybenzoic acid), the product of O-methylation. Decarboxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

Subgroup 3

The biphenyl substance in subgroup 3 [FL-no: 05.221] is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2. It is expected that the aldehyde group(s) will undergo oxidation to form the corresponding carboxylic acid which is likely to be conjugated and excreted. The reduction to alcohol may again be a minor pathway, but some steric hindrance may occur making this less likely than for the benzaldehyde derivatives in subgroup 2.

Based on experimental evidence and general knowledge of toxicokinetics of structurally related compounds, it is expected, that at the reported levels of intake as flavouring substances, the candidate substances are 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 45 candidate substances from chemical groups 23 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2a.

Step 1

Forty-one of the flavouring substances are classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978) into structural class I, four are classified into structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104].

Step 2

Step 2 requires consideration of the metabolism of the candidate substances. It can be anticipated that at the estimated levels of intake all candidate substances are expected to be metabolised to innocuous products. Accordingly, the evaluation of these substances proceeds via the A-side of the Procedure scheme.

Step A3

The estimated levels of the European daily *per capita* intake (MSDI) for the 41 candidate substances classified into structural class I are in the range of 0.0012 to 610 micrograms. For the four candidate substances [FL-no: 02.205, 05.066, 05.221 and 06.104] classified into structural class II, the intakes are 0.011, 1.2, 0.61 and 100 micrograms, respectively (Table 2a). These intakes are below the thresholds of concern of 1800 and 540 microgram/person/day for structural class I and II, respectively.

Based on results of the safety evaluation sequence of the Procedure, these 45 candidate substances do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

6. COMPARISON OF THE INTAKE ESTIMATIONS BASED ON THE MSDI APPROACH AND THE MTAMDI APPROACH

The estimated intakes for the 41 candidate substances in structural class I, based on the mTAMDI approach, range from 770 to 120000 microgram/person/day. For six of the substances [FL-no: 05.129, 05.142, 05.153, 05.158, 08.080 and 09.858] the mTAMDI values are below the threshold of concern of 1800 microgram/person/day for structural class I. For the remaining 35 substances in class I, the mTAMDI is above the threshold of concern.

The estimated intake of the four candidate substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II, based on the mTAMDI, are 3900, 1600, 7000 and 3900 microgram/person/day, respectively. These intakes are above the threshold of concern of 540 microgram/person/day for structural classes II.

Thus, for 39 candidate substances, for which the mTAMDI is above the threshold of concern, further information is required. This would include more reliable intake data and subsequently, if required, additional toxicological data.

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)
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol	0.037	3900	Class I	1800
05.026	o-Tolualdehyde	1.0	9100	Class I	1800
05.028	m-Tolualdehyde	0.85	9100	Class I	1800
05.029	p-Tolualdehyde	160	9100	Class I	1800

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)
05.129	2-Methoxybenzaldehyde	0.16	1400	Class I	1800
05.142	3,4-Dihydroxybenzaldehyde	8.5	1600	Class I	1800
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	0.74	1600	Class I	1800
05.158	3-Methoxybenzaldehyde	0.011	1600	Class I	1800
06.017	(Diethoxymethyl)benzene	1.7	3900	Class I	1800
08.080	Gallic acid	0.011	1600	Class I	1800
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	1.2	3200	Class I	1800
08.132	3-Hydroxybenzoic acid	610	120000	Class I	1800
08.133	3,4-Dihydroxybenzoic acid	610	120000	Class I	1800
09.152	Benzyl valerate	1.7	3900	Class I	1800
09.313	Benzyl 2-methylbutyrate	7.3	3900	Class I	1800
09.314	Benzyl crotonate	0.37	3900	Class I	1800
09.315	Benzyl dodecanoate	0.13	3900	Class I	1800
09.316	Benzyl hexanoate	0.75	3900	Class I	1800
09.317	Benzyl lactate	0.91	3900	Class I	1800
09.318	Benzyl octanoate	0.12	3900	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	0.0012	3900	Class I	1800
09.363	Ethyl 2-methoxybenzoate	5.5	3900	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	10	3900	Class I	1800
09.560	Hex-3(cis)-enyl anisate	0.12	3900	Class I	1800
09.570	Hex-3-enyl salicylate	0.13	3900	Class I	1800
09.581	Hexyl salicylate	0.018	3900	Class I	1800
09.611	4-Isopropylbenzyl acetate	0.012	3900	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	0.012	3900	Class I	1800
09.631	Methyl 4-methylbenzoate	0.0012	3900	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	0.12	3900	Class I	1800
09.693	Prenyl benzoate	0.012	4900	Class I	1800
09.696	Prenyl salicylate	0.011	3900	Class I	1800
09.762	Pentyl salicylate	0.24	3900	Class I	1800
09.779	Butyl benzoate	3.7	3900	Class I	1800
09.798	Ethyl vanillate	0.024	3900	Class I	1800
09.799	Methyl vanillate	0.011	3900	Class I	1800
09.825	Pentyl benzoate	1.1	3900	Class I	1800
09.835	Benzyl decanoate	0.35	3900	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	0.011	3900	Class I	1800
09.858	Phenylmethyl 2-methyl-2-butenoate	0.037	770	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	0.37	3900	Class I	1800
02.205	Piperonyl alcohol	0.011	3900	Class II	540
05.066	4-Ethoxy-3-methoxybenzaldehyde	1.2	1600	Class II	540
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde	0.61	7000	Class II	540
06.104	Vanillin propylene glycol acetal	100	3900	Class II	540

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, 2003u; EFFA, 2004d; EFFA, 2007d; EFFA, 2011e; EFFA, 2012m; Flavour Industry, 2008c) the combined estimated daily *per capita* intake as flavourings of the 41 candidate flavouring substances assigned to structural class I is 1400 microgram. This value does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. The combined estimated daily *per capita* intake as

flavourings of the four candidate flavouring substances assigned to structural class II is 100 microgram. This value does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

The candidate substances are structurally related to 77 supporting substances evaluated by the JEFCA at its 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. Production volumes in Europe were not reported for one of the supporting substances [FL-no: 09.754].

The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100 microgram/*capita*/day for structural class I and II, respectively, which exceed the thresholds of concern of 1800 and 540 microgram/*capita*/day for structural classes I and II, respectively. However, the supporting substances were evaluated by the JECFA at the 46th and 57th meeting, where it was noted that although the combined intakes exceed the thresholds for the structural classes, the substances are expected to be efficiently detoxicated and the available detoxication pathways would not be saturated.

The Panel agreed with this view and concluded that the contributions to the total combined intakes of the candidate substances of about 1400 and 100 microgram/*capita*/day for structural class I and II, respectively, would not alter the JECFA conclusion based on combined intakes of approximately 75000 and 7100 microgram/*capita*/day for the two classes. The Panel noted that a considerable proportion of this combined intake is accounted for by the supporting substance vanillin [FL-no: 05.018] and for this compound the JECFA has allocated an Acceptable Daily Intake (ADI) of 0 - 10 mg/kg body weight (bw) (JECFA, 1967a; JECFA, 2002b).

8. TOXICITY

8.1. Acute Toxicity

Data are available for 13 candidate substances and for 63 structurally related supporting substances evaluated by the JECFA (JECFA, 2002a). The LD_{50} values range from 500 to more than 5000 mg/kg body weight (bw) in four different animal species.

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Benzyl Derivatives (Subgroup 1)

There are no data available on short-term and long-term toxicity of candidate substances from subgroup 1 (benzyl derivatives). Data on benzyl derivatives are available for 10 supporting substances, which have been tested for subacute oral toxicity [FL-no: 05.110], for subchronic oral toxicity [FL-no: 09.051, 09.725, 09.812, 09.803 and 05.027] and for chronic toxicity and carcinogenicity [FL-no: 02.010, 09.014, 05.013 and 08.021].

Results from carcinogenicity studies on benzyl alcohol, benzyl acetate and benzaldehyde by administration via gavage showed that in some of the studies, in mice benign squamous cell hyperplasia and papillomas of the forestomach could be induced without progression into malignant carcinomas. In male mice benzyl acetate also induced adenomas (no carcinomas) in the liver. No tumorigenic effects were observed in rats. These substances are not genotoxic. The Panel considered the observed pathological changes in mouse forestomach and livers to be of no toxicological relevance for humans following dietary exposure at the indicated levels of use. This consideration is based on the observation that the spontaneous incidence of tumors is that high that the mouse carcinogenicity

study does not contribute to human risk assessment (Billington et al., 2010; Carmichael et al., 1997; EFSA, 2012). These substances have been evaluated by the JECFA (JECFA, 1996b). The JECFA concluded that "the data reviewed for compounds in this group were sufficient to demonstrate the lack of teratogenic, reproductive or carcinogenic potential". A group ADI of 0 - 5 mg/kg bw was allocated to these compounds. The SCF evaluated data on benzyl alcohol (SCF, 2002b) and concluded that it did not show compound-related effects with respect to carcinogenicity.

Four candidate esters in subgroup 1, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825], prenyl benzoate [FL-no: 09.693], yield benzoic acid and simple aliphatic alcohols upon hydrolysis. One of the substances in subgroup 1 is an acetal, (diethoxymethyl)benzene [FL-no: 06.017], which would be expected to yield benzaldehyde and ethanol upon hydrolysis, and in turn benzaldehyde is expected to be oxidized to benzoic acid. The SCF (2002) has established a group ADI of 5 mg/kg bw for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings, based on a developmental toxicity study in rats (SCF, 2002c)[8].

From two studies with the supporting substance [FL no: 05.027], a mixture of tolualdehyde isomers, a NOAEL of 250 mg/kg bw could be derived, based on the study by Brantom et al., 1972 (Brantom et al., 1972). The second study (Oser et al., 1965) only provide information that no toxicity was observed at one exposure level of *ca.* 40 mg/kg bw/day when administered to male and female rats for 90 days.

Hydroxy-/Alkoxy- Substituted Benzyl Derivatives (Subgroup 2)

Short- and long-term toxicity data on hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2) are available for five candidate substances and eight supporting substances. The candidate substances have been tested for subacute oral toxicity [FL-no: 05.142, 08.080, 08.087 and 08.133] and for subchronic oral toxicity [FL-no: 08.080, 08.133 and 09.367]. There are data available on chronic toxicity and carcinogenicity for one candidate substance [FL-no: 08.133] in a study designed to evaluate incidences of lesions (hyperplasia, papillomas, squamous cell carcinoma and sarcoma incidence) in the forestomach in rats. No lesions developed in the forestomach of the rats. Other organs (oesophagus, stomach, intestines, liver and kidney) were inspected grossly. Mean body weight was not different from the control, but relative liver and kidney weight was significantly increased, but not further evaluated.

For the supporting substances, data are available on subacute oral toxicity [FL-no: 09.796], on subchronic oral toxicity [FL-no: 05.015 and 09.751] and on chronic toxicity and carcinogenicity [FL-no: 09.754, 05.018, 09.749, 05.019 and 05.016].

Ethyl 4-hydroxybenzoate and other parabens were evaluated by SCF in 1994 (SCF, 1996). From subchronic and chronic toxicity tests conducted in rats, dogs and mice, an overall NOAEL of 1000 mg/kg bw/day was derived. This NOAEL value has been confirmed for ethyl- and methyl paraben by EFSA (EFSA, 2004b).

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

There are data available for one candidate substance [FL-no: 09.367] (subgroup 2) and for 12 supporting substances of which four belong to subgroup 1 [FL-no: 02.010, 05.013, 08.021 and 09.014] and eight to subgroup 2 [FL-no: 05.016, 05.017, 05.018, 05.019, 08.076, 08.112, 09.749 and 09.754].

[8] The CEF panel is aware that the benzoic acid is currently under reviewing in the ANS Panel.

For the candidate substance ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367], a NOAEL of 2600 mg/kg bw/day has been reported for developmental toxicity in rats (Moriyama et al, 1975), while a NOAEL of 460 mg/kg bw/day was found in the same study for maternal toxicity. From another study a NOAEL of 1043 mg/kg bw/day is available for reproductive toxicity in male rats (Oishi, 2004). Ethyl paraben has been evaluated as a food additive by the AFC panel, and the Panel considered 1000 mg/kg bw/day as the overall NOAEL, based on the absence of effects on sex hormones and on the male reproductive organs in juvenile rats at doses up to 1000 mg/kg bw/day in the above study (EFSA, 2004b).

As there are valid and sufficient studies available on the candidate substance ethyl paraben, the data on the supporting substance butyl paraben [FL-no: 09.754] were not considered for the evaluation of ethyl paraben.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for 12 candidate substances [subgroup 1: FL-no: 05.026, 05.028, 05.029, 09.631; subgroup 2: FL-no: 09.367, 05.129, 05.158, 08.080, 05.153, 08.087 and 08.133; subgroup 3: FL-no: 05.221], for 28 supporting substances (12 from subgroup 1 and 16 from subgroup 2) and for one related substance (vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] related to subgroup 2). Data from *in vivo* tests are available for two candidate substances from subgroup 2 [FL-no: 09.367 and 08.080] and for 10 supporting substances (three from subgroup 1 and seven from subgroup 2).

All the candidate substances [FL-no: 05.026, 05.028, 05.029, 05.129, 05.153, 05.221, 08.080, 08.087, 09.367 and 09.631] tested for bacterial gene mutations gave negative results. For six candidate substances [FL-no: 09.367, 05.129, 05.158, 08.080, 08.087 and 08.133] both positive and/or negative results were reported in various other *in vitro* test systems (Rec assay, chromosomal aberration test, sister chromatid exchange (SCE) and mammalian cell gene mutation assays (mouse lymphoma tests and silk worm)) for most of which the validity cannot be evaluated or are known to be of very limited relevance.

The same situation was observed for the supporting substances. All the available bacterial gene mutation assays on supporting substances gave negative results. Both positive and negative results were reported in other *in vitro* test systems (Rec assay, chromosomal aberration test, sister chromatid exchange (SCE) and mammalian cell gene mutation assay) for most of which, however, the validity cannot be evaluated.

The available *in vivo* studies on candidate substances reported negative results for ethyl 4-hydroxybenzoate [FL-no: 09.367] in a chromosome aberration assay in rat bone marrow cells and for gallic acid [FL-no: 08.080] in a bioassay in the rat liver. However, due to very limited details on method and results, the validity of these studies cannot be evaluated.

The Panel noted that the supporting substance benzyl acetate [FL-no: 09.014] was positive in an *in vivo* Comet assay, which may indicate a genotoxic activity at high dose levels. The study was considered of limited validity. However, all other *in vivo* studies with benzyl acetate were negative and several of these studies, among which an unscheduled DNA synthesis (UDS) test in the liver and a mouse bone marrow micronucleus test, were considered to be of good quality (NTP, 1993d). Additionally, in the long term carcinogenicity studies with benzyl acetate (Table IV.2), no carcinogenic effects were observed in mice and rats after administration via the diet (NTP, 1993d). In a previous study by NTP (1986) in which this substance was administered by gavage in corn oil, concern was raised, in particular about pancreatic tumours in rats, but for these tumours a confounding influence of the vehicle was suspected. In two other genotoxicity studies, specifically aiming at the

determination of benzyl acetate-induced DNA damage (UDS test and alkaline elution assay) in rat pancreas, no indications of a genotoxic effect were obtained, although these studies were of limited or inassessible validity. Taking all this information into account, the Panel considered the positive result from the *in vivo* Comet assay as insufficient grounds to preclude the evaluation of benzyl acetate via the Procedure.

Furthermore, all the studies carried out with 10 different supporting substances, among which were benzyl alcohol, benzyl acetate and benzaldehyde, give no indication of a genotoxic potential *in vivo* in several studies for different genetic endpoints and by different routes of administration.

Conclusion on genotoxicity:

While some of the *in vitro* studies indicated equivocal weak positive or positive results, considering the weight of evidence from candidate and supporting substances and the *in vivo* studies, the Panel concluded there was no safety concern with respect to genotoxicity of the substances in the present flavouring group.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

CONCLUSIONS

The present revision of FGE.20, FGE.20Rev4, includes the assessment of four additional substances, *o*- *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenoate [09.858].

So, the present FGE.20Rev4 deals in total with 45 benzyl alcohols, benzaldehydes, related acetals, benzoic acids and related esters and a hydroxy- and alkoxy-substituted biphenyl derivative. These flavouring substances belong to chemical groups 23 and 30 of Annex I of Regulation (EC) No 1565/2000.

Four flavouring substances can exist as optical isomers [FL-no: 06.104, 09.313, 09.317 and 09.852] and four substances can exist as geometrical isomers [FL-no: 09.314, 09.560, 09.570 and 09.858].

Forty-one candidate substances are classified into structural class I and four [FL-no: 02.205, 05.066, 05.221 and 06.104] are classified into structural class II according to the decision tree approach presented by Cramer et al., 1978.

Twenty-five 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 41 flavouring substances allocated to structural class I have intakes in Europe from 0.001 to 610 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day). The four substances in structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104] have estimated intakes of 0.011, 1.2, 0.61 and 100 microgram/*capita*/day, respectively. These intakes are below the threshold values of 540 microgram/person/day for structural class II.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 41 of the candidate substances belonging to structural class I is approximately 1400 microgram/*capita*/day and the combined intake of the four candidate substances belonging to structural class II is approximately 100 microgram/*capita*/day. These values are lower than the threshold of concern for structural class I and II substances. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100

microgram/*capita*/day for structural class I and II, respectively, which exceed the thresholds of concern. However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

For the substances in this group the available genotoxicity data do not preclude the evaluation of the candidate substances using the Procedure.

It is anticipated that the candidate substances in FGE.20Rev4 would be metabolised to innocuous products.

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 on the basis of the default MSDI approach the 45 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 770 to 120000 microgram/person/day for 41 flavouring substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for six flavouring substances [FL-no: 05.129, 05.142, 05.153, 05.158, 08.080 and 09.858]. The estimated intakes, based on the mTAMDI, of the four flavouring substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II were 3900, 1600, 7000 and 3900 microgram/person/day, respectively, which are above the threshold of concern for the structural class (540 microgram/person/day). The six substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products. Thus, on the basis of the mTAMDI, the estimated intakes for 39 flavouring substances considered in this Opinion, exceed the relevant threshold for their structural class to which the flavouring substance has been assigned. Therefore, for these 39 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 45 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 each of the 45 flavouring substances.

For these 45 flavouring substances the Panel concluded that they would present no safety concern at their estimated levels of intake based of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 20, REVISION 4

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

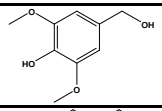
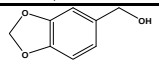
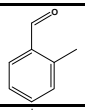
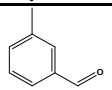
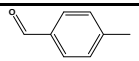
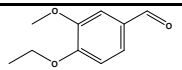
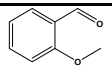
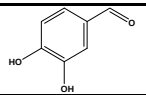
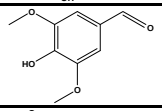
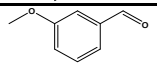
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		 530-56-3	Solid $C_9H_{12}O_4$ 184.19	Practically insoluble or insoluble Freely soluble	387 133 MS 95 %	n.a. n.a.	
02.205	Piperonyl alcohol		 10306 495-76-1	Solid $C_8H_8O_3$ 152.15	Very slightly soluble Freely soluble	161 (26 hPa) 55 MS 95 %	n.a. n.a.	
05.026	o-Tolualdehyde		3068 529-20-4	Liquid C_8H_8O 120.15	Soluble Soluble	200 MS 95 %	1.540-1.547 1.013-1.029	
05.028	m-Tolualdehyde		3068 620-23-5	Liquid C_8H_8O 120.15	Soluble Soluble	199 MS 95 %	1.540-1.549 1.013-1.029	
05.029	p-Tolualdehyde		3068 104-87-0	Liquid C_8H_8O 120.15	Soluble Soluble	204 MS 95 %	1.540-1.547 1.013-1.027	
05.066	4-Ethoxy-3-methoxybenzaldehyde		 703 120-25-2	Solid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	168 (17 hPa) 63 MS 95 %	n.a. n.a.	
05.129	2-Methoxybenzaldehyde		4077 10350 135-02-4	Solid $C_8H_8O_2$ 136.15	Practically insoluble or insoluble Freely soluble	238 38 MS 97 %	1.556-1.562 1.128-1.136	
05.142	3,4-Dihydroxybenzaldehyde		 10328 139-85-5	Solid $C_7H_6O_3$ 138.12	Slightly soluble Freely soluble	323 154 MS 98 %	n.a. n.a.	
05.153 1878	4-Hydroxy-3,5-dimethoxybenzaldehyde		 10340 134-96-3	Solid $C_9H_{10}O_4$ 182.18	Practically insoluble or insoluble Freely soluble	192 (19 hPa) 113 MS 95 %	n.a. n.a.	
05.158	3-Methoxybenzaldehyde		 10351 591-31-1	Liquid $C_8H_8O_2$ 136.15	Practically insoluble or insoluble Freely soluble	230 MS	1.549-1.555 1.116-1.122	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

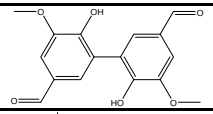
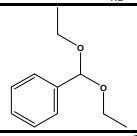
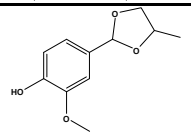
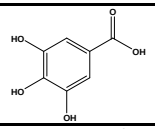
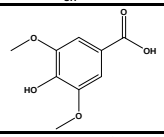
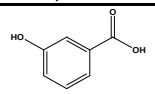
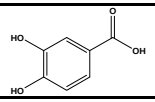
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
05.221 1881	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde		 2092-49-1	Solid $C_{16}H_{14}O_6$ 302.28	Practically insoluble or insoluble Soluble	 315 MS 91.4 %	n.a. n.a.	Secondary component is Vanillin at 5-7 % (EFFA, 2012k).
06.017	(Diethoxymethyl)benzene		 517 774-48-1	Liquid $C_{11}H_{16}O_2$ 180.25	Practically insoluble or insoluble Freely soluble	222 MS 95 %	1.475-1.481 0.903-0.909	
06.104 1882	Vanillin propylene glycol acetal		3905 68527-74-2	Liquid $C_{11}H_{14}O_4$ 210.23	Practically insoluble or insoluble Freely soluble	154 (0.1 hPa) NMR 97 %	1.537-1.543 1.190-1.206	Commercial compound: Vanillin propylene glycol acetal up to 80 % and with 18-20 % vanillin (EFFA, 2010a). Four diastereoisomers (RR, RS, SS & SR - or two trans forms (RR & SS) & two cis forms (RS & SR)). The composition will be 50-70 % trans (50/50 for RR/SS) and 30-50 % cis (50/50 for RS/SR)(EFFA, 2012u).
08.080	Gallic acid		 10170 149-91-7	Solid $C_7H_6O_5$ 170.12	Sparingly soluble Freely soluble	501 242 MS 95 %	n.a. n.a.	
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		 10111 530-57-4	Solid $C_9H_{10}O_5$ 198.18	Sparingly soluble Freely soluble	440 206 MS 95 %	n.a. n.a.	
08.132	3-Hydroxybenzoic acid		 99-06-9	Solid $C_7H_6O_3$ 138.12	Soluble Soluble	 202 IR NMR MS >99%	n.a. n.a.	
08.133	3,4-Dihydroxybenzoic acid		 99-50-3	Solid $C_7H_6O_4$ 154.12	Soluble Soluble	 221 IR NMR MS >99%	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

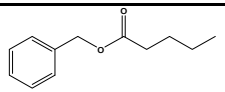
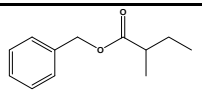
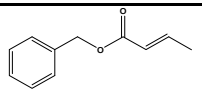
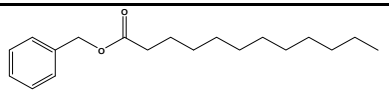
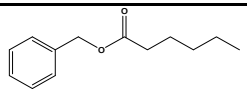
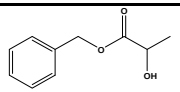
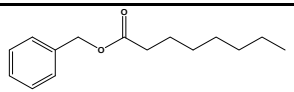
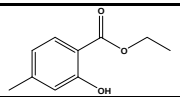
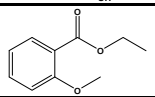
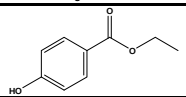
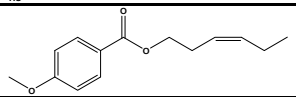
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.152	Benzyl valerate		470 10361-39-4	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	236 MS 95 %	1.487-1.493 0.990-0.996	
09.313	Benzyl 2-methylbutyrate		10523 56423-40-6	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	248 MS 99 %	1.486-1.495 0.982-0.994	Racemate.
09.314	Benzyl crotonate		65416-24-2	Liquid $C_{11}H_{12}O_2$ 176.21	Practically insoluble or insoluble Freely soluble	138 (16 hPa) MS 95 %	1.515-1.521 1.029-1.035	
09.315	Benzyl dodecanoate		140-25-0	Liquid $C_{19}H_{30}O_2$ 290.44	Practically insoluble or insoluble Freely soluble	210 (16 hPa) MS 95 %	1.479-1.485 0.937-0.943	
09.316	Benzyl hexanoate		10521 6938-45-0	Liquid $C_{13}H_{18}O_2$ 206.28	Practically insoluble or insoluble Freely soluble	270 MS 99 %	1.486-1.492 0.978-0.985	
09.317	Benzyl lactate		2051-96-9	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	134 (13 hPa) MS 95 %	1.512-1.518 1.120-1.144	Racemate.
09.318	Benzyl octanoate		10276-85-4	Liquid $C_{15}H_{22}O_2$ 234.34	Practically insoluble or insoluble Freely soluble	153 (8 hPa) MS 95 %	1.484-1.490 0.960-0.966	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		60770-00-5	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	254 MS 95 %	1.514-1.520 1.088-1.094	
09.363	Ethyl 2-methoxybenzoate		7335-26-4	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	235 MS 95 %	1.519-1.525 1.109-1.115	
09.367	Ethyl 4-hydroxybenzoate		120-47-8	Solid $C_9H_{10}O_3$ 166.18	Slightly soluble Freely soluble	298 118 MS 95 %	n.a. n.a.	
09.560	Hex-3(cis)-enyl anisate		121432-33-5	Solid $C_{14}H_{18}O_3$ 234.29	Practically insoluble or insoluble Freely soluble	363 73 NMR 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

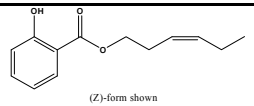
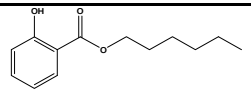
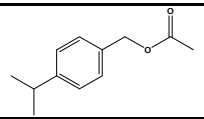
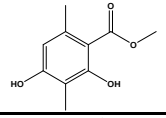
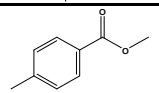
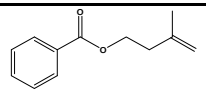
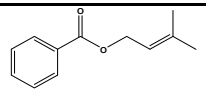
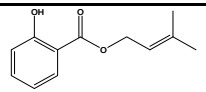
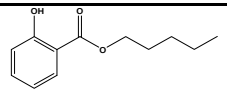
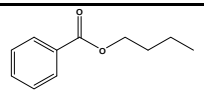
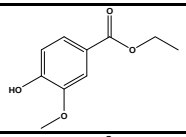
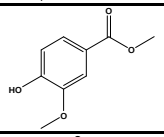
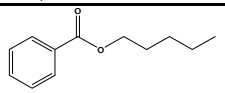
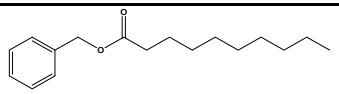
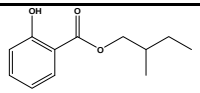
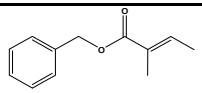
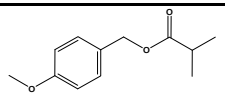
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.570	Hex-3-enyl salicylate	 (Z)-form shown	10685 65405-77-8	Solid $C_{13}H_{16}O_3$ 220.26	Practically insoluble or insoluble Freely soluble	394 139 MS 98 %	1.518-1.522 1.057-1.065	Register name to be changed to (Z)-Hex-3-enyl salicylate (EFFA, 2010a).
09.581	Hexyl salicylate		10695 6259-76-3	Liquid $C_{13}H_{18}O_3$ 222.28	Practically insoluble or insoluble Freely soluble	290 MS 99 %	1.501-1.507 1.029-1.040	
09.611	4-Isopropylbenzyl acetate		59230-57-8	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	250 MS 95 %	1.494-1.500 0.998-1.004	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		4707-47-5	Solid $C_{10}H_{12}O_4$ 196.20	Slightly soluble Freely soluble	246 143 MS 95 %	n.a. n.a.	
09.631	Methyl 4-methylbenzoate		99-75-2	Solid $C_9H_{10}O_2$ 150.18	Practically insoluble or insoluble Freely soluble	421 33 MS 95 %	n.a. n.a.	
09.656	3-Methylbut-3-enyl benzoate		5205-12-9	Liquid $C_{12}H_{14}O_2$ 190.24	Practically insoluble or insoluble Freely soluble	60 (0.1 hPa) MS 95 %	1.499-1.505 0.986-0.992	
09.693	Prenyl benzoate		4203 5205-11-8	Liquid $C_{12}H_{14}O_2$ 190.24	Practically insoluble or insoluble Freely soluble	60 (0.1 hPa) MS 95 %	1.505-1.511 0.982-0.988	
09.696	Prenyl salicylate		68555-58-8	Solid $C_{12}H_{14}O_3$ 206.24	Practically insoluble or insoluble Freely soluble	370 113 MS 95 %	n.a. n.a.	
09.762	Pentyl salicylate		613 2050-08-0	Liquid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble Freely soluble	268 MS 95 %	1.533-1.539 1.062-1.068	
09.779	Butyl benzoate		740 136-60-7	Liquid $C_{11}H_{14}O_2$ 178.23	Practically insoluble or insoluble Freely soluble	249 MS 95 %	1.493-1.499 1.003-1.009	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

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
09.798	Ethyl vanillate		2302 617-05-0	Solid $C_{10}H_{12}O_4$ 196.20	Practically insoluble or insoluble Freely soluble	292 44 MS 95 %	n.a. n.a.	
09.799	Methyl vanillate		2305 3943-74-6	Solid $C_9H_{10}O_4$ 182.18	Sparingly soluble Freely soluble	286 63 MS 95 %	n.a. n.a.	
09.825	Pentyl benzoate		2307 2049-96-9	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	260 MS 95 %	1.482-1.493 0.989-0.993	
09.835	Benzyl decanoate		42175-41-7	Solid $C_{17}H_{26}O_2$ 262.39	Practically insoluble or insoluble Freely soluble	400 76 MS 95 %	n.a. n.a.	
09.852	2-Methylbutyl 2-hydroxybenzoate		51115-63-0	Solid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble Freely soluble	366 117 MS 95 %	n.a. n.a.	Racemate.
09.858	Phenylmethyl 2-methyl-2-butenoate		3330 2184 67674-41-3	Liquid $C_{12}H_{14}O_2$ 190.24	Insoluble Soluble	250 IR 95 %	1.515-1.526 1.029-1.040	Mixture of (Z)- and (E)-isomer, 60-90 % E-form and 10-40 % Z-form (EFFA, 2012k).
09.895	4-Methoxybenzyl-2-methylpropionate			Solid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble Freely soluble	287 40 MS 95 %	1.499-1.505 1.057-1.063	CASrn is missing. CASrn in Register to be introduced 71172-26-4. Register name to be changed to 4-methoxybenzyl 2-methylpropionate.

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)

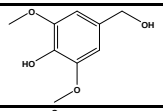
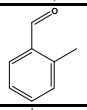
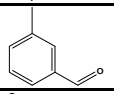
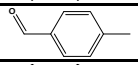
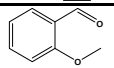
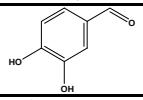
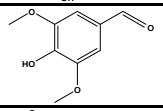
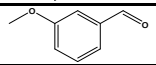
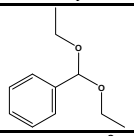
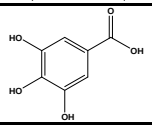
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		0.037	Class I A3: Intake below threshold	4)	6)	
05.026	o-Tolualdehyde		1.0	Class I A3: Intake below threshold	4)	6)	
05.028	m-Tolualdehyde		0.85	Class I A3: Intake below threshold	4)	6)	
05.029	p-Tolualdehyde		160	Class I A3: Intake below threshold	4)	6)	
05.129	2-Methoxybenzaldehyde		0.16	Class I A3: Intake below threshold	4)	6)	
05.142	3,4-Dihydroxybenzaldehyde		8.5	Class I A3: Intake below threshold	4)	6)	
05.153 1878	4-Hydroxy-3,5-dimethoxybenzaldehyde		0.74	Class I A3: Intake below threshold	4)	6)	
05.158	3-Methoxybenzaldehyde		0.011	Class I A3: Intake below threshold	4)	6)	
06.017	(Diethoxymethyl)benzene		1.7	Class I A3: Intake below threshold	4)	6)	
08.080	Gallic acid		0.011	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)

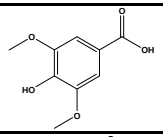
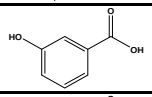
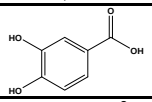
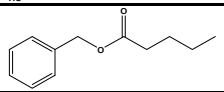
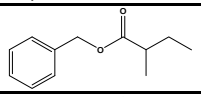
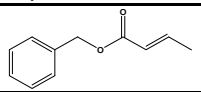
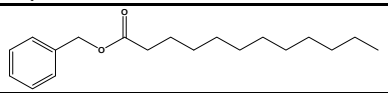
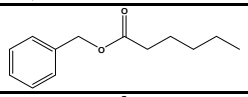
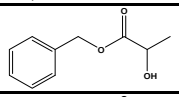
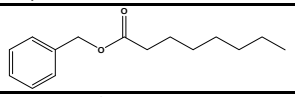
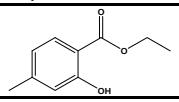
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		1.2	Class I A3: Intake below threshold	4)	6)	
08.132	3-Hydroxybenzoic acid		610	Class I A3: Intake below threshold	4)	6)	
08.133	3,4-Dihydroxybenzoic acid		610	Class I A3: Intake below threshold	4)	6)	
09.152	Benzyl valerate		1.7	Class I A3: Intake below threshold	4)	6)	
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	6)	
09.314	Benzyl crotonate		0.37	Class I A3: Intake below threshold	4)	6)	
09.315	Benzyl dodecanoate		0.13	Class I A3: Intake below threshold	4)	6)	
09.316	Benzyl hexanoate		0.75	Class I A3: Intake below threshold	4)	6)	
09.317	Benzyl lactate		0.91	Class I A3: Intake below threshold	4)	6)	
09.318	Benzyl octanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		0.0012	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)

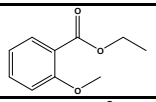
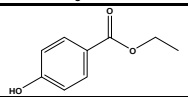
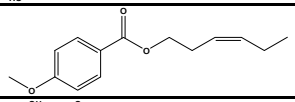
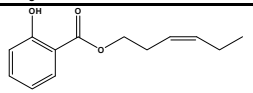
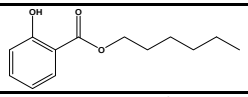
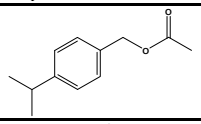
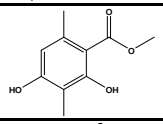
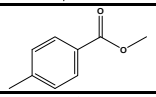
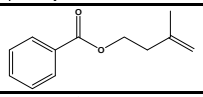
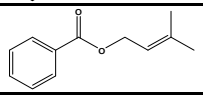
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.363	Ethyl 2-methoxybenzoate		5.5	Class I A3: Intake below threshold	4)	6)	
09.367	Ethyl 4-hydroxybenzoate		10	Class I A3: Intake below threshold	4)	6)	
09.560	Hex-3(cis)-enyl anisate		0.12	Class I A3: Intake below threshold	4)	6)	
09.570	Hex-3-enyl salicylate	 (Z)-form shown	0.13	Class I A3: Intake below threshold	4)	6)	
09.581	Hexyl salicylate		0.018	Class I A3: Intake below threshold	4)	6)	
09.611	4-Isopropylbenzyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.631	Methyl 4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.656	3-Methylbut-3-enyl benzoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.693	Prenyl benzoate		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)

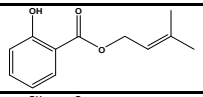
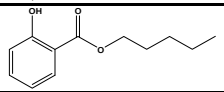
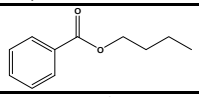
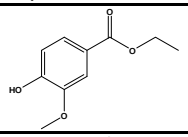
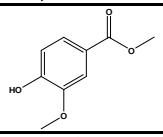
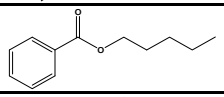
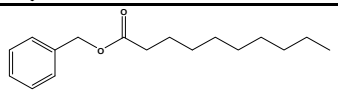
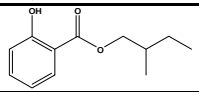
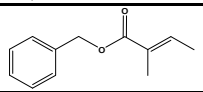
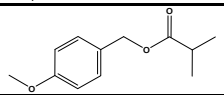
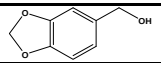
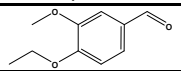
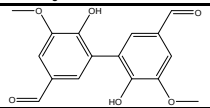
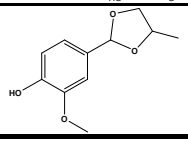
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.696	Prenyl salicylate		0.011	Class I A3: Intake below threshold	4)	6)	
09.762	Pentyl salicylate		0.24	Class I A3: Intake below threshold	4)	6)	
09.779	Butyl benzoate		3.7	Class I A3: Intake below threshold	4)	6)	
09.798	Ethyl vanillate		0.024	Class I A3: Intake below threshold	4)	6)	
09.799	Methyl vanillate		0.011	Class I A3: Intake below threshold	4)	6)	
09.825	Pentyl benzoate		1.1	Class I A3: Intake below threshold	4)	6)	
09.835	Benzyl decanoate		0.35	Class I A3: Intake below threshold	4)	6)	
09.852	2-Methylbutyl 2-hydroxybenzoate		0.011	Class I A3: Intake below threshold	4)	6)	
09.858	Phenylmethyl 2-methyl-2-butenoate		0.037	Class I A3: Intake below threshold	4)	6)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	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
02.205	Piperonyl alcohol		0.011	Class II A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
05.221 1881	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde		0.61	Class II A3: Intake below threshold	4)	6)	
06.104 1882	Vanillin propylene glycol acetal		100	Class II 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.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

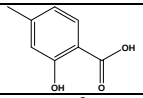
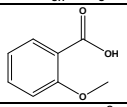
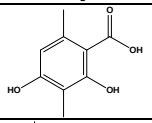
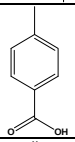
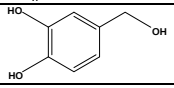
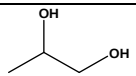
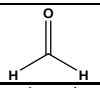
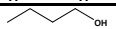
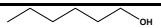
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	4-Methylsalicylic acid		Not evaluated as flavouring substance		Not in EU-Register
	o-Methoxybenzoic Acid		Evaluated as flavouring substance by JECFA (881)		Not in EU-Register
	2,4-Dihydroxy-3,6-dimethylbenzoic acid		Not evaluated as flavouring substance		Not in EU-Register
	p-Toluic acid		Not evaluated as flavouring substance		Not in EU-Register
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	3,4-Dihydoxybenzyl alcohol		Not evaluated as flavouring substance		Not in EU-Register
	Propylene glycol 925		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	Formaldehylde		Not evaluated as flavouring substance		Not in EU-Register
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.005	Hexan-1-ol 91		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

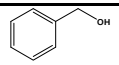
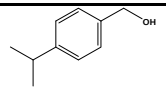
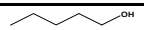
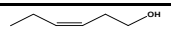
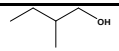
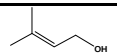
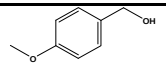
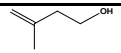
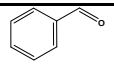
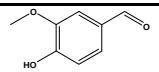
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.010	Benzyl alcohol 25		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.039	4-Isopropylbenzyl alcohol 864		No safety concern d) Category B c)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.056	Hex-3(cis)-en-1-ol 315		Category 1 a) No safety concern e) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern f) Category B c)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a) No safety concern g)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
02.109	3-Methylbut-2-en-1-ol 1200		No safety concern f)	Class I A3: Intake below threshold	This substance has been evaluated in FGE.202. it was concluded that there would be no safety concern with respect to genotoxicity or carcinogenicity.
02.128	p-Anisyl alcohol 871		No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.176	3-Methylbut-3-en-1-ol		FGE.06	Class I A3: Intake below threshold	
05.013	Benzaldehyde 22		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
05.018	Vanillin 889		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

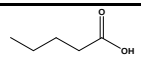
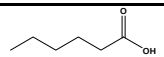
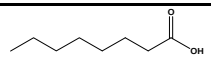
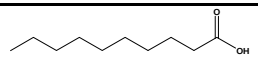
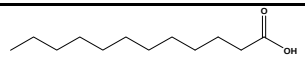
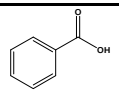
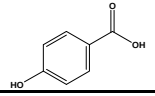
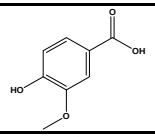
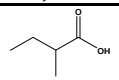
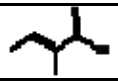
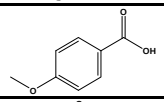
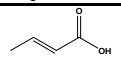
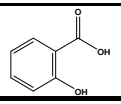
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.007	Valeric acid 90		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.010	Octanoic acid 99		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.011	Decanoic acid 105		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.012	Dodecanoic acid 111		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.021	Benzoic acid 850		No safety concern h) Deleted c)	Class I A3: Intake below threshold	Substances for which CoE Committee of Experts had no information as to real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992).
08.040	4-Hydroxybenzoic acid 957		No safety concern d) Category A c)	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
08.043	Vanillic acid 959		No safety concern d) Category A c)	Class I A3: Intake below threshold	
08.046	2-Methylbutyric acid 255		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.064	2-Methylcrotonic acid 1205		No safety concern f	Class I A3: Intake below threshold	
08.071	p-Anisic acid 883		No safety concern d)	Class I A3: Intake below threshold	
08.072	But-2-enoic acid (cis and trans) (E)-isomer shown		FGE.05	Class I A3: Intake below threshold	
08.112	Salicylic acid 958		No safety concern d)	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) (SCF, 1995).

b) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 2002b).

e) (JECFA, 2000a).

f) (JECFA, 2004a).

g) (JECFA, 1997a).

h) (JECFA, 2002c).

ND: Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

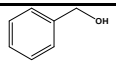
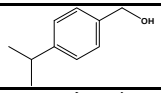
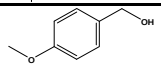
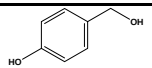
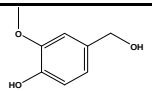
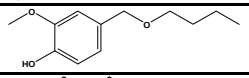
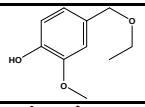
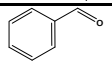
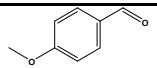
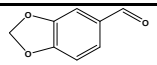
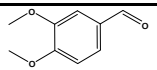
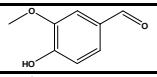
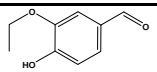
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.010	Benzyl alcohol		2137 58 100-51-6	25 JECFA specification (JECFA, 2001c).	13000	No safety concern a) Category A b)	GrADI: 0-5 (JECFA, 1997a).
02.039	4-Isopropylbenzyl alcohol		2933 88 536-60-7	864 JECFA specification (JECFA, 2001c).	0.24	No safety concern a) Category B b)	
02.128	p-Anisyl alcohol		2099 66 105-13-5	871 JECFA specification (JECFA, 2001c).	130	No safety concern a) Category A b)	
02.165	4-Hydroxybenzyl alcohol		3987 623-05-2	955 JECFA specification (JECFA, 2002d).	5.2	No safety concern a)	
02.213	Vanillyl alcohol		3737 690 498-00-0	886 JECFA specification (JECFA, 2001c).	5.4	No safety concern a) Category A b)	
04.093	Butyl vanillyl ether		3796 82654-98-6	888 JECFA specification (JECFA, 2001c).	1.4	No safety concern a)	
04.094	Ethyl 4-hydroxy-3-methoxybenzyl ether		3815 13184-86-6	887 JECFA specification (JECFA, 2001c).	20	No safety concern a)	
05.013	Benzaldehyde		2127 101 100-52-7	22 JECFA specification (JECFA, 2001c).	7900	No safety concern a) Category A b)	ADI: 0-5 (JECFA, 1997a).
05.015	4-Methoxybenzaldehyde		2670 103 123-11-5	878 JECFA specification (JECFA, 2001c).	370	No safety concern a) Category A b)	
05.016	Piperonal		2911 104 120-57-0	896 JECFA specification (JECFA, 2001c).	1500	No safety concern a) Category A b)	ADI: 0-2.5 (JECFA, 1968).
05.017	Veratraldehyde		3109 106 120-14-9	877 JECFA specification (JECFA, 2001c).	120	No safety concern a) Category A b)	
05.018	Vanillin		3107 107 121-33-5	889 JECFA specification (JECFA, 2001c).	47000	No safety concern a) Category A b)	ADI: 0-10 (JECFA, 1968).
05.019	Ethyl vanillin		2464 108 121-32-4	893 JECFA specification (JECFA, 2001c).	5400	No safety concern a) Category A b)	ADI: 0-3 (JECFA, 1995).

Table 3: Supporting Substances Summary

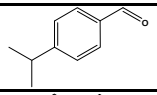
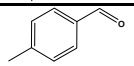
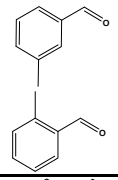
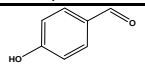
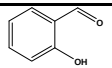
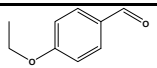
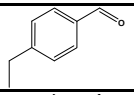
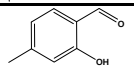
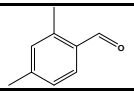
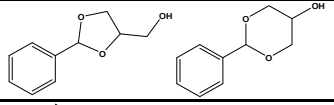
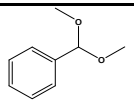
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
05.022	4-Isopropylbenzaldehyde		2341 111 122-03-2	868 JECFA specification (JECFA, 2001c).	110	No safety concern a) Category B b)	
05.027	Tolualdehyde	 	3068 115 1334-78-7	866 JECFA specification (JECFA, 2002d).	7.5	No safety concern a) Category A b)	CASrn does not specify position of methyl substituent, "Incompletely Defined Substance".
05.047	4-Hydroxybenzaldehyde		3984 558 123-08-0	956 JECFA specification (JECFA, 2002d).	55	No safety concern a) Category B b)	
05.055	Salicylaldehyde		3004 605 90-02-8	897 JECFA specification (JECFA, 2001c).	84	No safety concern a) Category B b)	
05.056	4-Ethoxybenzaldehyde		2413 626 10031-82-0	879 JECFA specification (JECFA, 2001c).	0.073	No safety concern a) Category B b)	
05.068	4-Ethylbenzaldehyde		3756 705 4748-78-1	865 JECFA specification (JECFA, 2001c).	0.37	No safety concern a) Category A b)	
05.091	2-Hydroxy-4-methylbenzaldehyde		3697 2130 698-27-1	898 JECFA specification (JECFA, 2001c).	0.61	No safety concern a) Category B b)	
05.110	2,4-Dimethylbenzaldehyde		3427 15764-16-6	869 JECFA specification (JECFA, 2001c).	0.37	No safety concern a)	
06.002	5-Hydroxy-2-phenyl-1,3-dioxane		2129 36 1319-88-6	838 JECFA specification (JECFA, 2001c).	13	No safety concern a) Category A b)	CASrn refers to benzaldehyde glyceryl acetate.
06.003	alpha,alpha-Dimethoxytoluene		2128 37 1125-88-8	837 JECFA specification (JECFA, 2001c).	0.12	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

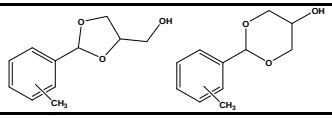
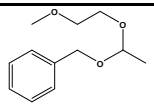
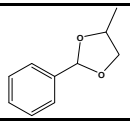
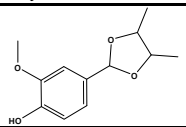
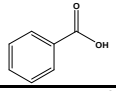
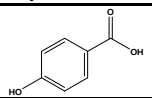
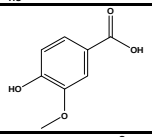
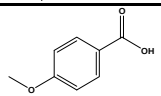
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
06.012	Tolualdehyde glyceryl acetal		3067 46 1333-09-1	867 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category A b)	CASrn refers to named substance.
06.019	1-Benzyloxy-1-(2-methoxyethoxy)ethane		2148 523 7492-39-9	840 JECFA specification (JECFA, 2001c).	1.2	No safety concern a) Category B b)	Racemate. Min. assay value: 98% (sum of parent compound and starting materials). The "starting materials" are methoxyethanol, acetaldehyde and benzyl alcohol which make up less than 10% combined of the mixture under anhydrous conditions (EFFA, 2010a).
06.032	4-Methyl-2-phenyl-1,3-dioxolane		2130 2226 2568-25-4	839 JECFA specification (JECFA, 2001c).	0.037	No safety concern a) Category A b)	
06.132	Vanillin butan-2,3-diol acetal (mixture of stereo isomers)		4023 63253-24-7	960 JECFA specification (JECFA, 2002d).	3.4	No safety concern a)	CASrn does not specify stereoisomers. Stereoisomeric composition to be specified.
08.021	Benzoic acid		2131 21 65-85-0	850 JECFA specification (JECFA, 2001c).	34	No safety concern c) Deleted b)	GrADI: 0-5 (JECFA, 1997a).
08.040	4-Hydroxybenzoic acid		3986 693 99-96-7	957 JECFA specification (JECFA, 2002d).	16	No safety concern a) Category A b)	
08.043	Vanillic acid		3988 697 121-34-6	959 JECFA specification (JECFA, 2002d).	24	No safety concern a) Category A b)	
08.071	p-Anisic acid		3945 10077 100-09-4	883 JECFA specification (JECFA, 2001c).	1.7	No safety concern a)	

Table 3: Supporting Substances Summary

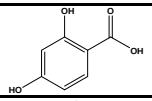
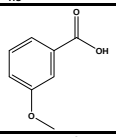
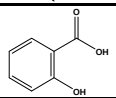
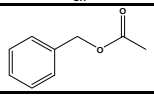
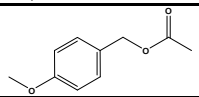
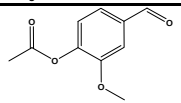
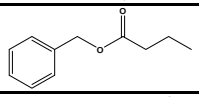
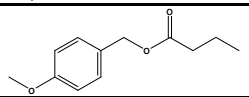
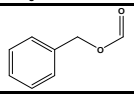
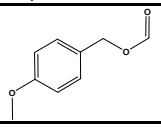
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.076	2,4-Dihydroxybenzoic acid		3798 89-86-1	908 JECFA specification (JECFA, 2001c).	5.5	No safety concern a)	
08.092	3-Methoxybenzoic acid		3944 586-38-9	882 JECFA specification (JECFA, 2001c).	0.012	No safety concern a)	
08.112	Salicylic acid		3985 10165 69-72-7	958 JECFA specification (JECFA, 2002d).	0.024	No safety concern a)	
09.014	Benzyl acetate		2135 204 140-11-4	23 JECFA specification (JECFA, 2001c).	1200	No safety concern a) Category B b)	GrADI: 0-5 (JECFA, 1997a).
09.019	p-Anisyl acetate		2098 209 104-21-2	873 JECFA specification (JECFA, 2001c).	50	No safety concern a) Category B b)	
09.035	Vanillyl acetate		3108 225 881-68-5	890 JECFA specification (JECFA, 2001c).	1.8	No safety concern a) Category B b)	
09.051	Benzyl butyrate		2140 277 103-37-7	843 JECFA specification (JECFA, 2001c).	100	No safety concern a) Category A b)	
09.058	p-Anisyl butyrate		2100 286 6963-56-0	875 JECFA specification (JECFA, 2001c).	29	No safety concern a) Category B b)	
09.077	Benzyl formate		2145 344 104-57-4	841 JECFA specification (JECFA, 2001c).	35	No safety concern a) Category A b)	
09.087	p-Anisyl formate		2101 354 122-91-8	872 JECFA specification (JECFA, 2002d).	39	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

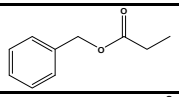
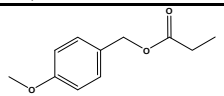
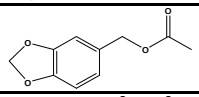
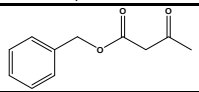
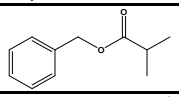
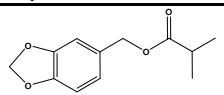
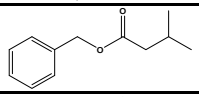
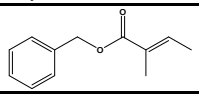
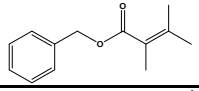
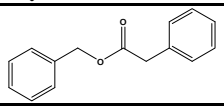
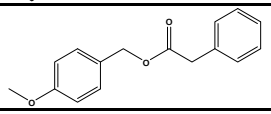
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.132	Benzyl propionate		2150 413 122-63-4	842 JECFA specification (JECFA, 2001c).	41	No safety concern a) Category A b)	
09.145	p-Anisyl propionate		2102 426 7549-33-9	874 JECFA specification (JECFA, 2001c).	0.42	No safety concern a) Category B b)	
09.220	Piperonyl acetate		2912 2068 326-61-4	894 JECFA specification (JECFA, 2001c).	34	No safety concern a) Category B b)	
09.406	Benzyl 3-oxobutyrate		2136 244 5396-89-4	848 JECFA specification (JECFA, 2001c).	0.24	No safety concern a) Category B b)	
09.426	Benzyl isobutyrate		2141 301 103-28-6	844 JECFA specification (JECFA, 2001c).	13	No safety concern a) Category B b)	
09.430	Piperonyl isobutyrate		2913 305 5461-08-5	895 JECFA specification (JECFA, 2001c).	0.085	No safety concern a) Category B b)	
09.458	Benzyl isovalerate		2152 453 103-38-8	845 JECFA specification (JECFA, 2001c).	12	No safety concern a) Category B b)	
09.494	Benzyl 2-methylcrotonate		3330 2184 37526-88-8	846 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category B b)	
09.508	Benzyl 2,3-dimethylcrotonate		2143 11868 7492-69-5	847 JECFA specification (JECFA, 2002d).	0.012	No safety concern a)	
09.705	Benzyl phenylacetate		2149 232 102-16-9	849 JECFA specification (JECFA, 2001c).	4.3	No safety concern a) Category B b)	
09.706	Anisyl phenylacetate		3740 233 102-17-0	876 JECFA specification (JECFA, 2001c).	0.0024	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

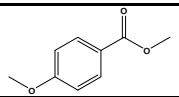
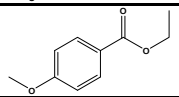
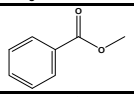
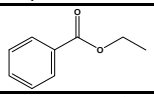
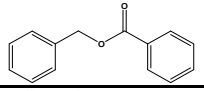
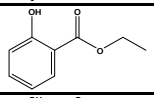
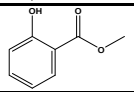
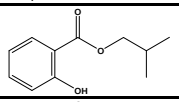
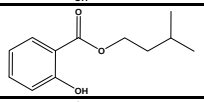
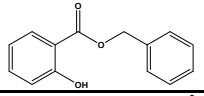
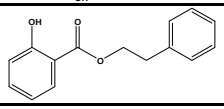
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.713	Methyl 4-methoxybenzoate		2679 248 121-98-2	884 JECFA specification (JECFA, 2001c).	0.97	No safety concern a) Category B b)	
09.714	Ethyl 4-methoxybenzoate		2420 249 94-30-4	885 JECFA specification (JECFA, 2001c).	9.1	No safety concern a) Category B b)	
09.725	Methyl benzoate		2683 260 93-58-3	851 JECFA specification (JECFA, 2001c).	40	No safety concern a) Category B b)	
09.726	Ethyl benzoate		2422 261 93-89-0	852 JECFA specification (JECFA, 2001c).	96	No safety concern a) Category B b)	
09.727	Benzyl benzoate		2138 262 120-51-4	24 JECFA specification (JECFA, 2001c).	1600	No safety concern a) Category A b)	GrADI: 0-5 (JECFA, 1980a).
09.748	Ethyl salicylate		2458 432 118-61-6	900 JECFA specification (JECFA, 2001c).	27	No safety concern a) Category B b)	
09.749	Methyl salicylate		2745 433 119-36-8	899 JECFA specification (JECFA, 2001c).	410	No safety concern a) Category A b)	ADI: 0-0.5 (JECFA, 1968).
09.750	Isobutyl salicylate		2213 434 87-19-4	902 JECFA specification (JECFA, 2001c).	0.97	No safety concern a) Category B b)	
09.751	Isopentyl salicylate		2084 435 87-20-7	903 JECFA specification (JECFA, 2001c)	41	No safety concern a) Category B b)	
09.752	Benzyl salicylate		2151 436 118-58-1	904 JECFA specification (JECFA, 2001c).	26	No safety concern a) Category B b)	
09.753	Phenethyl salicylate		2868 437 87-22-9	905 JECFA specification (JECFA, 2001c).	0.12	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

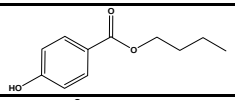
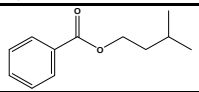
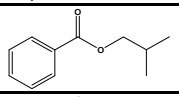
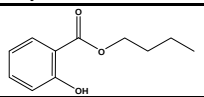
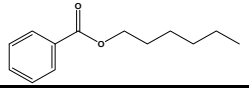
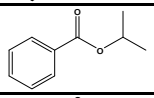
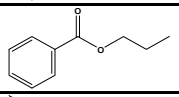
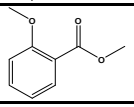
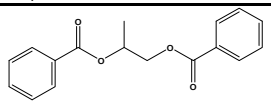
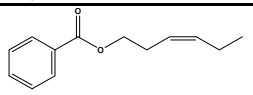
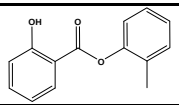
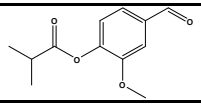
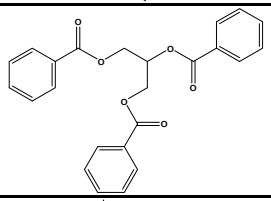
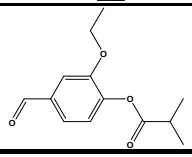
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.754	Butyl 4-hydroxybenzoate		2203 525 94-26-8	870 JECFA specification (JECFA, 2002d).	ND	No safety concern c) Deleted b)	
09.755	Isopentyl benzoate		2058 562 94-46-2	857 JECFA specification (JECFA, 2001c).	96	No safety concern a) Category B b)	
09.757	Isobutyl benzoate		2185 567 120-50-3	856 JECFA specification (JECFA, 2001c).	0.37	No safety concern a) Category B b)	
09.763	Butyl salicylate		3650 614 2052-14-4	901 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category B b)	
09.768	Hexyl benzoate		3691 645 6789-88-4	854 JECFA specification (JECFA, 2001c).	320	No safety concern a) Category B b)	
09.770	Isopropyl benzoate		2932 652 939-48-0	855 JECFA specification (JECFA, 2001c).	0.0037	No safety concern a) Category B b)	
09.776	Propyl benzoate		2931 677 2315-68-6	853 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category B b)	
09.796	Methyl 2-methoxybenzoate		2717 2192 606-45-1	880 JECFA specification (JECFA, 2001c).	49	No safety concern a) Deleted b)	
09.803	Propylene glycol dibenzoate		3419 10890 19224-26-1	862 JECFA specification (JECFA, 2002d).	13	No safety concern c)	CASrn refers to the racemate.
09.806	Hex-3-enyl benzoate	 (Z)-isomer shown	3688 11778 25152-85-6	858 JECFA specification (JECFA, 2001c).	6.7	No safety concern a)	CASrn refers to (Z)-isomer.
09.807	o-Tolyl salicylate		3734 617-01-6	907 JECFA specification (JECFA, 2001c).	28	No safety concern a)	

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
09.811	Vanillin isobutyrate		3754 20665-85-4	891 JECFA specification (JECFA, 2001c).	55	No safety concern a)	
09.812	Glyceryl tribenzoate		3398 10656 614-33-5	861 JECFA specification (JECFA, 2002d).	45	No safety concern c)	
09.933	Ethyl vanillin isobutyrate		3837 188417-26-7	953 JECFA specification (JECFA, 2001c).	0.61	No safety concern a)	

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, 2002b).

b) (CoE, 1992).

c) (JECFA, 2002c).

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 microgram/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 products[9] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[10] (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).*

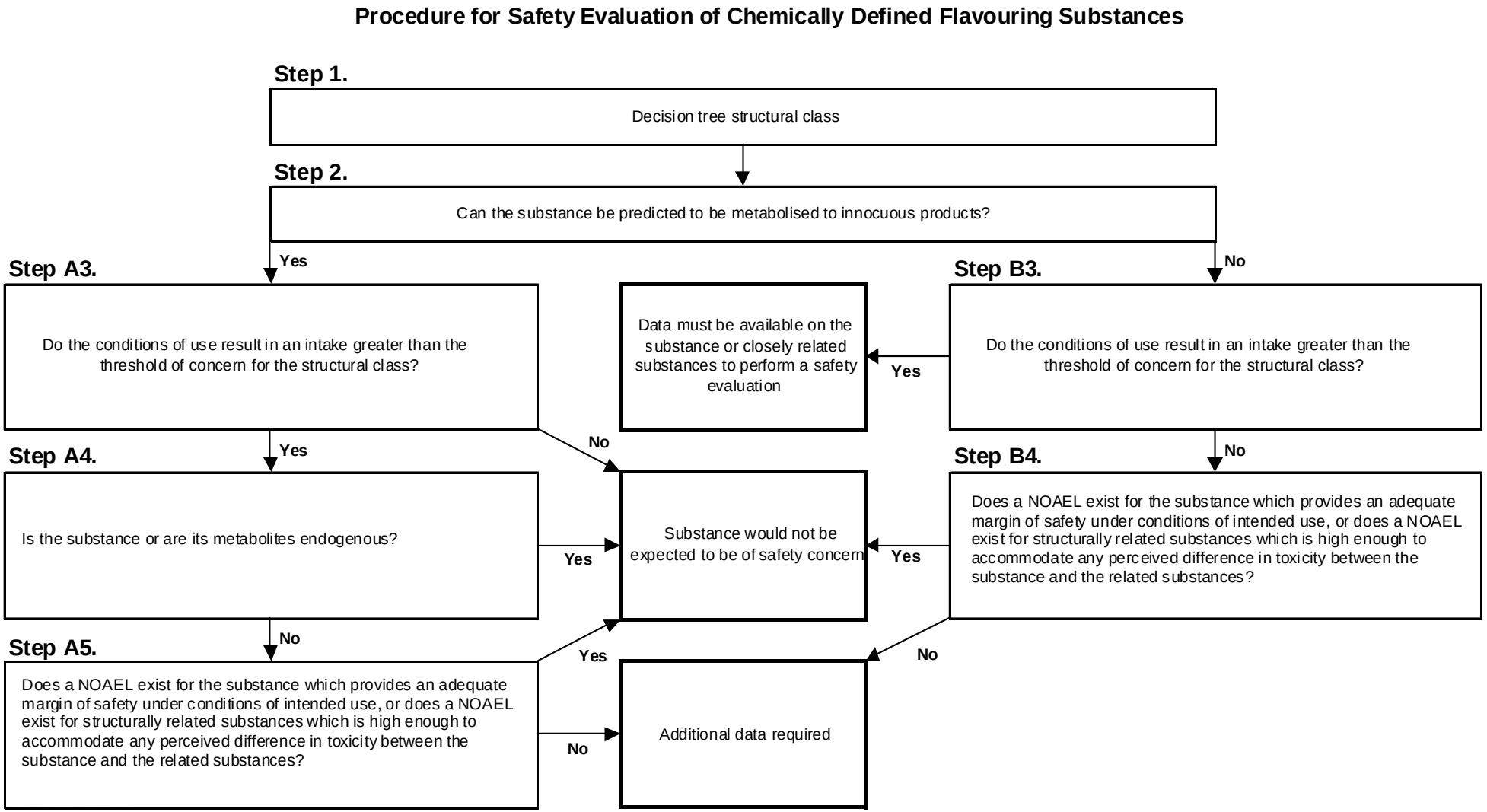


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 for 45 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.20Rev4 (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c).

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
02.164	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.205	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
05.026	15,03 - 17,74	- -	- -	- -	- -	56,26 - 111,2	- -	25,82 - 33,56	- -	- -	- -	- -	- -	- -	9,91 13,2 9	6,23 12,7	- -	- -
05.028	15,03 - 17,74	- -	- -	- -	- -	56,26 - 111,2	- -	25,82 - 33,56	- -	- -	- -	- -	- -	- -	9,91 13,2 9	6,23 12,7	- -	- -
05.029	15,03 - 17,74	- -	- -	- -	- -	56,26 - 111,2	- -	25,82 - 33,56	- -	- -	- -	- -	- -	- -	9,91 13,2 9	6,23 12,7	- -	- -
05.066	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.20Rev4 (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c).

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
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
05.129	3	2	3	2	-	5	2	-	1	1	-	-	2	3	2	5	5	2
	15	10	15	10	-	25	10	-	5	5	-	-	10	15	10	25	25	10
05.142	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
05.153	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
05.158	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
05.221	10	15	10	-	-	10	-	30	-	-	-	-	10	-	5	15	30	10
	40	20	20	-	-	20	-	50	-	-	-	-	30	-	15	30	50	20
06.017	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
06.104	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
08.080	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
08.087	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.132	-	-	-	-	-	500	-	-	-	-	-	-	-	-	300	300	-	-
	-	-	-	-	-	2000	-	-	-	-	-	-	-	-	500	500	-	-
08.133	-	-	-	-	-	500	-	-	-	-	-	-	-	-	300	300	-	-
	-	-	-	-	-	2000	-	-	-	-	-	-	-	-	500	500	-	-
09.152	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
09.313	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
09.314	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
09.315	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
09.316	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
09.317	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
09.318	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
09.362	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
09.363	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
09.367	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
09.560	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
09.570	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
09.581	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
09.611	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
09.623	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
09.631	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	34	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.656	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
09.693	7	5	10	7	-	10	5	10	2	2	-	-	5	20	5	10	-	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	100	25	50	-	25
09.696	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
09.762	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
09.779	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.20Rev4 (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c).

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
	35	25	50	35	-	50	25	50	11	10	-	-	25	50	25	50	100	25
09.798	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
09.799	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
09.825	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
09.835	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
09.852	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
09.858	-	-	-	-	-	-	-	5	-	-	-	-	5	-	-	-	-	-
	-	-	-	-	-	-	-	8	-	-	-	-	8	-	-	-	-	-
09.895	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

Three candidate substances [FL-no. 5.026, 05.028 and 05.029] 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 387/657.60 mg/kg for all three substances. 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 food categories and the use of chewing gum sum up to 9100 µg/person/day. 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
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 45 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c). The mTAMDI values are only given for the highest reported normal use levels.

TableII.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)**
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol	3900	Class I	1800
05.026	o-Tolualdehyde	9100	Class I	1800
05.028	m-Tolualdehyde	9100	Class I	1800
05.029	p-Tolualdehyde	9100	Class I	1800
05.129	2-Methoxybenzaldehyde	1400	Class I	1800
05.142	3,4-Dihydroxybenzaldehyde	1600	Class I	1800
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	1600	Class I	1800
05.158	3-Methoxybenzaldehyde	1600	Class I	1800
06.017	(Diethoxymethyl)benzene	3900	Class I	1800
08.080	Gallic acid	1600	Class I	1800

TableII.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)
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	3200	Class I	1800
08.132	3-Hydroxybenzoic acid	120000	Class I	1800
08.133	3,4-Dihydroxybenzoic acid	120000	Class I	1800
09.152	Benzyl valerate	3900	Class I	1800
09.313	Benzyl 2-methylbutyrate	3900	Class I	1800
09.314	Benzyl crotonate	3900	Class I	1800
09.315	Benzyl dodecanoate	3900	Class I	1800
09.316	Benzyl hexanoate	3900	Class I	1800
09.317	Benzyl lactate	3900	Class I	1800
09.318	Benzyl octanoate	3900	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	3900	Class I	1800
09.363	Ethyl 2-methoxybenzoate	3900	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	3900	Class I	1800
09.560	Hex-3(cis)-enyl anisate	3900	Class I	1800
09.570	Hex-3-enyl salicylate	3900	Class I	1800
09.581	Hexyl salicylate	3900	Class I	1800
09.611	4-Isopropylbenzyl acetate	3900	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	3900	Class I	1800
09.631	Methyl 4-methylbenzoate	3900	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	3900	Class I	1800
09.693	Prenyl benzoate	4900	Class I	1800
09.696	Prenyl salicylate	3900	Class I	1800
09.762	Pentyl salicylate	3900	Class I	1800
09.779	Butyl benzoate	3900	Class I	1800
09.798	Ethyl vanillate	3900	Class I	1800
09.799	Methyl vanillate	3900	Class I	1800
09.825	Pentyl benzoate	3900	Class I	1800
09.835	Benzyl decanoate	3900	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	3900	Class I	1800
09.858	Phenylmethyl 2-methyl-2-butenoate	770	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	3900	Class I	1800
02.205	Piperonyl alcohol	3900	Class II	540
05.066	4-Ethoxy-3-methoxybenzaldehyde	1600	Class II	540
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde	7000	Class II	540
06.104	Vanillin propylene glycol acetal	3900	Class II	540

ANNEX III: METABOLISM

III.1. Introduction

The flavouring group consists of 45 substances out of which 19 are benzyl derivatives (subgroup 1) and 25 are hydroxy- and alkoxy-ringsubstituted benzyl derivatives (subgroup 2) and one is a hydroxy- and alkoxy-substituted biphenyl derivative (subgroup 3).

Subgroup 1 (Benzyl derivatives)
Comprises three tolualdehyde isomers (*o*-, *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and 15 alkyl esters, ten of which contain benzyl alcohol in the alcohol moiety and straight or branched carboxylic acids as acid moiety [FL-no: 09.152 (benzyl valerate); 09.313 (benzyl 2-methylbutyrate); 09.314 (benzyl crotonate); 09.315 (benzyl dodecanoate); 09.316 (benzyl hexanoate); 09.317 (benzyl lactate); 09.318 (benzyl octanoate); 09.611 (4-isopropylbenzyl acetate), 09.835 (benzyl decanoate); 09.858 (phenylmethyl 2-methyl-2-butenoate)]. Five of the esters contain benzoic acid in the acid moiety [FL-no: 09.631 (methyl 4-methylbenzoate); 09.656 (3-methylbut-3-enyl benzoate); 09.693 (prenyl benzoate); 09.779 (butyl benzoate); 09.825 (pentyl benzoate)].

One substance in subgroup 1 is an acetal [FL-no: 06.017 ((diethoxymethyl)benzene)].

Two of the substances in subgroup 1 contain an alkyl substituent on the aromatic ring [FL-no: 09.611 (4-isopropylbenzyl acetate); 09.631 (methyl 4-methylbenzoate)]. Four compounds contain a double bond in an alkyl chain [FL-no: 09.314 (benzyl crotonate); 09.656 (3-methylbut-3-enyl benzoate); 09.693 (prenyl benzoate); 09.858 (phenylmethyl-2-methyl-2-butenoate)].

Subgroup 2 (Hydroxy- and alkoxy-substituted benzyl derivatives)
Includes two derivatives of benzyl alcohol [FL-no: 02.164 (4-hydroxy-3,5-dimethoxybenzyl alcohol) and 02.205 (piperonyl alcohol (3,4-methylenedioxybenzyl alcohol))]. Piperonyl alcohol may also be considered as a cyclic acetal.

Six substances are derivatives of benzaldehyde [FL-no: 05.066 (4-ethoxy-3-methoxybenzaldehyde); 05.129 (2-methoxybenzaldehyde); 05.142 (3,4-dihydroxybenzaldehyde); 05.153 (4-hydroxy-3,5-dimethoxybenzaldehyde); 05.158 (3-methoxybenzaldehyde) and 06.104 (vanillin propylene glycol acetal)].

Four are derivatives of benzoic acid [FL-no: 08.080 (gallic acid (3,4,5-trihydroxybenzoic acid)); 08.087 (4-hydroxy-3,5-dimethoxybenzoic acid); 08.132 (3-hydroxybenzoic acid); 08.133 (3,4-dihydroxybenzoic acid)].

The remaining 13 substances are esters of which one is an ester with alkoxy substituted benzyl alcohol as the alcohol moiety [FL-no: 09.895 (4-methoxybenzyl-2-methylpropionate)] and 12 are esters with substituted benzoic acid as the acid moiety [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(cis)-enyl anisate (hex-3(cis)-enyl 4-methoxybenzoate)); 09.570 (hex-3-enyl salicylate (hex-3-enyl 2-hydroxybenzoate)); 09.581 (hexyl salicylate (hexyl 2-hydroxybenzoate)); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate (3-methyl-but-2-enyl 2-hydroxybenzoate)); 09.762 (pentyl salicylate (pentyl 2-hydroxybenzoate)); 09.798 (ethyl vanillate (ethyl 3-methoxy-4-hydroxybenzoate)); 09.799 (methyl vanillate (methyl 3-methoxy-4-hydroxybenzoate)); 09.852 (2-methylbutyl 2-hydroxybenzoate (2-methylbutyl salicylate))].

Three of the esters in subgroup 2 contain a double bond in an alkyl chain [FL-no: 09.560 (hex-3(cis)-enyl anisate); 09.570 (hex-3-enyl salicylate; 09.696 (prenyl salicylate)].

Subgroup 3 (Biphenyl)
Contains one derivative of biphenyl [FL-no: 05.221 (6,6'-dihydroxy-5,5'dimethoxy-biphenyl-3,3'-dicarbaldehyde)].

III.2. Absorption, Distribution and Elimination

Subgroup 1 (Benzyl derivatives)

Candidate substances from subgroup 1

There are no studies submitted on the candidate substances from subgroup 1.

Supporting substances from subgroup 1

Several studies have been submitted demonstrating efficient absorption, metabolism and excretion of the supporting substances benzyl alcohol [FL-no: 02.010], benzaldehyde [FL-no: 05.013], benzoic acid [FL-no: 08.021] and benzyl acetate [FL-no: 09.014].

Benzyl alcohol [FL-no: 02.010]

As already stated, the benzyl alcohol is expected to be oxidised to benzoic acid, which will be conjugated with glycine and excreted as hippuric acid (Bridges et al., 1970). This has been demonstrated in a study by LeBel et al. (1988) in which similar intravenous doses (range 0.036 to 0.222 micromole/kg bw) of benzyl alcohol were given via medications to 14 full-term and nine pre-term infants, in order to estimate plasma levels of benzoic and hippuric acids. In the urine of full-term new-borns a larger proportion of benzyl alcohol was found as hippuric acid and a smaller proportion benzoic acid, which is in line with what Bridges et al suggest. However, the mean peak concentrations of benzoic acid in the plasma of pre-term babies were almost 10 times higher than in full-term new-borns and in the urine of pre-term babies a larger proportion of benzyl alcohol was found as benzoic acid and a smaller proportion as hippuric acid in contrary to what was found in full-term new-borns. The results suggest that hippuric acid formation is deficient in pre-term newborns (LeBel et al., 1988). Overall, the results suggest that humans metabolise benzyl alcohol to both benzoic acid and hippuric acid but hippuric acid formation is deficient in pre-term new-borns.

Five minutes after single intraperitoneal doses of 500 - 1100 mg/kg bw of benzyl alcohol administered to CD1 mice, benzyl alcohol was detected in plasma (McCloskey et al., 1986).

Benzaldehyde [FL-no: 05.013]

In the rabbit, approximately 83 % of given single oral doses of 350 or 750 mg/kg bw benzaldehyde was absorbed; it was found in the urine of both dose groups. The aldehyde was oxidised mainly to benzoic acid and excreted predominantly as hippuric acid (approximately 68 %). Other urinary metabolites detected were benzoylglucuronic acid (10 %), benzoyl glucuronide (3 %), free benzoic acid (1.5 %) and trace amounts of benzyl mercapturic acid (Laham et al., 1988).

Benzoic acid [FL-no: 08.021]

Following administration of 375 mg [carboxyl-14C]-benzoic acid/kg bw to rats (orally) and mice intraperitonelly (i.p.), 88 - 89 % of the radioactivity was recovered in the urine of rats within 24 hours with 91 - 94 % recovery after 72 hours, and only 1 - 6 % was present in the faeces. In mice, 92 - 98 % of the radioactivity was recovered in the urine of rats within 24 hours and only 1 - 10 % was present in the faeces. It was possible to conclude that after both route of administration more than 95 % of benzoic acid is absorbed, metabolised and rapidly excreted. The following metabolites were identified: hippuric acid (70.2 -

84.2 %), benzoyl glucuronide (0.7 - 1.8 %), benzoic acid (0.4 - 12.8 %) and 3-hydroxy-3-phenyl propionic acid (0.1 - 0.2 %) (Nutley, 1990).

Benzyl acetate [FL-no: 09.014]

A study on benzyl acetate metabolism in male Fischer 344 rats and male $B6C3F_1$ mice was performed. Ring-labelled ^{14}C-benzyl acetate was used for single dose studies and unlabelled benzyl acetate was used for repeated dose studies. For intravenous administration of single doses, three rats were injected with 5 mg 14C-benzyl acetate in the tail vein and three mice were similarly injected with 10 mg. For the single oral dose study, groups of three rats were given 5, 50 or 500 mg/kg bw and groups of three mice were given 10, 100 or 1000 mg/kg bw in corn oil by gavage. For the repeated dose studies, three rats and three mice were given unlabelled benzyl acetate in corn oil by gavage at 500 or 1000 mg/kg bw, respectively, once a day, 5 days a week for 2 weeks. Metabolites in urine were determined by High Performance Liquid Chromatography (HPLC). After administration of the radioactive compound, rats and mice were housed in metabolism cages and urine and faeces were collected during 24 hours. After intravenous administration, CO_2 and volatiles were collected.

Benzyl acetate was rapidly and almost completely absorbed, based on the high recovery of radioactivity (nearly 90 % of the dose) in the urine in 24 hours, following both intravenous or oral dosing in rats and mice. Little radioactivity (0.3 - 1.3 % of the dose) was recovered in the faeces. Elimination as CO_2 or volatiles was minimal following intravenous administration and was not determined after oral dosing. This clearance pattern was not affected by repeated oral dosing, indicating no potential for bioaccumulation, as supported also by the absence of radioactivity in tissues analysed at 24 hours after dosing. The major metabolite of benzyl acetate in the urine of rats and mice was hippuric acid, accounting for more than 90 % of the total metabolites excreted in urine of all dose groups. Mercapturic acid was detected as a minor metabolite in the urine of rats and mice (less than 1 %), but was not found in all dose groups and not in all animals of the dose groups where it was detected. Small amounts of unidentified metabolites were also present. The absorption, routes of metabolism and excretion of benzyl acetate were apparently unaffected by the size or number of doses administered in the metabolism study. There was no evidence to indicate a reduction or saturation of the metabolic capacity in tested animals in the tested dose range (Abdo et al., 1985).

Subgroup 2 (Hydroxy- and alkoxy-substituted benzyl derivatives)

Candidate substances from subgroup 2

Piperonyl alcohol (1-hydroxymethyl-3,4-methylenedioxybenzene) [FL-no: 02.205]

In a study of several methylenedioxyphenyl compounds, male Swiss-Webster mice were administered a dimethyl sulphoxide (DMSO) solution of radiolabelled piperonyl alcohol (1-hydroxymethyl-3,4-methylene-^{14}C-dioxybenzene) by oral gavage at a dose of 0.76 mg/kg bw. Total radiocarbon determinations were made on expired $^{14}CO_2$ at 0.5, 1, 2, 4 and 6 hours after dosing and at each six-hours interval thereafter, on urine and faeces samples taken at 12, 24 and 48 hours after treatment and on selected organs removed from the animals and the remaining carcass at 48 hours after treatment. Only the 12-hours samples were used for separation and characterisation of metabolites. Forty-eight hours after treatment, the distribution of radioactivity was as follows (averages of four experiments): CO_2, 3.0 %; urine, 93.3 %; faeces, 8.5 %; intestine, 0.2 %; liver, 0.1 %; carcass, 0.3 %. These data indicate that piperonyl alcohol is almost completely absorbed in the gastrointestinal (GI) tract, then metabolised and rapidly and almost completely excreted, mostly via the urine. Less than 10 % was excreted in the faeces. In all cases, the major metabolite was the glycine conjugate of piperonylic acid. Free piperonylic acid was not detected. Minor amounts of two unidentified metabolites were also present (Klungsoeyr and Scheline, 1984).

Gallic acid (3,4,5-trihydroxybenzoic acid) [FL-no: 08.080]

Gallic acid [FL-no: 08.080] was given orally to six week old male Wistar rats in order to determine the metabolic fate of the substance. After oral administration of 100 mg/kg bw, gallic acid was absorbed fairly quickly and reached the maximum concentration at 15 minutes in portal blood. The concentration was halved by 30 minutes and gallic acid had almost disappeared after six hours. The metabolite 4-O-methyl gallic acid also reached peak values within 15 minutes, and then decreased slowly. In the inferior vena cava, gallic acid and its metabolite were detected in approximately equal proportions and both reached peak values at 30 minutes after oral administration and decreased gradually until six hours after administration. The main metabolite in urine was 4-O-methyl gallic acid, but unchanged gallic acid was also found in urine. The ratio of 4-O-methyl gallic acid to total gallic acid metabolites in urine ranged from 0.55 to 0.76, indicating that a significant amount of gallic acid was excreted without being metabolised (Zong et al., 1999).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

Ethyl paraben [FL-no: 09.367] was given orally in capsules at a dose of 1 g/kg bw to groups of three fasted dogs and blood and urine were analysed at frequent predetermined intervals until 48 hours after dosing. Metabolites were detectable in the blood up to 24 hours post-ingestion. Recovery as urine metabolites was 66 % of the administered dose at 48 hours. Dogs were also administered a 100 mg/kg bw dose of ethyl paraben intravenously and then killed to determine the distribution of the parent material and its metabolites. The ester was detected only in the brain and pancreas, whereas high concentrations of metabolites were detected in the liver and kidneys (Jones et al., 1956).

Absorption, distribution, metabolism and excretion of ethyl paraben [FL-no: 09.367] were investigated in Wistar rats administered 100 mg by oral gavage. Animals were held in metabolism cages for the collection of urine (at approximately 15, 30, 60, 75, 90, 120, 150 and 210 minutes) and blood (at approximately 30, 60, 90, 120, 180, 240 and 360 minutes), and samples were analysed to establish the excretion kinetics. Metabolites were detected in the urine starting at 30 minutes after dosing, and their concentration increased steadily during the next three to six hours. Absorption of ethyl paraben was followed by metabolism and excretion of mainly free 4-hydroxybenzoic acid and its glucuronic and glycine conjugates. A small portion of the dose was excreted as sulphate conjugate (Derache and Gourdon, 1963).

^{14}C-labelled ethyl 4-hydroxybenzoate was orally given to four male cats in the diet at a single dose of 156 mg/kg bw. Essentially all (mean = 96.0 %) of the radioactiviy was excreted in urine within 72 hours as *p*-hydroxyhippuric acid and 4-hydroxybenzoic acid (Phillips et al., 1978).

Supporting substances from subgroup 2

Piperonal (3,4-methylenedioxybenzaldehyde) [FL-no: 05.016]

In male rats a 150 mg/kg bw dose of piperonal in propylene glycol was administered by gavage. Urine samples were collected at 24 and 48 hours. Recovery of urine metabolites made up 90 % of the given dose, and metabolite excretion occurred mainly within 24 hours. No unchanged compound was detected in the urine (Klungsoeyr and Scheline, 1984).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017]

A 1 g/kg bw oral dose of veratraldehyde was administered to rabbits by gavage and urine was collected for 24 hours. At least approximately 70 % of the aldehyde was absorbed as it was present in the urine, mainly as the corresponding acid and its conjugates (Scheline, 1972).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018]

Oral dosage of 100 mg/kg bw of vanillin to male albino rats resulted in an urinary excretion of most metabolites within 24 hours, mainly as glucuronide and sulphate conjugates, although vanillic acid was also

excreted as free acid and as glycine conjugate. After 48 hours, 94 % of the dose was excreted as different metabolites (Strand and Sheline, 1975).

A 100 mg dose of vanillin dissolved in water was given to an adult human and the urine was collected for 24 hours. During this period, an increase, from a background level, in the vanillic acid output in the urine level was measured, accounting for approximately 94 % of the vanillin dose (Dirscherl and Wirtzfeld, 1964).

4-Hydroxybenzoic acid [FL-no: 08.040]

Groups of four to eight rabbits were administered 100, 250, 500, 1000 or 1500 mg 4-hydroxybenzoic acid/kg bw by gavage. Urine was collected continuously and analysed for metabolites. Based on the total urinary recovery of the test material (84 to 104 %), the compound was almost completely absorbed, metabolised and excreted (Bray et al., 1947).

Concluding remarks on absorption, distribution and excretion

The results of these studies indicate that the benzyl derivatives in subgroup 1 as well as the hydroxy- and alkoxy-substituted benzyl derivatives in subgroup 2 are expected to be rapidly absorbed, metabolised and excreted, mainly in the urine.

III.3. Metabolism

III.3.1. Hydrolysis of Esters and Acetals

In general, esters containing an aromatic ring system are expected to be hydrolysed *in vivo* to the component acid and alcohol through the catalytic activity of carboxylesterases or esterases. In mammals, esterases occur in most tissues throughout the body but predominate in the hepatocytes (Heymann, 1980).

Subgroup 1 (Benzyl derivatives)

Candidate substances from subgroup 1

Benzyl 2-methylbutyrate (benzyl 2-methylbutanoate) [FL-no: 09.313]

Benzyl 2-methylbutyrate at a concentration of 40 microlitre/l (0.21 mM) was incubated in 0.5 M phosphate buffer at pH 7.5 and 37°C with a preparation of pancreatin for two hours. The extent of hydrolysis was 100 % as determined by gas-liquid chromatography. The supporting substance benzyl acetate [FL-no: 09.014] at a concentration of 70 microlitre/l (0.49 mM) was 50 % hydrolysed after 2 hours (Grundschober, 1977).

Supporting substances from subgroup 1

Benzyl acetate [FL-no: 09.014]

Neat benzyl acetate was spiked into control rat plasma (1 microlitre/0.5 ml), vortexed and incubated at room temperature for 0.5 - 36 minutes. Incubation was terminated by addition of acetonitrile. The plasma was centrifuged to precipitate plasma proteins and the clear plasma was analysed by (High Performance Liquid Chromatography) HPLC to determine benzyl acetate and benzyl alcohol. Benzyl acetate was found to be rapidly hydrolysed to benzyl alcohol. The half-life of benzyl acetate was about 4 minutes and 24 minutes after spiking virtually all benzyl acetate was hydrolysed to benzyl alcohol. Hydrolysis was partially inhibited by the esterase inhibitor sodium fluoride, which suggests that plasma esterases contribute to the rapid

hydrolysis. When benzyl acetate was administered to rats and mice in gavage and dosed feed studies, benzyl acetate was not detected in any plasma samples collected (Yuan et al., 1995).

In vivo metabolism studies in mice and rats clearly indicate that radiolabelled benzyl acetate is readily hydrolysed since more than 90 % of the radioactivity is demonstrated in the urine as benzoic or hippuric acid (Abdo et al., 1985).

Benzyl acetate was hydrolysed in pig liver homogenate. At pH 7.4 and 25° C the velocity was calculated to 27 micromole/min/mg, K_m 0.55 mM. (Greenzaid & Jenks, 1971 referred in (Heymann, 1980)).

Alkyl- and aryl-benzoates

The plasma half-lives ($t_{½}$) for the *in vitro* hydrolysis by plasma enzymes of a series of four alkyl benzoates (including supporting chemicals methyl benzoate [FL-no: 09.725], ethyl benzoate [FL-no: 09.726] and propyl benzoate [FL-no: 09.776]) and two aryl benzoates (including supporting substance benzyl benzoate [FL-no: 09.727]) in 80 % human blood plasma ranged from 24 to 210 minutes for the alkyl benzoates. By increasing chain length, an increasing enzymatic degradation was seen, except when going from methyl to ethyl. The butyl ester was the least resistant (t½ 24 minutes), while the ethyl ester was the most resistant to hydrolysis (t½ 210 minutes). The plasma half-lives were 19 and 15 minutes for phenyl benzoate and benzyl benzoate, respectively (Nielsen and Bundgaard, 1987).

An *in vitro* hydrolysis study demonstrated that benzyl phenylacetate was 100 % hydrolysed within two hours of incubation with a pancreatin solution, whereas the supporting substance benzyl acetate [FL-no: 09.014] was only 50 % hydrolysed after 2 hours incubation. Benzyl cinnamate and methyl phenylacetate were 80 and 70 % hydrolysed, respectively (Leegwater and Straten, 1974a).

Other related substances:

4-Methyl-2-phenyl-1,3-dioxolane (benzaldehyde propylene glycol acetal)

Benzaldehyde-related acetals readily hydrolyse to their component alcohols and benzaldehyde under acidic conditions. Hydrolysis of acetals in simulated gastric juice (pH 1.2) and simulated intestinal fluid (pH 7.5) was monitored by the formation rate of aldehyde liberated during treatment. Data show that non-cyclic acetals are completely hydrolysed at pH 1.2 but that hardly any hydrolysis occurs at pH 7.5. Benzaldehyde–propylene–glycol acetal (4-methyl-2-phenyl-1,3-dioxolane, MPD), a cyclic acetal, was hydrolysed to an extent of around 50 % after one hour in simulated gastric juice and no further hydrolysis was observed after five hours. Reflux of MPD for five hours in 0.1 N HCl also resulted in hydrolysis to an extent of 50 % of the theoretical maximum. Due to the same poor hydrolysis of MPD (to around 50 %), even after five hours reflux in 0.1 N HCl, the author questioned the chemical identity of the substance (Morgareidge, 1962a). The result of this study on hydrolysis of a cyclic benzaldehyde acetal is inconclusive.

Subgroup 2 (Hydroxy- and alkoxy-substituted benzylderivatives)

Candidate substances from subgroup 2

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

An *in vitro* assay demonstrated that ethyl paraben is efficiently hydrolysed by the liver and kidney esterases as 96 % hydrolysis was measured after three minutes in dog liver tissue suspension and 100 % hydrolysis after 30 minutes in dog kidney suspension (Jones et al., 1956).

Ethyl paraben [FL-no: 09.367] was 80 % hydrolysed to free 4-hydroxybenzoic acid within 60 minutes in perfused mouse liver, only 2.3 % intact ester was recovered. Ethyl paraben was not detected in the blood of six humans 1 - 4½ hours after oral intake of 10 to 20 mg/kg bw. When given orally to dogs at doses between

25 and 500 mg/kg bw, high serum concentrations of 4-hydroxybenzoic acid were reported and no ethyl paraben was detected in the blood except for the 500 mg/kg bw dose (Heim et al., 1957).

Studies were conducted with methyl, ethyl [FL-no: 09.367], propyl and butyl 4-hydroxybenzoate [FL-no: 09.754] (supporting substance) in dogs. The results showed significantly higher rates of test material recovery in the urine of dogs dosed orally, 1 g/kg bw orally or 50 mg/kg bw by the intravenously route for the methyl, ethyl and propyl esters (% of dose excreted within 48 hours, oral: 89.0, 66.0 and 57.6 %, respectively; i.v. 85, 70 and 94 %, respectively) as compared to the butyl ester (oral 48.2 %; i.v. 40.1 %). The methyl, ethyl and propyl esters showed 100 % hydrolysis within 3 minutes when incubated with liver homogenate, whereas the butyl ester was completely hydrolysed only after 30 - 60 minutes. This finding suggests that an increase in the alkyl chain length in the homologous series of alkyl esters make the esters more resistant to hydrolysis (Jones et al., 1956).

Vanillin propylene glycol acetal [FL-no: 06.104]

Under acidic conditions, pH 2.6, vanillin propylene glycol acetal [FL-no: 06.104] began to hydrolyse immediately with approximately 3 % of the acetal disappearing and 92 % hydrolysed within two hours. At pH 1.8, approximately 90 % of vanillin propylene glycol acetal hydrolysed immediately and 93 % hydrolysed within five minutes (Bennett, 1997).

Supporting substances from subgroup 2

Methyl salicylate (methyl 2-hydroxybenzoate) [FL-no: 09.749]

An oral dose of methyl salicylate equivalent to 500 mg/kg bw of salicylic acid was dissolved in 2 % methyl cellulose and administered to male rats. The plasma levels measured within 20 minutes of dosing showed complete hydrolysis of methyl salicylate. A similar experiment was conducted with male dogs. Capsules containing 320 mg methyl salicylate/kg bw were given orally to three fasted dogs in five repeated experiments. Blood drawn 1 and 4 hours after dosing showed 95 % hydrolysis of methyl salicylate to salicylic acid at both time intervals. Six humans were given a 0.42 ml dose of methyl salicylate administered in ginger ale. Blood was drawn by venipuncture 15 and 90 minutes later. In contrast to the other two species an appreciable portion of unhydrolysed methyl salicylate was found, 39 % after 15 minutes and 21 % after 90 minutes (Davison et al., 1961).

Other related substances

At low pH similar to that found in the stomach, a structurally related substance, vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248], is readily hydrolysed. In a hydrolysis study, 12 - 39 mM vanillin 3-(l-menthoxy)propane-1,2-diol acetal underwent 91 % hydrolysis at pH 2 within 45 minutes. At pH 3, approximately 86 % of vanillin 3-(l-menthoxy)propane-1,2-diol acetal was hydrolysed within 90 minutes. At pH 4, approximately 92 % of the acetal was hydrolysed within eight hours. At pH 5, approximately 12 % of the flavouring substance was hydrolysed within eight hours (Reitz, 1995).

Concluding remarks on hydrolysis

There is some information about hydrolysis of esters in *in vivo* as well as *in vitro* systems for some candidate and supporting substances in subgroups 1 and 2.

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo*.

Nine of the candidate substances in subgroup 1 [FL-no: 09.152 (benzyl valerate); 09.313 (benzyl 2-methylbutyrate); 09.314 (benzyl crotonate); 09.315 (benzyl dodecanoate); 09.316 (benzyl hexanoate); 09.317 (benzyl lactate); 09.318 (benzyl octanoate); 09.835 (benzyl decanoate) and 09.858 (phenylmethyl 2-

methyl-2-butenoate)] will yield benzyl alcohol and simple aliphatic carboxylic acids upon hydrolysis. One ester, 4-isopropylbenzyl acetate [FL-no: 09.611], will yield 4-isopropylbenzyl alcohol and acetic acid.

The acetal in subgroup 1, (diethoxymethyl)benzene [FL-no: 06.017], is expected to be efficiently hydrolysed to yield benzaldehyde and ethanol.

Four of the remaining esters in subgroup 1 are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, [FL-no: 09.656 (3-methylbut-3-enyl benzoate); 09.693 (prenyl benzoate); 09.779 (butyl benzoate); 09.825 (pentyl benzoate)]. One ester, [FL-no: 09.631] (methyl 4-methylbenzoate), will yield 4-methylbenzoic acid upon hydrolysis. The alcohol part of the candidate substance [FL-no: 09.656] (3-methylbut-3-enyl benzoate) includes a terminal double bond.

Of the 13 esters in subgroup 2, one ester, [FL-no: 09.895] (4-methoxybenzyl-2-methylpropionate)], will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) [FL-no: 02.128] upon hydrolysis. The remaining 12 esters in subgroup 2, [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(cis)-enyl anisate); 09.570 (hex-3-enyl salicylate); 09.581 (hexyl salicylate); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate); 09.762 (pentyl salicylate); 09.798 (ethyl vanillate); 09.799 (methyl vanillate); 09.852 (2-methylbutyl 2-hydroxybenzoate)], will yield hydroxy and/or alkoxy-substituted benzoic acid upon hydrolysis.

III.3.2 Metabolism Studies

Subgroup 1 (Benzyl derivatives)

Candidate substances from subgroup 1

In a series of *in vitro* studies with purified microsomal rat liver aldehyde dehydrogenase, Martini and Murray (1996) (Martini and Murray, 1996) demonstrated that *m*- and *p*-tolualdehyde [FL no: 05.028 and 05.029] are substrates for this enzyme, which converts these aldehydes into the corresponding toluic acid derivatives using NAD as a co-factor. The affinity (K_m) was much lower than for aliphatic aldehydes (~ 200 - 500 higher K_m), but V_{max} values were in the same order of magnitude. The *o*-tolualdehyde is a relatively poor substrate for which the V_{max} was at least 5 times lower that for the other two isomers. It was not studied to what extent this enzyme contributes to the overall oxidation of these three aldehydes *in vivo.*

The overall conversion of the three tolualdehyde isomers into the corresponding toluic acids was studied by Watanabe et al (1995)(Watanabe et al., 1995) in mouse liver microsomes. The oxidation rates were predominantly depending on enzymes using NADPH, rather than NAD, as co-factors with reaction rate ratios of 3, 1.1 and 1.4 (rate NADPH/NAD) for *o*-, *m*- and *p*-tolualdehyde, respectively. This would indicate that cytochrome P450 enzymes contribute for 50 to 75 % to the overall conversion rate. Using a reconstituted metabolic system and antibodies against *cyp*2C29, it was demonstrated that this enzyme contributed most. The enzymatic nature of the NAD-driven oxidation reaction was not further investigated but could have been a non-P450-related aldehyde dehydrogenase. The contribution of *cyp*2C29 to the overall tolualdehyde oxidation *in vivo* was not further investigated.

Seutter-Berlage et al. (1982) (Seutter-Berlage et al., 1982) studied the influence of various *o*- and *p*-ringsubstituents of benzaldehyde and benzyl alcohol derivatives on the excretion of urinary thioethers in rats after a single intraperitoneal injection with approximately 400 to 440 micromol per substance/animal. No increase in thioether elimination was observed with the candidate substance *p*-tolualdehyde [FL no: 05.029] and with the supporting substances benzaldehyde, *p*-hydroxybenzaldehyde and *p*-methoxybenzaldehyde [FL no: 05.013, 05.047 and 05.015]. However, with the candidate substances *o*-methoxybenzaldehyde and *o*-tolualdehyde [FL no: 05.129 and 05.026] increases in urinary thioester excretion were observed. It was

estimated that from the two candidate substances, approximately 2 and 7 % of the dose was eliminated as a mercapturic acid conjugate after reduction of the aldehyde group via a benzyl alcohol sulphate ester as intermediate. By comparison of the influence of the various ring substituents, it was speculated that a larger substituent at the ortho position would result in a larger part of the dose to participate in aldehyde reduction and subsequent sulphate conjugation followed by conjugation with glutathione (Seutter-Berlage et al., 1982). Similarly, converion of *o*-tolualdehyde into mercapturic acid conjugates has been reported by Van Doorn et al. (1981). After intraperitoneal dosing of rats with 1 mmol/kg bw (roughly equivalent to 250 micromol/animal) ca. 2 % of the dose was excreted as thioether conjugate via the urine (van Doorn et al., 1981).

Supporting substances from subgroup 1

Benzyl alcohol [FL-no: 02.010]

Five minutes after single intraperitoneal doses of 500 - 1100 mg/kg bw of benzyl alcohol administered to CD1 mice, benzyl alcohol was detected in plasma. At doses of 700 - 1100 mg/kg bw, plasma also contained measurable concentrations of benzaldehyde. Animals pre-treated with an alcohol dehydrogenase inhibitor (pyrazole) showed a 200 % increase in plasma benzyl alcohol levels, whereas pre-treatment with an aldehyde dehydrogenase inhibitor (disulphiram) resulted in a 368 % increase in plasma benzaldehyde levels as compared to control values (McCloskey et al., 1986).

Conjugation of benzyl alcohol with glutathione has been observed by Van Doorn et al (1981) (van Doorn et al., 1981), who estimated that after an intraperitoneal dose of 1 mmol/kg bw to rats, approximately 0.8 % of the dose was eliminated as mercapturic acid via the urine. From additional studies it was anticipated that the sulphate ester of the alcohol would participate as an intermediate in the ultimate conjugation to glutathione.

Benzaldehyde [FL-no: 05.013]

The metabolism of benzaldehyde was investigated in Sprague Dawley rats (5/group/sex) which were administered single oral doses of 400, 750 or 1000 mg/kg bw of pure benzaldehyde by gavage once daily for 13 consecutive days. Urine was collected for 24 hours after the 2nd, the 8th and the 13th dose and analysed for the presence of metabolites. The major metabolites were benzoic acid acid conjugates and benzylmercapturic acid. Although females in the mid- and high-dose groups exhibited a slight decrease in excretion of benzylmercapturic acid after the 8th dose, all groups showed increased urinary levels after the 13th doses. An increase in dose from 400 to 1000 mg/kg bw/day resulted in a 7- to 8-fold increase in benzylmercapturic acid excretion. The amount of benzylmercapturic acid excreted in urine collected for 24 hours ranged between 0.13 - 2.05 mg/rat, the higher amounts collected from the rats in the highest dose groups. Benzaldehyde is reduced to benzyl alcohol only to a minor extent; the alcohol sulphate conjugate may further react with glutathione to form benzyl mercapturic acid (Laham and Potvin, 1987).

In the rabbits orally dosed with 350 or 750 mg/kg bw, the aldehyde was oxidised mainly to benzoic acid and excreted predominantly as hippuric acid (approximately 68 % of the administered dose). Other urinary metabolites detected were benzoylglucuronic acid (10 %), benzoyl glucuronide (3 %), free benzoic acid (1.5 %), and trace amounts of benzylmercapturic acid (Laham et al., 1988).

4-Isopropylbenzaldehyde (cuminaldehyde) [FL-no: 05.022]

High doses (2000 mg) of *p*-isopropylbenzaldehyde were given orally to male rabbits. Urine was collected for three days post-treatment. The yield of urinary oxidation metabolites was higher than that of reduction metabolites. This was in contrast to *o*-isopropylbenzaldehyde, the reduction of which was more extensive and the corresponding acids were not found. *p*-Isopropylbenzaldehyde mainly undergoes a combination of oxidation of the aldehyde function and the oxidation of the alkyl-side chain to yield 9-hydroxycuminic acid and 8-hydroxycuminic acid. Cumyl alcohol (cuminyl alcohol, 4-isopropylbenzyl alcohol) and 2-carboxyphenylpropionic acid were minor urinary metabolites. It was concluded that oxidation or reduction

was controlled by the position of substituents, in that oxidation occurs with the *p*-isomer and reduction occurs with the o-isomer. In addition, stereoselective oxidation was found in the aromatic isopropyl group of the *p*-isomer (Ishida et al., 1989b).

Benzoic acid [FL-no: 08.021]

Ring labelled ^{14}C-benzoic acid was given orally at doses in the range of 1 - 400 mg/kg bw to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds and reptiles. Hippuric acid was the primary urinary metabolite in most species. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 hours in chickens and reptiles. Benzoyl glucuronide was predominant in bats. In humans, more than 99 % of ^{14}C was excreted as hippuric acid within 24 hours (Bridges et al., 1970).

Following oral administration of 375 mg [^{14}C]-benzoic acid/kg bw to rats, 91 - 94 % of the radioactivity was recovered in the urine of rats after 72 hours, whereas only 1 - 6 % was present in the faeces. The following metabolites were identified: hippuric acid (70.2 - 84.2 %), benzoyl glucuronide (0.7 - 1.8 %), benzoic acid (0.4 - 12.8 %) and 3-hydroxy-3-phenyl propionic acid (0.1 - 0.2 %) (Nutley, 1990).

Urinary hippuric acid is used as a biologial marker of toluene exposure. In order to investigate the types and quantities of beverages that increase urinary hippuric acid excretion, 137 healthy students were recruited and divided into quintiles based on their consumption of non-alcoholic beverages containing benzoic acid. HPLC was used to determine benzoic acid intake from beverages and urinary hippuric acid before, and 1.5 and 3 hours after consumption of various beverages. The range of benzoic acid in 13 beverages was 0 - 1.02 mg/ml and the benzoic acid intakes from the beverages for groups 1 - 5, respectively, were: 0.4 mg ± 0.5; 23.4 mg ± 9.8; 55.2 mg ± 2.3; 76.3 mg ± 4.0; 116.5 mg ± 16.5. Urinary hippuric acid geometric mean concentrations before consuming beverages in the five groups, respectively, were 0.276, 0.270, 0.207, 0.262 and 0.316 g/l; 1.5 hours after beverage consumption they were 0.210, 0.603, 1.026, 1.066 and 1.688 g/l and significantly increased ($p < 0.001$) after adjustment for urinary hippuric acid before ingestion. Three hours after beverage consumption, urinary hippuric acid geometric mean concentrations in the five groups, respectively, were 0.160, 0.232, 0.306, 0.287 and 0.337 g/l ($p < 0.001$). The authors concluded that beverages containing more than 100 mg benzoic acid may increase urinary hippuric acid significantly (Chang et al., 2000).

Benzyl acetate [FL-no: 09.014]

Following gavage administration of [methylene-^{14}C]-benzyl acetate to groups of three or more male Fischer 344 rats at a dose of 5, 250 or 500 mg/kg bw as the substance alone, in corn oil, or in propylene glycol, 70 - 89 % of the dose was excreted in the urine within 24 hours. Approximately 4 % of the radioactivity was detected in the faeces after 72 hours and about 1 % in the carcass after 72 hours. The elimination of benzyl acetate and metabolites, regardless of vehicle, was largely complete after three days. Urine was collected and urinary metabolites were assayed by Thin Layer Chromatography (TLC) and HPLC. In other animals, ^{14}C plasma levels were measured, and variation of metabolites in plasma were assayed. No benzyl acetate was detected in the plasma or urine at any time point. Small amounts of benzyl alcohol were detected in the plasma at early time points after administration of the neat substance or dissolved in propylene glycol. After administration of 500 and 250 mg/kg bw, unconjugated benzoic acid was the major plasma metabolite. After the 5 mg/kg bw dose, hippuric acid was the major plasma metabolite. At the higher dose levels, small amounts of radioactivity (< 5 % of total plasma ^{14}C) was present as unknown metabolites of high and moderate polarity, but not in all samples. At the 5 mg/kg bw dose, 20 % of plasma ^{14}C was present as the unknown polar metabolite, although this became less important with time. When propylene glycol was used as vehicle, benzylmercapturic acid was detected in plasma, but only at the 5 mg/kg bw dose. Hippuric acid was always the major urinary metabolite but the proportion of dose present as benzoyl glucuronide increased with dose. Low levels (1.0 - 3.6 %) of benzoic acid and benzylmercapturic acid (1.0 - 1.9 %) excreted in urine were not significantly affected by dose or vehicle (Chidgey and Caldwell, 1986).

Chidgey et al. (1986) suggest that formation of benzylmercapturic acid occurs via formation of benzyl sulphate. In a study designed to define the route of metabolism of benzyl acetate leading to the formation of benzylmercapturic acid, male Fischer 344 rats were dosed by gavage with [methylene-^{14}C]benzyl acetate (500 mg/kg bw) alone or together with pyrazole (200 mg/kg), pentachlorphenol (10 mg/kg bw) or both. Urine and faeces were collected and urinary metabolites were assayed by radio-TLC and HPLC. The excretion of ^{14}C was rapid in all cases, with most of the dose being excreted in urine within 24 hours. Co-administration of benzyl acetate with pyrazole, an inhibitor of alcohol dehydrogenase, caused an 11-fold increase in the excretion of benzylmercapturic acid and halved the percentage of the dose excreted as benzoyl glucuronide. Pretreatment with pentachlorophenol, an inhibitor of sulphotransferase activity *in vivo*, abolished the excretion of benzylmercapturic acid, while excretion of the mercapturate following treatment with both pyrazole and pentachlorophenol was higher than in control or pentacholorophenol treated rats, but much lower than in the animals given pyrazole alone. Taken together, these results suggest that the formation of benzylmercapturic acid involves the sulphate ester of benzyl alcohol as an obligatory intermediate and that formation of reactive metabolites of toxicological significance is unlikely (Chidgey et al., 1986).

Fischer 344 rats and C57BL/6N mice were administered [ring-UL-^{14}C]benzyl acetate at single oral doses of 5 or 500 mg/kg bw in rats or 10 mg/kg bw in mice, and urine and faeces were collected for 96 hours to determine the effects of age on disposition of benzyl acetate. Age groups studied were 3 to 4, 9 and 25 month-old rats and 2, 13 and 25 month-old mice. In rats, approximately 80 % of radioactivity was recovered in the urine in the first 24 hours for all age groups. The major urinary metabolite was hippuric acid (> 90 % of total urinary radioactivity) and benzylmercapturic acid (1 - 2 %) was the only other metabolite detected in the urine of rats. There were no age differences in the percentage of [^{14}C]benzyl acetate excreted as hippuric acid, but the amount of excreted benzyl mercapturic acid increased slightly in the 25 month-old rats as compared to younger rats. The percentage of radioactivity excreted in the faeces was slightly decreased in the 25 months old group. In mice, hippuric acid was the major urinary metabolite, constituting 93 - 96 % of the total dose after 96 hours. Less radioactivity was excreted in the urine of 25 month-old mice than in the younger groups. Faecal excretion was a minor route and the amount was similar for all age groups. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice (McMahon et al., 1989).

Benzyl acetate was administered to rats and mice in gavage and dosed feed studies. Gavage study groups of six male F344/N rats and twelve male B6C3F$_1$ mice were administered benzyl acetate in corn oil at 500 mg/kg (rat) and 1000 mg/kg (mouse). Blood samples were collected 5 min - 24 hours after dosing. Dosed feed studies groups of ten rats and ten mice, of the same strains as in the gavage study, were dosed with benzyl acetate in feed (10,800 ppm for rats and 2700 ppm for mice) *ad libitum* during the study. The concentrations in feed were estimated to provide a daily benzyl acetate dose of 648 mg/kg for rats and 900 mg/kg for mice. At day 7 and 8 blood samples were collected at five time points during 15 hours, with two animals from each species sampled at each time point. Benzyl acetate was not detected in any plasma samples collected in the studies. Except for the 5 and 10 minutes rat plasma samples and the 5 minutes mice plasma samples in the gavage study, no benzyl alcohol was detected in plasma. Concentrations of benzoic acid and hippuric acid in plasma rapidly increased to peak concentrations within 3 hours after gavage with the peak benzoic acid concentrations being much higher (about 10- to 20-fold) than the peak hippuric acid concentrations. Plasma concentrations of benzoic acid in the dosed feed studies were much lower (more than 100-fold) than the concentrations in the gavage studies, consistently with the mode of administration (bolus dose with gavage). Plasma concentrations of hippuric acid were comparable in both studies. The absence of benzyl acetate in plasma shows that benzyl acetate is rapidly hydrolysed to benzyl alcohol. The major metabolite of benzyl acetate, benzoic acid, is mainly dependent on the conjugation pathway involving Coenzyme A (CoA). This pathway would be saturated when plasma concentrations of benzoic acid are very high or when the CoA is depleted. Such conditions appear to have occurred after a bolus gavage dose of benzyl acetate to result in a brief peak in the plasma concentration of benzoic acid. When benzyl acetate was administered to rats and mice in dosed feed, it appears that the CoA conjugation pathway was never saturated and plasma concentration of benzoic acid remained low (Yuan et al., 1995).

Other related substances

Sodium benzoate

Male volunteers were given oral doses of 2000 to 5000 mg sodium benzoate. The 5000 mg dose group was given a 5000 mg dose of glycine one hour later and 2000 mg doses every two hours thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoyl glucuronide were detected at both doses. Co-administration of glycine with benzoate increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid (Amsel and Levy, 1969).

After administration of oral doses of 40, 80 and 160 mg/kg bw of sodium benzoate to humans, the mean plasma Area Under Curves (AUCs) of benzoic acid increased disproportionately to the dose, 3.7 and 12.0 times greater, respectively, for the higher dosages than for the lowest dose, while the mean AUCs for hippuric acid was proportional to dose. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans (Kubota et al., 1988; Kubota and Ishizaki, 1991).

Subgroup 2 (Hydroxy- and alkoxy-substituted benzyl derivatives)

Candidate substances from subgroup 2

Piperonyl alcohol (3,4-methylenedioxybenzyl alcohol) [FL-no: 02.205]

The metabolism of piperonyl alcohol [FL-no: 02.205] and piperonal (3,4-methylenedioxybenzaldehyde) [FL-no: 05.016] (supporting substance) was studied in male Wistar rats. Piperonyl alcohol dissolved in propylene glycol was administered by oral gavage at a dose of 1 mmol/kg bw (corresponding to 152 mg/kg bw) and urine samples were taken at 24 and 48 hours. Recovery of urinary metabolites were 90 %, and metabolite excretion occurred mainly within 24 hours. Piperonyl glycine was identified as the major metabolite (70 ± 5 %; 24-hour analysis expressed as a percent of administered dose) and piperonylic acid (17 ± 3 %) was the other important metabolite. Demethylenation of the methylenedioxy moiety led to the excretion of three cathecol derivatives, which accounted for 0.7 % of the dose, protocatechuic acid (0.4 ± 0.1 %), protocatechuyl alcohol (0.3 ± 0.1 %). Other minor metabolites were piperonyl alcohol (1.4 ± 0.5 %) and vanillyl alcohol (0.05 ± 0.03 %) (Klungsoeyr and Scheline, 1984).

In a study of several methylenedioxyphenyl (MDP) compounds, radiolabelled piperonyl alcohol (1-hydroxymethyl-3,4-methylene-^{14}C-dioxybenzene) was administered to male Swiss-Webster mice in a DMSO solution by oral gavage at a dose of 0.76 mg/kg bw. Total radiocarbon determinations were made on expired $^{14}CO_2$ at 0.5, 1, 2, 4 and 6 hours after dosing and at each 6-hour interval thereafter. Urine and faeces samples were taken at 12, 24 and 48 hours after treatment. The 12-hour urine samples were used for separation and characterisation of metabolites. At the end of the experiment, $^{14}CO_2$ excretion amounted to 3 % of the dose, indicating that demethylenation of piperonyl alcohol only occurs as a minor metabolic pathway. The major part of radioactivity was retrieved in urine, 93.3 %, and less than 10 % in faeces. The major urinary metabolite after administration of piperonyl alcohol was piperonyl glycine. Other MDP substances studied were safrole, dihydrosafrole, myristicin, Tropital, piperonyl butoxide, piperonal and piperonylic acid. The major metabolic pathway for piperonyl butoxide, safrole, dihydrosafrole and myristicin was demethylenation of the methylenedioxy moiety. As for piperonyl alcohol, oxidation and conjugation of the side chain is the major metabolic pathway for Tropital, piperonal and piperonylic acid. The authors discussed that the polar nature of these compounds or their ease of conversion to polar products may minimise their entrance to the lipid components of the microsomal enzymes so that no extensive demethylenation would occur (Kamienski and Casida, 1970).

Gallic acid (3,4,5-trihydroxybenzoic acid) [FL-no: 08.080]

Following administration of gallic acid [FL-no: 08.080] to rats, either in the diet at a concentration of 0.5 % or in single doses of 100 mg/rat via oral gavage, the major urinary excretion products were the unchanged parent substance and one metabolite which was concluded to be 4-O-methyl gallic acid. A minor metabolite, 2-O-methylpyrogallol, was excreted mainly as an acid-labile conjugate. When the same compound was given at 100 mg/rat as intraperitoneal injection, the results were similar to those obtained when the substance was given orally, however, a minor metabolite identified as pyrogallol was also present along with a trace of 2-O-methylpyrogallol. Rabbits administered a diet containing 0.5 % gallic acid also excreted 4-O-methyl gallic acid, pyrogallol, and possibly also 2-O-methyl pyrogallol. The data indicated that mostly free benzoic acid derivatives were excreted, although rabbits excreted an acid-labile conjugate of 4-O-methyl gallic acid. The results indicate that O-methylation and decarboxylation are the reactions involved in the metabolic conversion of gallic acid. The authors stated that this selective O-methylation would prevent the formation of the catechol configurations (Booth et al., 1959).

Scheline (1966a) reported that rats that were administered 100 mg/kg bw of gallic acid [FL-no: 08.080] by oral gavage excreted the parent substance and the free and acid-labile conjugates of its 4-O-methyl ether. Pyrogallol and 2-O-methylpyrogallol, the decarboxylated metabolites, were excreted in their conjugated forms. Dosing with 30 and 300 mg gallic acid/kg bw showed that excretion of decarboxylated metabolites increased with increasing dose. Intraperitoneal injection of gallic acid in four rats resulted in the urinary excretion of gallic acid and 3,5-dihydroxy-4-methoxybenzoic acid, but neither pyrogallol or 2-O-methylpyrogallol were detected in these urines. A study of the ability of rat intestinal contents to metabolise gallic acid showed that it was decarboxylated to pyrogallol when test substance was added to medium containing extracts of caecal or colon contents. Test substance was recovered essentially unchanged when small intestine contents were used (Scheline, 1966a).

In order to examine decarboxylation and demethylation of some phenolic benzoic acid derivatives by rat caecal contents, test substances were incubated for 22 hours in medium containing ceacal contents. Solutions together with appropriate standards were then examined by TLC. Gallic acid [FL-no: 08.080] gave rise to pyrogallol which was present in five out of eight samples. When pyrogallol was absent after incubation, large amounts of resorcinol were observed on the chromatograms. Dehydroxylation to resorcinol was also seen when pyrogallol itself was incubated with caecal extract. Unchanged gallic acid was found in 4 out of 8 samples. Pyrogallol was not dehydroxylated to catechol in these experiments. The main findings of the study that covered 27 phenolic benzoic acid derivatives was that decarboxylation only occurred when a free hydroxyl group was present in the *para* position (Scheline, 1966b).

The metabolic fate of gallic acid [FL-no: 08.080] in peripheral blood, liver and urine after oral administration was studied in six-week-old male Wistar rats in order to determine the most appropriate route of administration for the treatment of liver cancer, i.e. the route that gives the highest concentration of gallic acid in liver. Gallic acid was given orally to the rats at 50, 100 or 500 mg/kg bw (The number of animals is not reported, but results from the 100 mg/kg bw group are shown as the mean of 4 - 6 animals). Blood samples were taken from the portal vein and the inferior vena cava at 5, 15, 30, 60, 180 and 360 minutes after administration and urine was collected at the same time points. Intestinal contents were collected and, in order to avoid contamination due to enterohepatic circulation, bile duct was ligated before oral administration of 100 mg/kg bw. Animals were sacrificed and the entire intestinal contents were collected. The liver was removed from terminated animals after perfusion with saline to eliminate blood contamination. For analysis, samples from serum, urine and liver were processed and then analysed by HPLC. Gallic acid reached its peak concentration in the portal vein 15 minutes after oral administration of 100 mg/kg bw. After 30 minutes it had decreased to half the concentration and had almost disappeared after 6 hours. The only metabolite detected in the blood and urine was identified as 4-O-methyl gallic acid. 4-O-Methyl gallic acid also reached peak concentration in the portal vein after 15 minutes, and then decreased slowly. In the inferior vena cava both gallic acid and 4-O-methyl gallic acid reached peak concentration at 30 minutes after oral administration of 100 mg gallic acid/kg bw. In the portal vein, gallic acid was detected at about twice the concentration of 4-O-methyl gallic acid. In the inferior vena cava, 4-O-methyl gallic acid and gallic acid

were detected in approximately equal proportions and in about the same amount as 4-O-methyl gallic acid in the portal vein. 4-O-Methyl gallic acid, but not gallic acid, was found in the liver homogenate prepared after thorough perfusion with saline. The main metabolite of gallic acid in urine was 4-O-methyl gallic acid and its concentration was about 100 times higher than in the inferior vena cava. Gallic acid was also found in urine at a higher concentration than in the inferior vena cava, but at lower concentration than 4-O-methyl gallic acid in urine. In contrast to previously published studies (Booth et al., 1959; Scheline, 1966a), this study (Zong et al., 1999) did not detect pyrogallol as a metabolite in blood or urine. The authors attribute this discrepancy to the earlier studies using TLC for determination of metabolites, but without proper determination of structures, and also comment that the time for collecting urine under unstable conditions may have led to the decomposition of gallic acid to pyrogallol (Zong et al., 1999).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

Absorption, distribution, metabolism and excretion of ethyl paraben [FL-no: 09.367] were investigated in Wistar rats administered 100 mg by oral gavage. Animals were held in metabolism cages for the collection of urine (at approximately 15, 30, 60, 75, 90, 120, 150 and 210 minutes) and blood (at approximately 30, 60, 90, 120, 180, 240 and 360 minutes), and samples were analysed to establish the excretion kinetics. Metabolites were detected in the urine starting at 30 minutes after dosing, but no unchanged ethyl paraben was identified. *p*-Hydroxyhippuric acid appeared in the urine 30 minutes after dosing and its concentration increased steadily during the next three hours. The glucuronide and ethereal sulphate metabolites only appeared between 30 and 75 minutes post-ingestion. A continuous increase of free 4-hydroxybenzoic acid in the blood occurred during the first hour post-dosing, but its concentration then decreased over the next hour and plateaued for the remaining four hours of sample collection. Maximum urinary excretion of free 4-hydroxybenzoic acid occurred at 90 minutes post-dosing, whereas excretion of the glucuronic and glycine conjugates increased until the end of the collection period at 210 minutes post-dosing. In summary, absorption of ethyl paraben was followed by metabolism and excretion of mainly free 4-hydroxybenzoic acid and its glucuronic and glycine conjugates. A small portion of the dose was excreted as sulphate conjugate (Derache and Gourdon, 1963).

The ^{14}C-labelled ethyl 4-hydroxybenzoate was orally given to four male cats in the diet at a concentration that provided a single dose of 156 mg/kg bw, equivalent to 130 mg/kg bw of the parent acid. Urine was collected at 24-, 48- and 72-hour intervals, total faeces were collected at 72-hour, and all samples were assayed for total radioactivity. The radioactive metabolites present in the 24-hour samples were isolated and identified. Essentially, all (mean = 96.0 %) of the radioactiviy was excreted within 72 hour with the breakdown expressed as mean values for the four animals as follows: 24-hour urine, 85.8 %; 48-hour urine, 3.6 %; 72-hour urine, 0.8 %; 72-hour faeces, 5.8 %. The 24-hour urine samples revealed two metabolites. Metabolite I contained between 54 and 69 % of the administered radioactivity and had a similar retention volume as *p*-hydroxyhippuric acid. Metabolite II contained between 31 and 46 % of the administered radioactivity and had a similar retention volume as 4-hydroxybenzoic acid. Additional evaluations confirmed the identity of the suggested metabolites (Phillips et al., 1978).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367] and supporting substances 4-hydroxybenzoic acid [FL-no 08.040] and butyl 4-hydroxybenzoate (butyl paraben) [FL-no: 09.754]

Groups of three fasted dogs were administered single doses of 1 g/kg bw of 4-hydroxybenzoic acid ([FL-no 08.040], supporting substance) or its methyl, ethyl ([FL-no: 09.367], candidate substance), propyl and butyl ([FL-no: 09.754], supporting substance) esters orally or 50 mg/kg bw by intravenous injection. Blood and urine samples were collected at fixed intervals until 48 hours. Recovery of total test material as metabolites in urine after the oral and intravenous doses was 60 - 95 % for the acid and the methyl, ethyl and propyl esters. For the candidate substance ethyl 4-hydroxybenzoate [FL-no: 09.367], recovery of total material was 66 % of the oral and 70 % of the intravenous dose. Metabolites were detectable in the blood up to 24 hours post-ingestion. Of the dose, 12.3 % was excreted as free acid and 32.5 % as the glucuronic acid conjugate. Recovery of the butyl ester was 48 % after oral and 40 % after intravenous dosing. After oral dosing about 5

% of the butyl ester was excreted as free 4-hydroxybenzoic acid and 27.5 % as the glucuronic acid conjugate. Other conjugates were not determined. After intravenous dosing, 11.3 % of the given dose was recovered as free acid in urine and 20.1 % as the glucuronic acid conjugate. The test material was mainly excreted within 24 hours after dosing. The low rate of recovery seen with both dosing methods was attributed to incomplete hydrolysis of the butyl ester in the body. *In vitro* incubation of the butyl ester with freshly prepared liver homogenate showed complete hydrolysis within 30 - 60 minutes. Studies conducted with related benzoate esters, methyl and ethyl *p*-hydroxybenzoate, showed significantly higher rates of test material recovery when given to dogs by the oral and intravenous route, and showed 100 % hydrolysis within 3 minutes when incubated with liver homogenate. This finding suggests that an increase in the length of the alkyl rest in the homologous series of alkyl esters make the esters more resistant to hydrolysis and may result in the activation of other metabolic and excretion pathways (Jones et al., 1956).

Supporting substances from subgroup 2

p-Anisyl alcohol (4-methoxybenzyl alcohol) [FL-no: 02.128]

In an *in vitro* study, *p*-anisyl alcohol (4-methoxybenzyl alcohol) [FL-no: 02.128] was incubated with rat caecal extract. Analysis after approximately 46 hours showed the presence of unchanged compound and anisic acid. No observation of O-demethylation was observed (Scheline, 1972).

Vanillyl alcohol (4-hydroxy-3-methoxybenzyl alcohol) [FL-no: 02.213] and vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018]

In an *in vivo* study conducted in male albino rats, vanillyl alcohol [FL-no: 02.213] and vanillin [FL-no: 05.018] were administered by gavage in doses of 100 or 300 mg/kg bw. Urinary metabolites were collected over the first 24 - 48 hours period and analysed qualitatively. Vanillyl alcohol was mainly excreted as vanillyl alcohol or vanillic acid and related conjugates. The aldehyde intermediate was also detected. Conjugated fractions of vanillin, guaiacol, catechol, 4-methylguaiacol and 4-methylcatechol were also identified in smaller quantities. Oral dosage of 100 mg/kg bw of vanillin resulted in an urinary excretion of most metabolites within 24 hours, mainly as glucuronide and sulphate conjugates, although vanillic acid was also excreted as free acid and as glycine conjugate. After 48 hours, 94 % of the dose was accounted for as follows: vanillin 7 %, vanillyl alcohol 19 %, vanillic acid 47 %, vanilloylglycine 10 %, catechol 8 %, 4-methyl catechol 2 %, guaiacol 0.5 % and 4-methyl guaiacol 0.6 %. Vanillin and its primary reduction and oxidation metabolites were also excreted in appreciable amounts in the bile. Bile collected five hours after two rats were given 100 and 300 mg/kg bw oral doses of vanillin contained glucuronide conjugates of vanillin (6 %), vanillyl alcohol (8 %) and vanillic acid (9 %). The results show that both oxidative and reductive pathways exist for the metabolism of vanillin, although the oxidative metabolism dominates. At a dose level of 100 mg/kg bw, 57 % of the dose of vanillin was excreted as free vanillic acid or its conjugates and in total oxidation products amounted to approximately 65 - 70 % of the dose. The reduction pathway accounted for a little more than 20 % of the dose (Strand and Sheline, 1975).

In Sprague-Dawley albino rats, 100 mg vanillin [FL-no: 05.018]/kg bw was given by intraperitoneal injection and 24-hour urine was collected and analysed. The main urinary metabolite was conjugated vanillic acid which accounted for 41 % of the administered dose, while free vanillic acid accounted for 6 %. In addition, there was a trace of catechol. Vanillyl alcohol, a reductive product, represented 10 % of the administered dose. The presence of the urinary glycine conjugate of vanillic acid was not reported in this study. The oxidative path of metabolism was found to predominate, however, the importance of the minor reductive pathway may be magnified by inhibition of the oxidative process, as was achieved by administration of disulphiram in the study (Wong and Sourkes, 1966).

An experiment was conducted with the aim to determine whether man is capable of oxidising vanillin to vanillic acid. A 100 mg dose of vanillin dissolved in water was given to an adult human and the urine collected for 24 hours. Examination revealed an increase in the vanillic acid output in the urine from a

background level of 0.3 mg/24 hours to 96 mg/24 hours. The observed increase accounted for approximately 94 % of the vanillin dose (Dirscherl and Wirtzfeld, 1964).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017]

A 1 g/kg bw oral dose of veratraldehyde (3,4-dimethoxybenzaldehyde) was administered to rabbits by gavage and urine was collected for 24 hours. Approximately 70 % of the aldehyde was accounted for in urine, mainly as the corresponding acid, veratric acid (28 %), and its glucuronic acid (38 %) or sulphate (3 - 7 %) conjugate. To a small extent, veratric acid was decarboxylated and O-demethylated to yield catechol (Sammons and Williams, 1941). Presumably, veratric acid may enter the enterohepatic circulation where gut microflora decarboxylate the acid to yield catechol (*o*-hydroxyphenol). The observation that catechol was formed as a minor metabolite when veratraldehyde was incubated with rat caecal extract illustrates this decarboxylation pathway in gut bacteria (Scheline, 1972).

4-Hydroxybenzaldehyde [FL-no: 05.047] and salicylaldehyde (2-hydroxybenzaldehyde) [FL-no: 05.055]

In rabbits, 96 % of a single oral dose of 400 mg/kg bw 4-hydroxybenzaldehyde was excreted in the urine within 24 hours as 4-hydroxybenzoic acid and its glycine, glucuronic acid and sulphate conjugates (Bray et al., 1952b).

A single dose of 400 mg/kg bw salicylaldehyde (was administered to a fasted rabbit in three or six experiments. Approximately 75 % of the dose was excreted as ether soluble acids in the urine collected over 24 hours, and 27 % and 3 % accounted for as glucuronic acid and sulphate conjugates, respectively (Bray et al., 1952b).

In a corresponding study, approximately 94 % of a single oral dose of 250 or 500 mg/kg bw salicylaldehyde administered to two groups of four rabbits was excreted unchanged or as the glucuronic acid and sulphate conjugates, while the major part was excreted as the unchanged acid (Bray et al., 1948).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017], vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018] and vanillic acid (4-hydroxy-3-methoxy benzoic acid) [FL-no: 08.043]

Veratraldehyde [FL-no: 05.017], vanillin [FL-no: 05.018] or vanillic acid [FL-no: 08.043] were given orally by gavage to six rabbits at a dose of approximately 1 g/kg bw. Urine was collected for 5 hours. After administration of veratraldehyde, approximately 70 % of the material was recovered in the urine as free corresponding acid (28 %) and its glucuronic acid (38 %) or sulphate (3 - 7 %) conjugate. Approximately 69 % of vanillin was oxidised to vanillic acid, of which 44 % was recovered as free acid and 25 % conjugated acid. About 14 % of the dose was excreted as the glucuronic acid conjugate of vanillin. In the case of vanillic acid, 56 % was excreted as free vanilllic acid and 27 % as conjugated, as glucuronide conjugate or ethereal sulphate. Less than 5 % was demethylated (Sammons and Williams, 1941).

4-Hydroxybenzoic acid [FL-no: 08.040]

Groups of four to eight rabbits were administered doses of 100, 250, 500, 1000 or 1500 mg/kg bw 4-hydroxybenzoic acid by gavage. Urine was collected continuously and analysed for metabolites. Total urinary recovery of the test material was in the range of 84 to 104 %, with ether soluble acids comprising 64 to 75 % of the total. Glucuronic acid and sulphate conjugates were also detected in the urine at 10 to 35 % and 4 to 7 %, respectively. The levels of all the metabolites returned to background levels within 24 hours after dosing (Bray et al., 1947).

Results from four experiments showed that between 2.2 and 5.4 % was excreted in the urine within 24 hours as the corresponding hippurate of a 0.41 mmole 4-hydroxybenzoic acid dose administered by intraperitoneal injection to female albino rats (Teuchy et al., 1971).

Other related substances

Methyl 4-hydroxybenzoate (methyl paraben)

Methyl 4-hydroxybenzoate (methyl paraben) was administered to three male rabbits by oral gavage at 800 mg/kg bw as a 12 % sodium salt solution, and the 24-hours urine was analysed. Three major metabolites, 4-hydroxybenzoic acid, *p*-hydroxyhippuric acid and *p*-carboxyphenyl glucuronide, as well as two minor metabolites, *p*-hydroxybenzoyl glucuronide and *p*-carboxyphenyl sulphate, were identified (Tsukamoto and Terada, 1962).

Concluding remarks on metabolism

The esters in subgroup 1,benzyl derivatives, will upon hydrolysis yield benzyl alcohol or benzoic acid along with alkyl carboxylic acids or alcohols. The metabolic fate of alkyl carboxylic acids and alcohols has been discussed in previous FGEs, and will not be discussed further in this evaluation. Benzyl alcohol, benzylaldehyde and benzoic acid have been evaluated by the JECFA (JECFA, 1996b), as has 4-isopropylbenzyl alcohol (JECFA, 2002a), which is the alcohol moiety of candidate substance [FL-no: 09.611] (4-isopropylbenzyl acetate). Benzaldehyde and the benzyl alcohols are expected to be oxidized to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids.

The candidate substance [FL-no: 09.314 (benzyl crotonate)] will yield crotonic acid as acid moiety, this substance has been discussed in the previous FGE.05Rev2 (EFSA, 2010f). In addition, crotonic acid has been evaluated by the SCF (SCF, 2002a).

The alcohol part of the candidate substance [FL-no: 09.656] (3-methylbut-3-enyl benzoate) includes a terminal double bond, a structure that has been discussed in FGE.06Rev3 (EFSA, 2011w) and will not be further discussed in this FGE.

The candidate substance (diethoxymethyl)benzene [FL-no: 06.017] is an acetal. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde is expected to be oxidized to benzoic acid and subsequently conjugated with glycine or glucuronic acid and eliminated via the urine. The same biotransformations will occur with the three tolualdehyde isosomers ([FL no: 05.026, 05.028 and 05.029].

Subgroup 2, hydroxy- and alkoxy-substituted benzyl derivatives, includes 13 esters of which one [FL-no: 09.895] (4-methoxybenzyl-2-methylpropionate) will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) [FL-no: 02.128] upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxybenzyl alcohol is expected to be excreted in the urine either unchanged or as glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative [FL-no: 02.164] (4-hydroxy-3,5-dimethoxybenzyl alcohol).

The remaining 12 esters in subgroup 2 will yield hydroxy- and/or alkoxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine as the glucuronic acid, glycine or sulphate conjugate or at a minor extent unchanged. The same metabolic route is proposed for the candidate acids [FL-no: 08.087] (4-hydroxy-3,5-dimethoxybenzoic acid), [FL-no: 08.132] (3-hydroxybenzoic acid) and [FL-no: 08.133] (3,4-dihydroxybenzoic acid).

The candidate substance piperonyl alcohol [FL-no: 02.205] (3,4-methylenedioxybenzyl alcohol) is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as a glycine conjugate. Demethylenation of the methylenedioxy moiety does seem to be only a very minor metabolic path for this compound.

For the six candidate aldehydes in subgroup 2, the main metabolic pathway is presumed to be oxidation to the corresponding acids, followed by glycine and glucuronic acid conjugation and excretion. The reduction

to alcohols is a minor metabolic route and the oxidative pathway clearly dominates. To a minor extent O-demethylation followed by conjugation may occur.

The main metabolite of gallic acid (3,4,5-trihydroxy-benzoic acid) [FL-no: 08.080] is expected to be 4-O-methyl gallic acid, i.e. the product of O-methylation. Decarboxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

The biphenyl substance in subgroup 3 is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2.

III.4. Summary and Conclusions

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo* to their component alcohols and acids. Nine of the 15 esters from subgroup 1 (benzyl derivatives) will yield benzyl alcohol which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester [FL-no: 09.611] (4-isopropylbenzyl acetate) will yield 4-isopropylbenzyl alcohol. This substance has been previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidized to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids.

Subgroup 1

Nine of the 15 esters from subgroup 1 will yield benzyl alcohol, which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester, 4-isopropylbenzyl acetate [FL-no: 09.611], will yield 4-isopropylbenzyl alcohol, previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidised to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids. Of the remaining five candidate esters in subgroup 1, four are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825] and prenyl benzoate [FL-no: 09.693]. One ester, methyl 4-methylbenzoate [FL-no: 09.631], will yield 4-methylbenzoic acid upon hydrolysis. Benzoic acid will mainly be conjugated with glycine and excreted as hippuric acid. Conjugation with glycine may be a saturable process and with increasing levels of exposure glucuronide conjugation may become relatively more important.

One of the substances, (diethoxymethyl)benzene [FL-no: 06.017], in subgroup 1 is an acetal. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by the JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid and subsequently conjugated with glycine or glucuronic acid and eliminated via the urine. The same biotransformations will occur with the three tolualdehyde isomers ([FL no: 05.026, 05.028 and 05.029]). Additionally, for the tolualdehyde isomers reduction of the aldehyde function to yield the corresponding alcohol has also been demonstrated. These alcohols can be converted into the corresponding sulphate esters which in their turn can further react with glutathione to give benzylmercapturic acids. This metabolic pathway is more important for *o*-touladehyde than for the other two isomers, but at any rate only a limited fraction of the dose (< 10 %) will be eliminated via this route.

Subgroup 2

Subgroup 2 (hydroxy- and alkoxy-substituted benzyl derivatives) includes 13 esters of which one, 4-methoxybenzyl-2-methylpropionate [FL-no: 09.895], will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxybenzyl alcohol is expected to be excreted in the urine either unchanged or as a

glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative 4-hydroxy-3,5-dimethoxybenzyl alcohol [FL-no: 02.164].

The remaining 12 esters in subgroup 2 [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(cis)-enyl anisate; hex-3(cis)-enyl-4-methoxybenzoate); 09.570 (hex-3-enyl salicylate; hex-3-enyl-2-hydroxybenzoate); 09.581 (hexyl salicylate; hexyl 2-hydroxybenzoate); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate; 3-methyl-but-2-enyl 2-hydroxybenzoate); 09.762 (pentyl salicylate; pentyl 2-hydroxybenzoate); 09.798 (ethyl vanillate; ethyl 3-methoxy-4-hydroxybenzoate); 09.799 (methyl vanillate; methyl 3-methoxy-4-hydroxybenzoate); 09.852 (2-methylbutyl 2-hydroxybenzoate; 2-methylbutyl salicylate)] will yield hydroxy- and/or alkoxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine unchanged or as the glucuronic acid, glycine or sulphate conjugate. The same metabolic route is proposed for the candidate acids [FL-no: 08.087] (4-hydroxy-3,5-dimethoxybenzoic acid), [FL-no: 08.132 (3-hydroxybenzoic acid)] and [FL-no: 08.133] (3,4-dihydroxybenzoic acid).

The main metabolic pathway for the acetal [FL-no: 06.104 (vanillin propylene glycol acetal)], after hydrolysis to the aldehyde and for the five candidate aldehydes in subgroup 2 [FL-no: 05.066 (4-ethoxy-3-methoxybenzaldehyde); 05.129 (2-methoxybenzaldehyde); 05.142 (3,4-dihydroxybenzaldehyde); 05.153 (4-hydroxy-3,5-dimethoxybenzaldehyde); 05.158 (3-methoxybenzaldehyde)] is presumed to be oxidation to the corresponding acids, followed by conjugation and excretion. The reduction to alcohols is a minor metabolic route and the oxidative pathway dominates clearly. For 2-methoxybenzaldehyde it has been shown that this reductive metabolic pathway leads to the formation of sulphate conjugates, which are converted into glutathione conjugates. The latter are eliminated via the urine as mercapturic acids. To a minor extent O-demethylation followed by conjugation may occur.

The candidate substance piperonyl alcohol (3,4-methylenedioxybenzyl alcohol) [FL-no: 02.205] is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as glycine conjugate. Demethylenation of the methylenedioxy moiety is a very minor metabolic path for this compound, according to published literature.

The main metabolite of gallic acid (3,4,5-trihydroxy-benzoic acid) [FL-no: 08.080] is expected to be 4-O-methyl gallic acid the product of O-methylation. Decarboxilation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

Subgroup 3

The biphenyl substance in subgroup 3, [FL-no: 05.221 (6,6'-dihydroxy-5,5'dimethoxy-biphenyl-3,3'-dicarbaldehyde)], is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2. It is expected that the aldehyde group(s) will undergo oxidation to form the corresponding carboxylic acid which is likely to be conjugated and excreted. The reduction of the alcohol groups may again be a minor pathway, but some steric hindrance may occur making this less likely than for the benzaldehyde derivatives in subgroup 2.

Based on experimental evidence and general knowledge of toxicokinetics of structurally related compounds it is expected that at the reported levels of intake as flavouring substances, the candidate substances in FGE.20Rev4 would be rapidly and efficiently absorbed, metabolised to innocuous products and excreted.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 13 candidate substances of the present flavouring group evaluation from chemical groups 23 and 30, and for 63 supporting substances evaluated by the JECFA at the 57th meeting (JECFA, 2002a). The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Benzyl alcohol [02.010])	Rabbit	NR	Oral	1040	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	2979	(Ciba-Geigy Corp., 1945)	
	Rat	NR	Oral	2080	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	1230	(Jenner et al., 1964)	
	Rat	M, F	Oral	1570	(Damment, 1980)	
	Rat	NR	Oral	3100	(Smyth et al., 1951a)	
	Mouse	NR	Gavage	1580	(Jenner et al., 1964)	
	Mouse	NR	Oral	1150	(Carter et al., 1958)	
(Benzyl formate [09.077])	Rat	M, F	Gavage	1.7 (1.4-2.1) ml/kg bw (1840; 1510-2270)[10]	(Shelanski and Moldovan, 1971d)	
(Benzyl acetate [09.014])	Rabbit	NR	Oral	2640	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	2490	(Jenner et al., 1964)	
	Rat	NR	Oral	3690	(Graham and Kuizenga, 1945)	
(Benzyl propionate [09.132])	Rat	NR	Oral	3300	(Moreno, 1973u)	
(Benzyl butyrate [09.051])	Rat	NR	Oral	1850	(Moreno, 1973v)	
	Rat	M, F	Gavage	2330	(Jenner et al., 1964)	
(Benzyl isobutyrate [09.426])	Rat	M, F	Oral	2850	(Owen, 1971)	
(Benzyl isovalerate [09.458])	Rat	NR	Oral	5000	(Moreno, 1974j)	
Benzyl dodecanoate [09.315]	Rat	NR	Oral	> 5000	(Moreno, 1975m)	
(Benzyl 2-methylcrotonate [09.494])	Rat	NR	Oral	> 5000	(Moreno, 1979d)	
(Benzyl benzoate [09.727])	Cat	NR	Oral	2240	(Graham and Kuizenga, 1945)	
	Rabbit	NR	Oral	2016	(Draize et al., 1948)	
	Rabbit	NR	Oral	1680	(Graham and Kuizenga, 1945)	
	Rabbit	NR	Oral	1800	(Lehman, 1955)	
	Guinea pig	NR	Oral	1120	(Draize et al., 1948)	
	Guinea pig	NR	Oral	1000	(Lehman, 1955)	
	Rat	NR	Oral	1904	(Draize et al., 1948)	
	Rat	NR	Oral	2800	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	1700	(Lehman, 1955)	
	Mouse	NR	Oral	1568	(Draize et al., 1948)	
	Mouse	NR	Oral	1400	(Lehman, 1955)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Benzyl phenylacetate [09.705])	Rat	M, F	Oral	> 5000	(Owen, 1971)	
(Benzaldehyde [05.013])	Guinea pig	M, F	Gavage	1000	(Jenner et al., 1964)	
	Rat	M, F	Gavage	1300	(Jenner et al., 1964)	
	Rat	NR	Oral	2850	(Sporn et al., 1967)	
	Rat	M, F	Gavage	1300	(Taylor et al., 1964)	
	Mouse	NR	Diet	1250	(Schafer and Bowles, 1985)	
(alpha,alpha- Dimethoxytoluene [06.003])	Rat	NR	Oral	1220	(Moreno, 1977z)	
(5-Hydroxy-2-phenyl-1,3-dioxane [06.002])	Rat	NR	Oral	3749	(Levenstein, 1974g)	
	Rat	NR	Oral	2750	(Moreno, 1980k)	
(4-Methyl-2-phenyl-1,3-dioxolane [06.032])	Rat	M, F	Gavage	3000	(Lewis and Palanker, 1979b)	
(Benzoic acid [08.021])	Mouse	NR	Oral	1250	(Schafer and Bowles, 1985)	
	Mouse	NR	Oral	1996	(Sado, 1973)	
	Mouse	NR	Gavage	1950	(Shell Oil Company, 1982)	
(Methyl benzoate [09.725])	Rabbit	NR	Oral	2170	(Graham and Kuizenga, 1945)	
	Guinea pig	NR	Gavage	4100	(Kravets-Bekker and Ivanova, 1970)	
	Rat	NR	Oral	2170	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	1350	(Jenner et al., 1964)	
	Rat	NR	Gavage	3500	(Kravets-Bekker and Ivanova, 1970)	
	Rat	M, F	Oral	3420	(Smyth et al., 1954)	
	Mouse	NR	Gavage	3330	(Jenner et al., 1964)	
	Mouse	NR	Gavage	3000	(Kravets-Bekker and Ivanova, 1970)	
Methyl 4-methylbenzoate [09.631]	Rat	M	Gavage	2987	(Dashiell and Hinckle, 1981)	
	Rat	NR	Oral	3300	(Moreno, 1977aa)	
(Ethyl benzoate [09.726])	Rabbit	NR	Oral	2630	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	2100	(Graham and Kuizenga, 1945)	
	Rat	M, F	Oral	6480	(Smyth et al., 1954)	
Butyl benzoate [09.779]	Mouse	M, F	Gavage	3450	(Bier, 1979)	
	Rat	M, F	Gavage	5140[1]	(Smyth et al., 1954)	
(Hexyl benzoate [09.768])	Rat	NR	Oral	12300	(Smyth et al., 1951a)	
(Isopropyl benzoate [09.770])	Rat	NR	Oral	3730	(Smyth et al., 1951a)	
(Isobutyl benzoate [09.757])	Rat	M, F	Gavage	3685	(Levenstein, 1973e)	
(Isopentyl benzoate [09.755])	Rat	NR	Oral	6330	(Weir and Wong, 1971b)	
(Hex-3-enyl benzoate [09.806])	Rat	NR	Oral	> 5000	(Moreno, 1976u)	
(4-Isopropylbenzyl alcohol [02.039])	Rat	NR	Oral	1020	(Moreno, 1973z)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
4-Isopropylbenzyl acetate [09.611]	Rat	NR	Oral	1450	(Moreno, 1978i)	
(4-Ethylbenzaldehyde [05.068])	Rat	M, F	Oral	1970	(Costello, 1984)	
(Tolualdehydes (mixed *o, m, p*) [05.027])	Rat	NR	Oral	2250	(Moreno, 1973w)	
(Tolualdehyde glyceryl acetal [06.012])	Rat	NR	Oral	3400	(Moreno, 1972i)	
(4-Isopropylbenzaldehyde [05.022])	Rat	M, F	Gavage	1390	(Jenner et al., 1964)	
(2,4-Dimethylbenzaldehyde [05.110])	Rat	M, F	Gavage	between 1750 and 5000	(deGroot et al., 1974)	Death of 3/5 male and 3/5 female rats after single dose of 5000 mg/kg bw. No death after repeated doses of 1750 mg/kg bw in 5 male and 5 female rats.
(4-Hydroxybenzaldehyde [05.047])	Rat	NR	Oral	3980	(Dow Chemical Company, 1992b)	
(4-Hydroxybenzoic acid [08.040])	Mouse	NR	Oral	2200	(Sokol, 1952)	
(Salicylic acid [08.112])	Mouse	NR	Oral	908	(Sado, 1973)	
	Rat	NR	Gavage	1050	(Hasegawa et al., 1989)	
Ethyl 4-hydroxybenzoate [09.367]	Rat	F	Gavage	4300	(CTFA, 1980b)	
	Mouse	NR	Oral	8000	(Sokol, 1952)	
	Mouse	NR	Oral	6008	(Sado, 1973)	
	Rabbit	NR[2]	Oral	5000	(Sabalitschka and Neufeld-Crzellitzer, 1954)	
	Dog	NR[2]	Oral	5000	(Sabalitschka and Neufeld-Crzellitzer, 1954)	
(Butyl 4-hydroxybenzoate [09.754])	Mouse	NR	Oral	13200	(Sado, 1973)	
	Mouse	NR	Oral	> 5000	(Sokol, 1952)	
(*p*-Anisyl alcohol [02.128])	Mouse	NR	Oral	1780	(Draize et al., 1948)	
	Rat	NR	Oral	1340	(Draize et al., 1948)	
(Anisyl formate [09.087])	Rat	NR	Oral	1770	(Levenstein, 1975j)	
(Anisyl acetate [09.019])	Rat	M, F	Oral	2250	(Weir and Wong, 1971b)	
(*p*-Anisyl propionate [09.145])	Rat	NR	Oral	3330	(Wohl, 1974d)	
(*p*-Anisyl butyrate [09.058])	Rat	NR	Oral	3400	(Moreno, 1976v)	
(Anisyl phenylacetate [09.706])	Rat	M, F	Gavage	M: 5417 F: 4641	(Reagan and Becci, 1984d)	
	Rat	NR	Oral	> 5000	(Moreno, 1977ab)	
(Veratraldehyde [05.017])	Rat	NR	Oral	2000	(Moreno, 1974k)	
	Rat	M	Oral	2040	(Field, 1979a)	
	Mouse	M	Oral	3200	(Field, 1979b)	
(4-Methoxybenzaldehyde [05.015])	Rat	NR	Oral	3210	(BASF, 1981)	
	Rat	M, F	Gavage	1510	(Jenner et al., 1964)	
	Guinea pig	M, F	Gavage	1260	(Jenner et al., 1964)	
	Rat	M, F	Gavage	1510	(Taylor et al., 1964)	
2-Methoxybenzaldehyde [05.129]	Rat	M	Gavage	2.4 –2.8 ml/kg (2705 –3156)[3]	(Field, 1979b)	
	Rat	NR	Oral	2500	(Moreno, 1977ac)	
	Mouse	M	Gavage	2.4 ml/kg (2705)[3]	(Field, 1979b)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(4-Ethoxybenzaldehyde [05.056])	Rat	NR	Oral	2100	(Moreno, 1977ad)	
(Methyl 2-methoxybenzoate [09.796])	Rat	NR	Oral	3800	(Moreno, 1982l)	
(Methyl 4-methoxybenzoate [09.713])	Rat	NR	Oral	> 5000	(Levenstein, 1975k)	
(Ethyl 4-methoxybenzoate [09.714])	Rat	NR	Oral	2240	(Levenstein, 1975l)	
Gallic acid [08.080]	Mouse	M, F	Oral	> 5000	(Rajalakshsmi et al., 2001)	
	Rabbit	NR	Gavage	5000[4]	(Dollahite et al., 1962)	
(Vanillin [05.018])	Mouse	M	Gavage	1000	(Inouye et al., 1988)	
	Rabbit	NR	Gavage	2600	(Deichmann and Kitzmiller, 1940)	
	Rat	M, F	Gavage	1580	(Taylor et al., 1964)	
	Rat	M, F	Gavage	1580	(Jenner et al., 1964)	
	Rat	M, F	Gavage	3978[5] 3925[6]	(Lheritier, 1992)	
	Rat	M	Gavage	3830	(Monsanto Co., 1955b)	
	Rat	M, F	Oral	3300	(Monsanto Co., 1976)	
	Rat	NR	Oral	4370	(Makaruk, 1980)	
	Guinea pig	M, F	Gavage	1400	(Jenner et al., 1964)	
(Vanillin isobutyrate [09.811])	Rat	M, F	Gavage	4755	(Mallory et al., 1983)	
(Salicylaldehyde [05.055])	Rat	NR	Oral	520	(Moreno, 1977af)	
	Rat	M	Gavage	566	(Eastman Kodak Co., 1991b)	
	Mouse	M	Gavage	504	(Eastman Kodak Co., 1991b)	
(2-Hydroxy-4-methylbenzaldehyde [05.091])	Rat	M, F	Gavage	1520	(Mondino, 1982)	
	Rat	M, F	Oral	1520	(Peano and Berruto, 1982)	
(Methyl salicylate [09.749])	Mouse	M	Gavage	1390	(Ohsumi et al., 1984)	
	Rat	NR	Gavage	1250	(Giroux et al., 1954b)	
	Rat	M, F	Oral	M: 3049 F: 2642	(Hazleton Laboratories, 1982c)	
	Rat	M, F	Gavage	887	(Jenner et al., 1964)	
	Rat	NR	Oral	1220	(Nivikov et al., 1994)	
	Mouse	M	Oral	1110	(Davison et al., 1961)	
	Guinea pig	M, F	Gavage	1060	(Jenner et al., 1964)	
	Mouse	M, F	Gavage	1440	(NTP, 1984a)	
(Ethyl salicylate [09.748])	Rat	NR	Oral	1320	(Moreno, 1976x)	
(Butyl salicylate [09.763])	Rat	NR	Oral	1836	(Levenstein, 1975m)	
(Isobutyl salicylate [09.750])	Rat	NR	Oral	1560	(Moreno, 1973aa)	
(Isopentyl salicylate [09.751])	Rat	NR	Oral	4100	(Moreno, 1982m)	
	Rat	M, F	Oral	> 5000	(Hazleton Laboratories, 1982c)	
Hexyl salicylate [09.581]	Rat	NR	Oral	> 5000	(Moreno, 1975n)	
Hex-3-enyl salicylate [09.570]	Rat	NR	Oral	5000	(Moreno, 1975o)	
Prenyl salicylate [09.696]	Rat	NR	Oral	3200	(Moreno, 1978k)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Benzyl salicylate [09.752])	Rat	M	Gavage	2227	(Fogleman and Margolin, 1970)	
(Phenethyl salicylate [09.753])	Rat	NR	Oral	> 5000	(Moreno, 1973ab)	
(*o*-Tolyl salicylate [09.807])	Rat	M, F	Oral	1.81 ml/kg (1810)[7]	(Sterner and Chibanguza, 1983)	
(2,4-Dihydroxybenzoic acid [08.076])	Mouse	NR	Intraperitonel	> 800	(Grady et al., 1976)	Highest dose applied was not lethal.
(Ethyl 4-hydroxy-3-methoxybenzyl ether [04.094])	Rat	M, F	Oral	> 2000	(Dufour, 1994)	
(Butyl vanillyl ether [04.093])	Rat	M, F	Gavage	M: 5104 F: 4734	(Buch, 1989)	
(Ethyl vanillin [05.019])	Rat	M, F	Gavage	> 2000	(Jenner et al., 1964)	
	Rat	M	Gavage	4470	(Rhone-Poulenc Inc., 1992b)	
	Rat	M, F	Oral	3500[8]	(Monsanto Co., 1991a)	
	Rat	M, F	Oral	3500[9]	(Monsanto Co., 1991b)	
	Rabbit	NR	Gavage	2000	(Deichmann and Kitzmiller, 1940)	
(Ethyl vanillin isobutyrate [09.933])	Rat	M, F	Oral	> 2000	(Sanders and Crowther, 1997)	
(Piperonyl acetate [09.220])	Rat	NR	Oral	2100	(Moreno, 1973ac)	
(Piperonal [05.016])	Rat	M, F	Gavage	2700	(Jenner et al., 1964)	
	Rat	M, F	Gavage	2700	(Taylor et al., 1964)	
	Rat	M, F	Gavage	2700	(Hagan et al., 1965)	
Prenyl benzoate [09.693]	Rat	NR	Oral	4700	(Moreno, 1978j)	
Prenyl salicylate [09.696]	Rat	NR	Oral	3200	(Moreno, 1978k)	
3,4-Dihydroxybenzoic acid [08.133]	Mice	NR	Oral	> 800	(Grady et al., 1976)	

' NR: Not Reported.

[1] Dose range-finding study.

[2] Article published in German. Data point not verified.

[3] Calculation based on a specific gravity of 1.127 g/ml.

[4] Dosed as a 10 % solution.

[5] Calculated using Bliss' method.

[6] Calculated using Litchfield and Wilcox's method.

[7] Calculation based on an assumed specific gravity of 1.0 g/ml.

[8] Administered as a 10 % solution in corn oil.

[9] Administered as a 20 % solution-suspension in corn oil.

[10] Calculated based on a specific gravity of 1.081 g/ml.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for five candidate substances of the present flavouring group evaluation from chemical group 23 and 30, and for 18 supporting substances evaluated by the JECFA at the 46th and 57th meetings (JECFA, 1996b; JECFA, 2002a). The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Benzyl alcohol [02.010])	Rat; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	100	(NTP, 1989a)	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 800 mg/kg bw/day in males.
	Rat; M, F 100	Gavage	0, 200, 400 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1989a)	Fully described NTP study. Survival in both dose group of females was 50 % that of controls. This was, as concluded by NTP, primarily due to an increased number of gavage-related deaths. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity.
	Mouse; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	100	(NTP, 1989a)	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 400 and 800 mg/kg bw/day in males.
	Mouse; M, F 100	Gavage	0, 100, 200 mg/kg bw/day	103 weeks	200	(NTP, 1989a)	Fully described NTP study. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity.
(Benzyl acetate [09.014])	Rat; M, F 20	Gavage	0, 62.5, 125, 250, 500, 1000 mg/kg bw/day	13 weeks	250	(NTP, 1986c)	Fully described NTP study. Clinical signs of toxicity in females at 500 mg/kg and in males and females at 1000 mg/kg. Decreased body weight in males at 1000 mg/kg. Deaths at highest dose (1 F/2 M). At necropsy thickened stomach walls in surviving animals (2/8 M, 4/9 F). Hippocampal necrosis in both sexes at 1000 mg/kg (8/8 M, 4/9 F).
	Rat; M 30	Oral	0, 20000, 35000, 50000 mg/kg in the diet (0, 1500, 2700, 3800 mg/kg bw/day)[25, 26]	13 weeks	ND[7]	(Abdo et al., 1998)	Published non-GLP study of good quality. Benzyl acetate caused an increase in mortality, incidence of abnormal neural behavioral signs along with astrocyte hypertrophy and neuronal necrosis in the cerebellum, hippocampus and pyriform cortex of the brain at 35000 mg/kg feed and more. Body weight was statistically significant reduced from 20000 mg/kg feed. These effects were reduced significantly by glycine but not by *L*-alanine.
	Rat; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 230, 460, 900, 1750, 3900 mg/kg bw/day for males	13 weeks	460	(NTP, 1993d)	Fully described NTP study. High mortality at highest dose (9/10 F, 9/10 M). Statistically significant decreases in final body weights (over 10 %) observed at 25000 mg/kg feed. Clinical signs of intoxication at 50000 mg/kg feed. At the highest dose level degeneration and necrosis of neurons and glial cells in the cerebellum and hippocampus, renal tubular degeneration and

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			0, 240, 480, 930, 1870, 4500 mg/kg bw/day for females)				histopathological changes in skeletal thigh muscles. Testicular tubular atrophy in a few males at 12500 mg/kg feed.
	Rat; M, F 120	Oral	0, 3000, 6000, 12000 mg/kg in the diet (equal to 0, 130, 260, 510 mg/kg bw/day in males and 0, 145, 290, 575 mg/kg bw/day in females)	103 weeks	260	(NTP, 1993d)	Fully described NTP study. Slightly reduced mean body weight and feed consumption at the highest dose. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity in male and female Fischer 344/N rats.
	Rat; M, F 100	Gavage	0, 250, 500 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1986c)	Fully described NTP study. No observable adverse effects on mean body weight gain and survival. NTP conclusion on carcinogenicity: Benzyl acetate increased the incidence of acinar-cell adenomas of the endocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. No evidence of carcinogenic activity for female rats.
	Mouse; M, F 20	Gavage	0, 125, 250, 500, 1000, 2000 mg/kg bw/day for females and 0, 62.5, 125, 250, 500, 1000 mg/kg bw/day for males	13 weeks	500	(NTP, 1986c)	Fully described NTP study. High mortality (8/10) in females at 2000 mg/kg due to gavage error. Clinical signs of toxicity were observed at 1000 mg/kg bw/day. Hippocampal necrosis in one female at 1000 mg/kg.
	Mouse; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equal to 0, 425, 1000, 2000, 3700, 7900 mg/kg bw/day for males and 0, 650, 1280, 2980, 4300, 9400 mg/kg bw/day for females)	13 weeks	ND[7]	(NTP, 1993d)	Fully described NTP study. Statistically significant, dose-related decreases in final body weights (over 10 %) observed in all treated animals. Hippocampal necrosis in one male and three females of highest dose group.
	Mouse; M, F 120	Oral	0, 330, 1000, 3000 mg/kg in the diet (equal to 0, 37, 112, 346 mg/kg bw/day in males and 0, 42, 132, 382 mg/kg bw/day in	103 weeks	ND[7]	(NTP, 1993d)	Fully described NTP study. Decreased mean body weights (9-13 %) in all treated mice except for females at 330 mg/kg feed (statistics not reported). Statistically significant, dose-related incidence and severity of non-neoplastic lesions of the nasal mucosa and glands in all treated animals. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity in male and

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			females)				female B6C3F$_1$ mice.
	Mouse; M, F 100	Gavage	0, 500, 1000 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1986c)	Fully described NTP study. No observable adverse effects on mean body weight gain and survival. NTP conclusion on carcinogenicity: For male and female B6C3F$_1$ mice there was evidence of carcinogenicity, in that benzyl acetate caused an increased incidence of hepatocellular neoplasms particularly adenoma, and squamous cell neoplasms (papillomas; no progression into carcinomas) of the forestomach.
(Benzyl butyrate [09.051])	Rat; M, F 12	Oral[1]	0, 26.5 mg/kg bw/day[24]	12 weeks	26.5	(Oser, 1957)	Unpublished non-GLP study with limited details on study protocol and results.
(Benzaldehyde [05.013])	Rat; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	200	(Kluwe et al., 1983; NTP, 1990c)	Fully described NTP study. High mortality in males (6/10) and death of three females in the highest group. Death of one female at 400 mg/kg. Reduced terminal body weights (26 %) in males at highest dose. Treatment-related lesions in the brain, forestomach, liver and kidney in both sexes at 800 mg/kg including necrosis and degeneration of cerebellum, necrosis of neurons of the hippocampus, hyperplasia and/or hyperkeratosis of sqamous epithelium of the forestomach, degeneration and/or necrosis of liver and kidney.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 100	Gavage	0, 200, 400 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1990c)	Fully described NTP study. Significantly reduced survival of the high dose group rats after one year and significant dose-related trend to reduced survival in the treated groups of males. Body weight not affected. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity for male or female F344/N rats.
	Mouse; M, F 20	Gavage	0, 75, 150, 300, 600, 1200 mg/kg bw/day	13 weeks	300	(Kluwe et al., 1983; NTP, 1990c)	Fully described NTP study. High mortality in males (9/10) and death of one female in the highest group. Reduced mean body weight at 600 mg/kg in males, but not in females. Mild to moderate renal tubular degeneration in all males at 1200 mg/kg and in one male at 600 mg/kg.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Mouse; M, F	Gavage	0, 200, 400 mg/kg bw/day for males, 0, 300, 600 mg/kg bw/day for females	103 weeks	ND[7]	(NTP, 1990c)	Fully described NTP study. No significant effects on body weight and survival observed in any group. Increased incidences of squamous cell papillomas of the forestomach in both exposure groups with dose-related increased incidences in forestomach hyperplasia. No progression into malignant carcinomas was observed. NTP conclusion on carcinogenicity: Some evidence of carcinogenic activity for male or female $B6C3F_1$ mice.
(Methyl benzoate [09.725])	Rat; NR 13	Gavage	0, 0.005, 0.05 mg/kg bw/day	6 months	0.005	(Kravets-Bekker and Ivanova, 1970)	Published non-GLP study of limited quality. Unusual study design and parameters analysed. Limited report of experimental details and results.
(Benzoic acid [08.021])	Mouse; M, F 100	Gavage	0, 80 mg/kg bw/day	3 months	ND[7]	(Shtenberg and Ignat'ev, 1970)	Published non-GLP study of limited quality. Insufficient details on methods and results provided. Reduced weight gain with normal food intake in treated animals.
	Mouse; M, F 50	oral (paste)	0, 40 mg/kg bw/day	17 months	40[2]	(Shtenberg and Ignat'ev, 1970)	Published non-GLP study of limited quality. Insufficient details on methods and results provided.
(Glyceryl tribenzoate [09.812])	Rat; M, F 30	Oral	0, 120, 600, 2600 mg/kg bw/day	90 days	600	(Carson, 1972a)	Unpublished non-GLP study carried out in accordance with OECD Guideline 408. Decreased body weight gain (by 23 %) in high dose males with normal food intake.
(Propylene glycol dibenzoate [09.803])	Rat; M, F 30	Oral	0, 130, 630, 2500 mg/kg bw/day	90 days	2500[2]	(Carson, 1972b)	Unpublished non-GLP study carried out in accordance with OECD Guideline 408.
(Tolualdehydes (mixed o, m, p) [05.027])	Rat; M, F 30	Oral	36 mg/kg bw/day for males, 43 mg/kg bw/day for females	90 days	36[2]	(Oser et al., 1965)	Published non-GLP study. Very limited details provided. The test substance is only indicated as "tolualdehydes", without any incication of isomerical composition
	Rat; M, F 30	Gavage	0, 50, 250, 500 mg/kg bw/day	13 weeks	250	(Brantom et al., 1972)	Published non-GLP study of good quality, carried out in accordance to OECD Guideline 408. Decreased relative pituitary weight in females at 500 mg/kg bw/day. Reduced weight and relative weight of small intestine in all treated groups. However, this effect was not dose-related and not reproduced in a second study. The test substance was an approx. equimolar mixture of the three isomers.
(2,4-Dimethylbenzaldehyde [05.110])	Rat; M, F 10	Gavage	0, 0.175, 1.75 mg/kg bw/day	2 weeks	1.75[2]	(deGroot et al., 1974)	Unpublished non-GLP study with limited parameters analysed and limited report of results. Increased relative liver weight in high-dose males without histopathological changes.
Ethyl 4-hydroxybenzoate [09.367]	Rat; M, F 24	Oral	0, 2, 8 % in the diet (0, 1050, 5700 mg/kg bw/day)	12 weeks	1050	(Matthews et al., 1956)	Published non-GLP study of very limited quality. Insufficient endpoints analysed. High mortality at highest dose.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Rat; NR 10 or 11	Oral	0, 0.2, 1, 2 % in the diet (0, 100, 500, 1000 mg/kg bw/day)[18]	25 weeks	1000	(Sado, 1973)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
(Butyl 4-hydroxybenzoate [09.754])	Rat; NR 10 or 18[3]	Gavage	0, 0.25, 50 mg/kg bw/day	13 - 15 weeks	50[2]	(Ikeda and Yokoi, 1950)	Published non-GLP study in Japanese with English translation. Validity cannot be evaluated due to incomplete report of data.
	Rat; M, F 24	Oral	0, 2, 8 % in the diet (0, 1050, 5700 mg/kg bw/day)	12 weeks	1050	(Matthews et al., 1956)	Published non-GLP study of very limited quality. Insufficient endpoints analysed. High mortality (100 % in males) at highest dose.
	Mouse; M, F 20	Oral	0, 0.6, 1.25, 2.5, 5, 10 % in the diet (0, 900, 1850, 3750, 7500, 15000 mg/kg bw/day)	6 weeks	900	(Inai et al., 1985)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported. Copy partly unreadable.
	Mouse; M, F 100	Oral	0, 0.15, 0.3, 0.6 % in the diet (0, 225, 450, 900 mg/kg bw/day)	102 weeks	900[2]	(Inai et al., 1985)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported. Copy partly unreadable.
(4-Methoxybenzaldehyde [05.015])	Rat; M, F 20	Oral	0, 7.3 mg/kg bw/day	12 weeks	7.3[4, 2]	(Trubek Laboratories Inc., 1958f)	Unpublished study of poor quality with insufficient study protocol and report of data; Eugenol: JECFA evaluation; NOAEL 250 mg/kg bw/day in rat (diet), ADI 2.5 mg/kg bw (See footnote 4).
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	15 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
(Methyl 2-methoxybenzoate [09.796])	Rat; M, F 10	Oral	0, 94 mg/kg bw/day	14 days	94[2]	(Van Miller and Weaver, 1987)	Unpublished GLP-study.
3,4-Dihydroxybenzaldehyde [05.142]	Rat; M, F 10	Oral	0, 1.5 % in the diet	4 weeks	1500[15]	(Shibata et al., 1990)	Published non-GLP study of limited quality. Experimental details and results insufficiently

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			(0, 1500 mg/kg bw/day)[16]				reported.
Gallic acid [08.080]	Rat; M, F 20	Oral	0, 0.2, 0.6, 1.7, 5 % in the diet	13 weeks	0.6 % in the diet (M: 119 F: 128)[17]	(Niho et al., 2001)	Published non-GLP study of good quality. Centrilobular liver cell hypertrophy, reflected in a significant increase in liver weight, which was observed in animals of both sexes from 1.7 %.
	Rat; M 5	Oral	0, 2 % in the diet (0, 2000 mg/kg bw/day)[16]	4 weeks	2000[2, 6]	(Hirose et al., 1987)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
	Mouse; M, F 12	Oral	0, 1000 mg/kg bw/day	28 days	1000[2]	(Rajalakshsmi et al., 2001)	Published non-GLP study of acceptable quality.
(Vanillin [05.018])	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M 5	Oral	0, 2, 5 % in the diet (0, 1000, 2500 mg/kg bw/day)[21]	1 year	2500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	ND	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. No NOAEL could be derived.
	Rat; NR 8	Oral	20 mg/kg bw/day	126 days	20[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 24	Oral	0, 0.5, 1, 2 % in the diet (0, 250, 500, 1000 mg/kg bw/day)[21]	2 years	1000[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; NR 12	Gavage	300 mg/kg bw twice a week	14 weeks	300[2, 5]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
							No adequate controls.
	Rat; M 10	Oral	0, 0.1, 0.5, 1.0 % in the diet (0, 40, 214, 437 mg/kg bw/day)[20]	26 weeks	437[2]	(Monsanto Co., 1955a)	Unpublished non-GLP study of limited quality. Insufficient analysis of clinical-chemical parameters. Results of microscopic examination not reported.
	Rabbit; NR 3	Oral	240 mg/kg bw/day	56 or 126 days[19]	240[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rabbit; NR 1	Oral	83 mg/kg bw/day for 14 days 103 mg/kg bw/day for 61 days	14 or 61 days	ND	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. The animal treated with 83 mg/kg bw/day died due to glycerol poisoning (solvent), the animal treated with 103 mg/kg bw/day suffered from anemia, diarrea and showed a reduced wt gain.
	Dog; M, F 2	Capsule	0, 25, 100 mg/kg bw/day	26 weeks	100	(Monsanto Co., 1955a)	Unpublished non-GLP study of limited quality. Insufficient analysis of clinical-chemical parameters. Results of microscopic examination not reported.
4-Hydroxy-3,5-dimethoxybenzoic acid [08.087]	Rat; M 5	Oral	0, 2 % in the diet (0, 2000 mg/kg bw/day) [16]	4 weeks	2000[2, 6]	(Hirose et al., 1987)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
(Methyl salicylate [09.749])	Rat; M, F 20	Oral	0, 0.1, 1.0 % in the diet (0, 50, 500 mg/kg bw/day)[21]	17 weeks	500[2]	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Preliminary study in extensive toxicological evaluation. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; M, F 6	Oral	0, 2 % in the diet (0, 1000 mg/kg bw/day)[21]	Up to 71 days	1000[7]	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Supplemental study in extensive toxicological evaluation to analyse bone effects. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; NR	Oral	1.12, 2 % in the diet (560, 1000 mg/kg bw/day)[21, 23]	10 weeks	< 560	(Harrison et al., 1963)	Only abstract available. No details reported. Study carried out to further investigate the increase of cancellous bone reported by Webb & Hansen, 1963). Effects confirmed at levels of 2 % and 1.12 % but not at lower (unspecified) levels.
	Rat; M, F 10	Oral	0, 0.2, 0.36, 0.63, 1.13, 2.0 % in the diet (0, 100, 180, 320, 560, 1000 mg/kg bw/day)[21]	11 weeks	180	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Decreased weight gain at 320 mg/kg, decreased weight gain and increased bone density from 560 mg/kg.
	Rat; M 5	Oral	0.6, 2.0 % in the diet (300, 1000 mg/kg	12 weeks	300	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			bw/day)[21]				parameters. No control animals used. Hundred % mortality in high dose group after 6 weeks with bone lesions. No such effects at 300 mg/kg.
	Rat; M, F 15	Oral	0, 2.0 % in the diet (0, 1000 mg/kg bw/day)[21]	11 weeks	ND[7]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Changes in bone density and 20 % mortality in treated animals. No such effects in controls.
	Rat; M 10[8]	Oral	0, 0.6, 2.0 % in the diet (0, 300, 1000 mg/kg bw/day)[21]	6 weeks	ND[9]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Deaths occurred among the rats at the high dose ad libitum and in all members of the pair-fed groups (incl. controls).
	Rat; M, F 20	Oral	0, 0.6., 0.9, 1.2, 2.0 % in the diet (0, 300, 450, 600, 1000 mg/kg bw/day)[21]	11 weeks	450	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Weekly X-ray evaluation of animals for progression of bone changes. Bone changes at 1000 mg/kg from week 2, at 600 mg/kg from week 5.
	Dog; M, F 2	Oral	50, 100, 250, 500, 800, 1200 mg/kg bw	Up to 59 days	250	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Preliminary study in extensive toxicological evaluation. Study protocols fully described, results not reported in detail but only summarised in text.
	Dog; M, F 6	Oral	0, 150, 300, 500, 800 mg/kg bw/day[22]	7.5 months[10]	ND[7]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Hundred % mortality at highest dose, only 2 surviving animals at 500 mg/kg. At lower doses no effects on body weight and hematological parameters. Increased liver and kidney weight at all doses but not after recovery diet. No NOEL could be derived.
	Dog; M, F 8, 12[11]	Oral	0, 50, 100, 170 mg/kg bw/day	6 months[12]	170[2]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. No adverse effects at any dose.
	Rat; M, F 50	Oral	0, 0.1, 0.5, 1.0, 2.0 % in the diet (0, 50, 250, 500, 1000 mg/kg bw/day)[21]	2 years	50	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; M, F 50	Oral	0.07, 0.21 % in the diet (0, 35 100)[21]	2 years	100[2]	(Packman et al., 1961)	Only abstract available with very limited report of study details and results.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Dog; M, F 4	Oral	0, 50,150, 350 mg/kg bw/day	2 years	50	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarised in text.
(Isopentyl salicylate [09.751])	Rat; M, F 30	Oral	0, 0.005, 0.05, 0.5 % in the diet (0, 4.7, 46, 420 mg/kg bw/day in males and 0, 4.8, 47, 480 mg/kg bw/day in females)	13 weeks	5	(Drake et al., 1975)	Published non-GLP study of good quality. Reduced body weight gain at 0.5 % associated with reduced food intake. Increased relative kidney weight without any histophathological changes at 0.05 and 0.5 % in the diet.
	Rat; M, F 10	Oral	0. 0.5 % in the diet (0, 420 mg/kg bw/day in males and 0, 480 mg/kg bw/day in females)[13]	98 days	420	(Drake et al., 1975)	Published non-GLP study of good quality. Reduced body weight gain at 0.5 % associated with reduced food intake. Only body weight gain analysed.
(Ethyl vanillin [05.019])	Rat; M 5	Oral	0, 2, 5 % in the diet (0, 1000, 2500 mg/kg bw/day)[21]	1 year	2500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	64[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. No NOAEL could be derived.
	Rat; NR 8	Oral	20 mg/kg bw/day	126 days	20[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; NR 12	Gavage	300 mg/kg bw twice a week	14 weeks	300[2, 5]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M, F 40	Oral	0, 500, 1000, 2000 mg/kg bw/day	13 weeks	500	(Hooks et al., 1992)	Unpublished report summarised by the JECFA (1996a). Study has been reported to be designed in accordance with toxicological principles for the safety assessement of food additives established by the US FDA in 1982. Limited report of experimental details and results.
	Rat; M, F 24	Oral	0, 0.5, 1, 2 % in the diet (0, 250, 500, 1000 mg/kg bw/day)[21]	2 years	1000[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rabbit; NR 3	Oral	240 mg/kg bw/day	56 or 126 days[19]	240[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Rabbit; NR 1	Oral	15-49 mg/kg bw/day (15, 15, 32, 41, 49 mg/kg day for 15, 31, 17, 31, 49 days, respectively)	15 - 49 days	41[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. The animal treated with 15 mg/kg bw/day for 15 days died due to glycerol poisoning (solvent). The animal treated with 49 mg/kg bw/day for 49 days suffered from anemia, diarrea and showed a reduced weight gain.
(Piperonal [05.016])	Rat; M, F 20	Oral	0, 16 mg/kg bw	12 weeks	16[14, 2]	(Trubek Laboratories Inc., 1958f)	Unpublished study of poor quality with insufficient study protocol and report of data; Eugenol: JECFA evaluation; NOAEL 250 mg/kg bw/day in rat (diet), ADI 2.5 mg/kg bw (See footnote 4).
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	15 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F NR	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F NR	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F 20 - 60	Oral	0, 0.1, 0.5 % in the diet (0, 50, 250 mg/kg bw/day)[21]	1.5 - 2 years	250[2]	(Bär and Griepentrog, 1967)	Published study with incomplete report of experimental details and results.
3,4-Dihydroxybenzoic acid [08.133]	Rat; M, F 10	Oral	0, 1.5 % in the diet (0, 750 mg/kg bw/day)	4 weeks	750	(Shibata et al., 1990)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
	Rat, M 10	Oral	0, 50 mg/kg bw/day	2 weeks	50	(Guglielmi et al., 2003)	
	Rat, M	Oral	0, 1000 ppm (0,	28 weeks	50	(Tanaka et al.,	

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	11		50 mg/kg bw/day)			1993a; Tanaka et al., 1993b)	
	Rat, M 16	Oral	0, 2000 ppm (0, 100 mg/kg bw/day)	32 weeks	100	(Tanaka et al., 1994)	
	Rat, M 15	Oral	0, 2000 ppm (0, 100 mg/kg bw/day)	41 weeks	100	(Hirose et al., 1995)	
	Rat, M 15	Oral	0, 1.5 % in the diet (0, 530 mg/kg bw/day)	51 weeks	< 530	(Hirose et al., 1992)	It was noted that the relative liver and kidney weight was significantly increased but this was not further evaluated.

[1] Six aromatic esters (ethyl benzoate, 0.15 ppm; isobutyl benzoate, 25 ppm; benzyl acetate, 18.7 ppm; benzyl butyrate, 25 ppm; ethyl methylphenyl glycidate, 25 ppm; and glycidate M-116, 25 ppm) were blended in the diet.

[2] This study was performed at either a single dose level or multiple dose levels that produced no adverse effects. Therefore, this dose level is the highest dose tested that produced no adverse effects.

[3] Five rats were used per group for low dose. Nine rats were used per group for high dose.

[4] Rats were fed a test mixture containing 123 ppm of eugenol, 10 ppm of 4-methoxybenzaldehyde and 22 ppm of piperonal.

[5] Compound was administered two times per week.

[6] Study evaluated histological changes in the rat forestomach.

[7] This study was performed at a single dose level or at multiple dose levels that produced adverse effects. No NOEL could be derived from that study.

[8] Two groups fed ad libitum (2.0 and 0.6 %) and one group (0.6 %) pair-fed.

[9] The 0.6 % pair-fed rats showed adverse effects; however, those fed ad libitum did not.

[10] Two animals from the 150 mg/kg/day group and three animals from the 300 mg/kg/day group were sacrificed after 6.5 months. Additionally, three animals from the 300 mg/kg/day group discontinued feeding at 6.5 months and recovered for 1.5 months, before being sacrificed.

[11] There were 8 dogs used for the 50 and 100 mg/kg bw doses and 12 dogs used for 167 mg/kg bw.

[12] All animals were fed the substance for six months and then sacrificed, with the exception of two dogs from the high dose group. These animals were fed the substance for four months, placed on control diets for two months, and then sacrificed with the other animals at six months.

[13] Pair-fed study.

[14] Rats were fed a test mixture containing 123 ppm of eugenol, 10 ppm of 4-methoxybenzaldehyde and 22 ppm of piperonal.

[15] This study evaluated the cell proliferation in the rat forestomach at a single concentration, 1.5 %. There was a statistically significant weight reduction in the females; however, it was deemed to be associated with a palatability problem.

[16] Calculated based on general assumptions for bw and food intake in weenling rats (e.g. 2 % in diet resulting in 2000 mg/kg bw/day).

[17] Calculated by using mean values of body weight and food intake in the 0.2 % group.

[18] Estimated based on FDA (1993). Priority-based assessment of food additives (PAFA) database. Center for food safety and applied nutrition. page 58.

[19] One animal was treated for 56 days and two animals for 126 days.

[20] Calculated based on a final bw of 453, 444, 427g and a total consumption of test compound of 18.2, 95.0 and 186.8 mg/animal/day, respectively, in the three dose groups.

[21] Calculated based on general assumptions for bw and food intake (e.g. 1 % in diet resulting in 500 mg/kg bw/day). As estimated by JECFA (2002a).

[22] The dogs were given one-half of the dose in the morning and the other half in the afternoon for six days/week.

[23] Also lower dose levels applied, however, not reported in more detail.

[24] Benzyl butyrate administered in the diet in a mixture of six aromatic esters (ethyl benzoate 0.15 mg/kg, isobutyl benzoate 25 mg/kg, benzyl acetate 18.7 mg/kg, benzyl butyrate 25 mg/kg, ethyl methylphenylglycidate 25 mg/kg, glycidate 25 mg/kg) providing intakes of 26.5 mg benzylbutyrate/kg bw/day and a total of 126 mg/kg bw/day of the mixture.

[25] Calculated based on a final bw of 200 g and a daily intake of 15.2 g/rat, as reported for the 20000 mg/kg feed group, for all groups.

[26] Additional groups with either 50000 mg benzyl acetate/kg feed plus 27000 mg glycine/kg feed or 32000 mg *L*-alanine/kg feed (supplemental *L*-alanine and glycine were equimolar). The *L*-alanine group served as amino-nitrogen control.

Developmental and reproductive toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 23 and for twelve supporting substances evaluated by the JECFA at the 46th and 57th meetings (JECFA, 1996b; JECFA, 2002a). Supporting substance listed in brackets.

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(Benzyl alcohol [02.010])	Developmental toxicity: Gestation days 6 - 15	Mouse; F 50	Gavage	0, 550 mg/kg bw/day	Maternal: 550 Foetal: 550	(JECFA, 1996a)	Unpublished study carried out by NIOSH of assumed good quality. Only summary available. In a preliminary experiment clinical signs of maternal toxicity were observed at 1320 mg/kg and reduced number of viable fetuses at 720 mg/kg.
	Developmental toxicity: Gestation days 6 - 13	Mouse; F 50	Gavage	0, 750 mg/kg bw/day	Maternal: ND[1] Foetal: ND[1]	(Hardin et al., 1987)	Published study carried out by NIOSH of assumed good quality. Increased mortality, clinical signs of matermal toxicity, reduced maternal body weight-gain and reduced pup weight in treated group.
(Benzyl acetate [09.014])	Developmental toxicity: Gestation days 6 - 15	Rat; F 20	Gavage	0, 10, 100, 500, 1000 mg/kg bw/day	Maternal: 1000 Foetal: 500	(Ishiguro et al., 1993)	Published non-GLP study. At highest dose, slight, but not significant maternal toxicity observed with slightly reduced maternal weight-gain. Also significantly reduced fetal body weight at highest dose and increased incidence of skeletal internal variations.
	Reproductive toxicity: Sperm morphology and vaginal cytology: 13 weeks	Mouse; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 425, 1000, 2000, 3700, 7900 mg/kg bw/day for males and 0, 650, 1280, 2980, 4300, 9400 mg/kg bw/day for females)	Reproductive toxicity: Females: 3700 Males: 7900	(Morrissey et al., 1988; NTP, 1993d)	Published report of screening test (SMVCE assay) carried out at end of fully described NTP-study. Statistically significant, dose-related decreases in final body weights (over 10 %) observed in all treated animals. Mean length of estrous cycle significantly greater in high dose than in controls. No effects on male reproductive endpoints.
	Reproductive toxicity. Sperm morphology and vaginal cytology: 13 weeks	Rat; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 230, 460, 900, 1750, 3900 mg/kg bw/day for males 0, 240, 480, 930, 1870, 4500 mg/kg bw/day for females)	Reproductive toxicity: Females: 4500 Males: 3900	(Morrissey et al., 1988; NTP, 1993d)	Published report of screening test (SMVCE assay) carried out at end of fully described NTP-study. Statistically significant decreases in final body weights (over 10 %) observed at 25000 mg/kg feed. Clinical signs of intoxication at 50000 mg/kg feed. No effects on male and female reproductive endpoints.
(Benzaldehyde [05.013])	Reproductive toxicity 32 weeks (every other day)	Rat; M, F 10	Gavage	2 mg/animal (equivalent to 5 mg/kg bw/day)	ND[1]	(Sporn et al., 1967)	Published non-GLP study of limited quality. Study in Romanian with English summary only. Reduced number of pregnant females among treated animals (no statistics presented).

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(Benzoic acid [08.021])	Developmental toxicity Gestation day: 9	Rat; F 7	Gavage	510 mg/kg bw/day[2]	Maternal: NR Foetal: 510	(Kimmel et al., 1971)	Published non-GLP study of limited validity due to inadequate study design. Study evaluated the influence of benzoic acid on the teratogenic effects of acetylsalicylic acid. No teratogenicity was observed with a single dose of benzoic acid. Pretreatment with benzoic acid increased the teratogenicity of acetylsalicylic acid by increasing the salicylate concentration in the embryo and serum.
	Four generation reproduction study: Continuously in diet	Rat; M, F 40	Oral	0, 0.5, 1.0 % in the diet (0, 275, 550 mg/kg bw/day)[3]	550	(Kieckebusch and Lang, 1960)	Published non-GLP study of acceptable quality. Limited male reproduction parameters analysed.
(Salicylic acid [08.112])	Developmental toxicity Gestation days: 8 - 14	Rat; F 20	Oral	0, 0.06, 0.1, 0.2, 0.4 % in the diet (0, 46.4, 77.4, 165.4, 330 mg/kg bw/day) (0, 50.7, 77.4, 165.4, 205.9 mg/kg bw/day)	Maternal: 165.4 Foetal: 77.4 Postnatal: 77.4	(Tanaka et al., 1973a)	Published non-GLP study of acceptable quality. No maternal mortality. At highest dose reduced maternal body weight, signs of clinical toxicity (salivation, piloerection), no alive fetuses in 9/15 dams, reduced litter size. External and skeletal anomalies in fetuses at 0.2 % and more. Internal anomalies at 0.4 %. Skeletal anomalies in postnatal animals at 0.4 %.
	Developmental toxicity Gestation days: 8 - 14	Rat; F 20	Gavage	0, 75, 150, 300 mg/kg bw/day	Maternal: 75 Foetal: 75 Postnatal: 75	(Tanaka et al., 1973b)	Published non-GLP study of acceptable quality. At highest dose 3 animals died, reduced maternal body weight, signs of clinical toxicity (salivation, piloerection), At 150 mg/kg and more significantly reduced uterus weight. No live fetuses at highest dose. At 150 mg/kg and more reduced litter size and fetal weight, internal, skeletal and external anomalies.
Ethyl 4-hydroxybenzoate [09.367]	Developmental toxicity Gestation days: 8 - 15	Rat; F 5 to 12	Oral	0, 0.1, 1, 10 % in the diet (0, 50, 460, 2600 mg/kg bw/day)[4]	Maternal: 460 Foetal: 2600	(Moriyama et al., 1975)	Published non-GLP study of good quality. Sufficient parameters analysed and detailed report of experimental design and results. Reduced terminal maternal body weight at high dose.
	Postnatal development Gestation days: 8 - 15	Rat; F 5 to 12 (46 to 73 fetuses nursed for 1 month)	Oral	0, 0.1, 1, 10 % in the diet (0, 50, 460, 2600 mg/kg bw/day)[4]	Maternal: NR Neonatal: 2600	(Moriyama et al., 1975)	Published non-GLP study of good quality. Sufficient parameters analysed and detailed report of experimental design and results.
	Reproductive toxicity 8 weeks	Rat; M 8	Oral	0, 0.1, 1.0 % in the diet (0, 103, 1043 mg/kg bw/day)	1043	(Oishi, 2004)	Published study of good quality. No effects on weights of reproductive

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
							organs, on sperm counts in the testes and epididymides and sperm morphology. No effect on serum testosterone, LH, FSH
	Uterotrophic assay 3 days	Mouse; F (immature) 7-10	SC Oral	0, 100 mg/kg bw/day 0, 1000 mg/kg bw/day	100 1000	(Hossaiani et al., 2000)	Published non-GLP study of good quality. No estrogenic response observed at the dose levels tested.
	Uterotrophic assay 3 days	Rat; F (immature) 11-16	SC	0, 6, 18, 60, 180 mg/kg bw/day	60	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight (wet and dry) at the highest dose (ED_{50} for uterotrophic effect: uterine wet weight 68 mg/kg, uterine dry weight 94 mg/kg).
	Uterotrophic assay 3 days	Mouse; F (immature and ovarectomized adult) 10-25	SC	0, 0.6, 6.0, 18, 60, 180 mg/kg bw/day[5]	0.6	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight in immature mice at 6 mg/kg and more and in ovarectomized mice at 18 mg/kg and more (ED_{50} 74 mg/kg and 25 mg/kg, respectively)
	Uterotrophic assay 3 days	Mouse; F (adult ovarectomized) 6	SC	0, 60, 180 mg/kg bw/day	ND[1]	(Lemini et al., 2004)	Published study of good quality with full report of experimental details and results. Uterotrophic effect with estrogenic histological changes in uteri in both dose groups.
(Butyl *p*-hydroxybenzoate [09.754])	Reproductive toxicity 8 weeks	Rat; M 8	Oral	0, 0.01, 0.10, 1.0 % in the diet (0, 10, 100, 1000 mg/kg bw/day)	ND[1]	(Oishi, 2001)	Published non-GLP study of good quality. Reduced cauda epididymal sperm reserve and daily sperm production in testis significantly reduced in all treated groups. Dose-dependently reduced serum testosterone concentration and absolute and relative epididymis weight (significant at 0.1 % or more).
	Reproductive toxicity 10 weeks	Mouse; M 8	Oral	0, 0.01, 0.10, 1.0 % in the diet (0, 14, 146, 1500 mg/kg bw/day)	ND[1]	(Oishi, 2002)	Published non-GLP study of good quality. Absolute and relative weight of epididymides significantly increased at 1.0 %. Dose-dependent decrease of both round and elnongated spermatid counts. Significantly decreased elongated spermatid counts in all treated groups. Dose-dependant decrease of serum testosterone concentrations (significant at 1.0 %).
	Uterotrophic assay 3 days	Rat; F (ovarectomized) 5	Oral SC	4, 40 , 400, 800, 1200 mg/kg bw[6] 40, 200, 400, 600, 800, 1000, 1200 mg/kg bw/day[6]	1200 40	(Routledge et al., 1998)	Published study of good quality. No estrogenic activity found after oral administration (small, statistically insignificant increase in uterus wet weight) in immature rats. Positive

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
							response after s.c. administration. In the same study also an *in vitro* estrogenic activity test in yeast was carried out in which butyl paraben was found to be weakly estrogenic.
	Uterotrophic assay 3 days	Mouse; F (immature) 10	SC	0, 100 mg/kg bw/day	100	(Hossaiani et al., 2000)	Published non-GLP study of good quality. No estrogenic response observed at the dose level tested.
	Uterotrophic assay 3 days	Rat; F (immature) 10	SC	0, 100, 400, 600 mg/kg bw/day	400	(Hossaiani et al., 2000)	Published non-GLP study of good quality. A weak estrogenic response with a significantly increased relative uterus weight was observed at 600 mg/kg. At 100 and 400 mg/kg the uterus wet weight was significantly increased, but not the relative uterus weight.
	Uterotrophic assay 3 days	Rat; F (immature) 11-16	SC	0, 7, 21, 70, 210 mg/kg bw/day	21	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine wet weight at 70 mg/kg and more, and uterine dry weight at 210 mg/kg (ED_{50} for uterotrophic effect: uterine wet weight 87 mg/kg, 338 mg/kg uterine dry weight).
	Uterotrophic assay 3 days	Mouse; F (immature and ovarectomized adult) 6-16	SC	0, 0.7, 7.0, 21, 70, 210 mg/kg bw/day[5]	0.7	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight in immature mice at 7 mg/kg and more and in ovarectomized mice at 21 mg/kg an more (ED_{50} 65 mg/kg and 22 mg/kg, respectively).
	Uterotrophic assay 3 days	Mouse; F (adult ovarectomized) 6	SC	0, 70, 210 mg/kg bw/day	ND[1]	(Lemini et al., 2004)	Published study of good quality with full report of experimental details and results. Uterotrophic effect with estrogenic histological changes in uteri in both dose groups.
	Developmental toxicity Gestation days: 6 - 19	Rat; F 25	Gavage	0, 10, 100, 1000 mg/kg bw/day	Maternal: 100 Foetal: 1000	(Daston, 2004)	Published study of good quality with full report of experimental details and results. Maternal food consumption and weight gain significantly decreased at highest dose.
(Methyl salicylate [09.749])	Reproductive toxicity: continuous breeding (RACB) 18 weeks[7]	Mouse; M, F 40	Gavage	0, 100, 250, 500 mg/kg bw/day	250	(NTP, 1984a)	Fully described NTP-study. Significant slight decrease in mean number of litters, number of pups per litter, mean number of pups born alive per litter and mean live pup weight at highest dose. The experiment was unable to discriminate which sex was affected in reproduction.
(Veratraldehyde [05.017])	Reproductive and	Rat; F	Gavage	0, 80, 400, 800 mg/kg bw/day	Maternal: ND[1]	(Vollmuth et al., 1990)	Unpublished study with limited report of

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
	developmental toxicity: 1 week before mating until 4 days post parturition	10			Foetal: 800		results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(Vanillin [05.018])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 125, 250, 500 mg/kg bw/day	Maternal: 250 Foetal: 500	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(2,4-Dihydroxybenzoic acid [08.076])	Teratogenicity: Gestation day 9	Rat; F 10	SC	0, 380 mg/kg bw/day	380	(Koshajki and Schulert, 1973)	Published study of limited quality. Limited report of experimental details and results. Insufficient endpoints analysed.
	Teratogenicity Gestation day 11	Rat; F Not Reported	SC	0, 428 mg/kg bw/day (plus 214 mg/kg bw/day after 2 hours)	ND[1]	(Saito et al., 1982)	Published study of limited quality. Unusual study design and limited report of experimental details and results. No effect on plasma Ca level in dams after a single dose. Reduced plasma Ca levels in dams and malformations and foetotoxicity after the additional dose. No effects on maternal reproductive parameters.
(Ethyl vanillin [05.019])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 200, 1000, 2000 mg/kg bw/day	Maternal: ND[1] Foetal: 2000	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(Piperonal [05.016])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 250, 500, 1000 mg/kg bw/day	Maternal: 500 Foetal: 500	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.

[1] This study was performed at a single dose level or at multiple dose levels that produced adverse effects. No NOEL could be derived from that study.

[2] Study evaluated the effects of Aspirin (acetylsalicylic acid) when administered to rats during gestation; however, two dose groups used benzoic acid (510 mg/kg) as a pre-treatment and one dose group was administered only benzoic acid. Benzoic acid alone had no effect.

[3] Calculated based on the reported daily intake of 150 mg per animal, equal to 0.45 mMol per 100 g bw, in animals of the high dose group and a molecular weight of 122.12.

[4] Calculated from data on bw and food consumption presented in the article.

[5] The lowest dose was used in immature mice only.

[6] The number of dose groups was comprised from two separate experiments. In the first experiment dose levels of 40 and 400 mg/kg/day were investigated, while in the second experiment dose levels of 800 and 1200 mg/kg/day were explored.

[7] Reproductive assessment by continuous breeding (RACB) consisted of a 7-day premating phase, a 98-day cohabitation period and a 21-day segregation period.

In vitro mutagenicity/genotoxicity data are available for 12 candidate substances of the present flavouring group evaluation from chemical groups 23 and 30 and for 28 supporting substances evaluated by the JECFA at the 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a) and for one related substance. Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Benzyl alcohol [02.010])	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported, the study is considered valid.
	Ames test (plate incorporation method)	*S. typhimurium* TA100	1000 µg/plate	Negative[2]	(Ball et al., 1984)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[2]	(Rogan et al., 1986)	
	Ames test (pre-incubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	6666 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmole/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	50,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate	Negative[2]	(Milvy and Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate	Negative[1]	(NTP, 1989a)	Valid study in accordance with OECD Guideline 471 (except that only four strains were used). Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Mutation assay	*Escherichia coli* WP2 *uvr*A	1000 to 8000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	10 µg/disc	Positive	(Kuroda et al., 1984b)	Study published in Japanese with English abstract. Data extracted from figure. Validity of the study cannot be evaluated. Inhibition of growth was reported.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm≤ D<8 mm). was reported (D=5 mm). No information on the use of metabolic activation.
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg /ml[4] (three	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			concentrations, max. concentration inducing 50 % cell-growth inhibition)			metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Equivocal[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 473. Lowest effective dose was 4000 µg/ml with and without S9. No dose-response observed. Positive results were not reproducible in all trials. Absence of cytotoxicity reported up to the highest dose.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Negative[2] Positive[3]	(NTP, 1989a)	Valid study in accordance with OECD Guideline 473. A positive result was reported only in the presence of S9 at relatively high concentrations of 4000 µg/ml in 3 of 4 tests carried out with harvest times between 12 and 18 hours. No data on cytotoxicity reported.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 5000 µg/ml	Positive	(NTP, 1989a)	Valid study in accordance with OECD Guideline 479. Dose-related increase in frequency of SCE at concentrations from 500 - 1250 µg/ml (without metabolic activation) and 500 - 4000 µg/ml (with metabolic activation). No data on cytotoxicity reported. Number of chromosomes per cell reduced at 4000 µg/ml with S9.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 1250 µg/ml[2] 16 to 4000 µg/ml[3]	Positive[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 479. Significant increase (20 %) in SCE only at the highest doses. No dose-response observed. No second trial using high concentrations to reproduce the positive effects performed. Absence of cytotoxicity reported up to the highest dose.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Up to 5000 µg/ml	Questionable	(McGregor et al., 1988a; Myhr et al., 1990)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 476 (however, no colony sizing performed). Positive responses observed in some experiments at concentrations of 3500 and higher. No dose-response was observed. The highest concentration was letal in some experiments. Positive and negative responses could not be reproduced in all experiments.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	150 to 5000 µg/ml	Negative[3] Positive[2]	(NTP, 1989a)	Valid study in accordance with OECD Guideline 476. In one of three trials without S9 a positive result (relative mutant fraction ≥ 1.6) was reported at 4500 µg/ml with relative total growth of 20 %. The concentration of 5000 µg/ml was letal in this trial, whereas in another one of three trials without S9 3500 µg/ml was letal.
	Mutation assay	*E. coli* WP2 *uvr*A	Not reported	Negative	(Kuroda et al., 1984a)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Cytotoxicity assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	DNA damage assay	Rat hepatocytes	10 mM	Negative	(Storer et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	DNA damage assay	*E. coli* P3478	50 µl/disc	Negative[1]	(Fluck et al., 1976)	
(Benzyl formate [09.077])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm ≤ D < 8 mm) was reported (D=4 mm). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	500 to 4000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
(Benzyl acetate [09.014])	Ames test (pre-incubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (pre-incubation and plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 ≤ D < 8) was reported (D could not clearly be determined). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	250 to 2000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells; Human lymphoblast TK6 cells	Mouse cells 0, 250, 500, 1000 µg/ml; Human cells 0, 500, 1000, 1250, 1500 µg/ml	Negative[2] Positive[3]	(Caspary et al., 1988)	Published non-GLP study in accordance with OECD Guideline 476 (except that no colony sizing was performed). Thus, the study is considered not fully valid. The lowest significantly effective doses in the presence of S9 were 500 µg/ml in mouse cells and 1500 µg/ml in human cells. Cytotoxicity was reported above 500 µg/ml with and without S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0-1600 µl/ml (6 concentrations)	Positive[2]	(McGregor et al., 1988a)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP. The study was not in accordance with OECD Guideline 476 (no colony sizing performed, only in the absence of metabolic activation) and thus not considered valid. The lowest significantly effective doses was 900 µg/ml at which the relative total growth was 50 %. The highest dose was lethal. A positive response was observed in two of three experiments. No dose-response was observed.
	Mammalian cell gene	Mouse lymphoma L5178Y cells	Not reported	Negative[2]	(Rudd et al., 1983)	Study carried out within a larger NTP project. Only abstract

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	mutation test			Positive[3,]		available. Validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	Not reported	Negative[2] Inconclusive[3]	(Honma et al., 1999a)	Published collaborative study on 40 chemicals. Protocol was in accordance with OECD Guideline 476, except that no colony sizing was performed. As the results are insufficiently reported, their validity cannot be evaluated. In the presence of S9 metabolic activation one laboratory achieved a statistically significant dose-dependant result, but did not induce mutations greater than three times the spontaneous response. The second laboratory did not obtain a positive response.
	Chromosomal aberration test	Chinese hamster ovary cells	160 - 1600 µg/ml[2]; 500 - 5000 µg/ml[3]	Negative[1]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster lung fibroblast cells	2400 µg/ml	Negative[1]	(Matsuoka et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Chinese hamster ovary cells	50 - 500 µg/ml[2]; 500 - 5000 µg/ml[3]	Negative[1,]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Unscheduled DNA synthesis test	Rat hepatocytes	Not reported	Negative	(Mirsalis et al., 1983)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Micronucleus test	Human lymphocytes and hepatoma cell line *Hep G2*	500 µM	Negative[1]	(Kevekordes et al., 2001)	
(Benzyl propionate [09.132])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Benzyl benzoate [09.727])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (pre-incubation and plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
(Benzaldehyde [05.013])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	37,500 nl/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	50 to 300 µl/plate	Negative[1]	(Rockwell and Raw, 1979)	Assay of urine samples from rats given benzaldehyde by oral gavage.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell and Raw, 1979)	Samples assayed prior to administration to rats.
	Ames test	*S. typhimurium* TA98; TA100; TA2637	2000 mg/plate	Negative[1]	(Nohmi et al., 1985)	Article published in Japanese. Data reported from English summary.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (pre-incubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD Guideline 471.
	Ames test	*S. typhimurium* TA100; TA102; TA104	3333 µg/plate	Negative[1]	(NTP, 1990c)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
	Ames test (pre-incubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki and Endo, 1978)	
	Ames test (pre-incubation method)	*S. typhimurium* TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (pre-incubation method)	*S. typhimurium* TA100	2000 nM/plate	Negative[1]	(Vamvakas et al., 1989)	
	Ames test (pre-incubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (pre-incubation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Ames test (pre-incubation method)	*S. typhimurium* TA97a; TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1998)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1537; TA7001; TA7002; TA7003; TA7004; TA7006; Mix of TA7001–7006 TA7005	1000 µg/ml	Negative[1] Negative[2]; Positive[3]	(Gee et al., 1998)	
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	Not reported	Negative[2] Positive[3]	(Matsui et al., 1989)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	251 nl/ml	Negative	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	12.5 to 800 nl/ml	Negative[2] Positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (12.5-800, 25-600, 400-600 nl/ml) were used in three independent experiments within which positive responses were observed. A 2.8 to 5.2-fold increase in mutant frequency was observed in the presence of S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0 to 800 µg/ml (6 concentrations)	Positive[2]	(McGregor et al., 1991)	Published summary report including detailed method and results from study on 27 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 476 (however, no colony sizing performed). Statistically significant increase in mutant fraction at the highest non-lethal concentration (400 µg/ml) in two experiments. Concentration of 640 and 800 µg/ml were lethal. Thus, significant increases in mutant fraction were close to toxic doses. No dose-response was observed. Since a positive response was observed without S9, no experiment was carried out with S9.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y +/- cells	600 µg/ml	Negative[2]	(Bigger and Clarke, 1991)	
	Chromosomal aberration test	Chinese hamster cells	0, 800, 1000, 1200 µg/ml	Positive[2] Weak positive[3]	(Sofuni et al., 1985)	Article published in Japanese. Data extracted from English summary and tables. Validity of the study cannot be evaluated. Cytotoxicity was observed at the two maximum concentrations tested. In the presence and in the absence of S9 a positive response was only observed at cytotoxic concentrations. Polyploidization (11 %) was reported at non-cytotoxic concentrations.
	Chromosomal aberration test	Chinese hamster ovary cells	50 - 500 µg/ml[2]; 160-1600 µg/ml[3]	Negative[1]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0053 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Information is only given for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Sister chromatid exchange assay	Chinese hamster ovary cells	5-160 µg/ml[2]; 160-1600 µg/ml[3]	Positive[2] Positive[3]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on a preliminary assay. Although some deatails of results are not reported the study is considered valid. Weakly positive results with metabolic activation were observed at the highest concentration which was cytotoxic and resulted in 50 % growth reduction.
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 1000 µM (up to 106 µg/ml)	Negative[3]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Human lymphocytes	0 - 2 mM (0-212 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Benzoic acid [08.021])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative[1]	(Anderson and Styles, 1978)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1536	3.6 µg/plate	Negative[1]	(Cotruvo et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Ames test	*S. typhimurium* TA100	Up to 1000 µg/plate	Negative	(Rapson et al., 1980)	Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[3]	(McCann et al., 1975)	
	Ames test (preincubation	*S. typhimurium* TA92; TA94; TA98;	Up to 10,000 µg/plate (6	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD Guideline 471.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	method)	TA100; TA1535; TA1537	concentrations)			Although some details of results are not reported the study is considered valid.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	100 µg/plate	Negative[2]	(Milvy and Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA1535; TA1537; TA1538	0.5 % (5 mg/ml)	Negative[1]	(FDA, 1975b)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Umu mutation assay	*S. typhimurium* TA1535/ pSK1002	1607 µg/ml	Negative[1]	(Nakamura et al., 1987)	
	Rec assay (liquid method)	*B. subtilis* M45 (rec^-), H17 (rec^+)	Not reported	Positive	(Nonaka, 1989)	Only abstract available. Details on method and results not reported. Use of metabolic activation not reported. The validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Positive	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) of sufficient quality to be taken into account in the evaluation. A weak positive result (D > 2 mm) was observed at concentrations of 4 mg/disc or more. At 5 mg/disc D = 2.9 mm.
	Mutation assay	*S. cerevisiae* D3	0.18 %	Negative[1]	(Cotruvo et al., 1977)	
	Mutation assay	*S. cerevisiae* D4	0.15 %	Negative[1]	(FDA, 1975b)	
	Indirect DNA repair test	*E. coli* PQ37	400 µg/ml	Negative	(Glosnicka and Dziadziuszko, 1986)	Genotoxicity measured as ability to induce ß-galactosidase.
	SOS Chromotest	*E. coli* PQ37	50 µg	Negative[1]	(Kevekordes et al., 1999)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1500 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition)[4]	Equivocal[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Total incidence of cells with aberrations was 8 %. Negative response for polyploidization.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-244 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	In vitro Micronucleus assay	Mouse lymphoma L5178Y cells	1000 µg/ml	Negative[1]	(Nesslany and Marzin, 1999)	
(Methyl benzoate [09.725])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	0 to 666 µg/plate (-S9); 0 to 6666 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD Guideline 471.
	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Methyl 4-methylbenzoate [09.631]	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537;	0 to 333 µg/plate (-S9); 0 to 3333 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD Guideline 471.
(Isopentyl benzoate [09.755])	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
(4-Isopropylbenzyl alcohol [02.039])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell and Raw, 1979)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell and Raw, 1979)	Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
o-Tolualdehyde [05.026]	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA100; TA1535; TA1537	10 - 666 µg/plate	Negative[1]	(Zeiger et al., 1988)	
m-Tolualdehyde [05.028]	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative[1]	(Florin et al., 1980)	
p-Tolualdehyde [05.029]	Ames test (preincubation method)	*S. typhimurium* TA104	0.8 µmol/plate	Negative[2]	(Marnett et al., 1985a)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.8 nmol -0.8 mmol/plate	Negative[1]	(Aeschbacher et al., 1989)	
(Tolualdehydes (mixed *o, m, p*) [05.027])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	18,750 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. Test substance only indicated as "tolualdehyde" without specification of isomeric composition. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	1000 µg/ml[4]	Negative[2]	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Test substance only indicated as "tolualdehyde" without specification of isomeric composition. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	300 µg/ml (+S9), 600 µg/ml (-S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Test substance only indicated as "tolualdehyde" without specification of isomeric composition.
	Ames test (pre-incubation method)	*S. typhimurium* TA104	0.8 µM/plate	Negative[1]	(Marnett et al., 1985a)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102	0.8 mM/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA100; TA1535; TA1537	666 µg/plate	Negative[1]	(Zeiger et al., 1988)	
(4-Isopropylbenzaldehyde [05.022])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell and Raw, 1979)	
	Ames test (plate method)incorporation	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell and Raw, 1979)	Assay of urine samples from rats given 4-isopropyl benzaldehyde (cuminaldehyde) by gavage.
	Umu test	*S. typhimurium* TA1535/ pSK1002	1 µmole/ml	Negative	(Miyazawa et al., 2000)	Results indicated that 4-isopropylbenzaldehyde (cuminaldehyde) was positive for antimutagenicty, but not genotoxic.
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 333 µM (up to 50	Negative[2]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay		µg/ml)			designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
(4-Hydroxybenzoic acid [08.040])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
(Salicylic acid [08.112])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	Not reported	Negative[2]	(McCann et al., 1975)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Positive	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study, with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation. A weak positive result (D > 2 mm) was observed at concentrations of 2 mg/disc or more. At 5 mg/disc D = 4.7 mm.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Mutation assay	*S. cerevisiae* rad18	Up to 0.1 mM (up to 13.8 µg/ml; 8 concentrations)	Positive	(Zetterberg, 1979)	Published non-GLP study with limited report of experimental details and result. Use of metabolic activation not reported. The validity of the study cannot be evaluated. The dose level tested was clearly cytotoxic. An increase in mutant frequency was not evident until 95 - 99 % of cells were killed.
Ethyl 4-hydroxybenzoate [09.367]	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	Up to 250 µg/ml	Positive	(Ishidate et al., 1978)	Published non-GLP study in Japanese with English summary and tabulated results. Some important details of method and results are not available. There is no

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						information on the use of metabolic activation. The substance was tested up to the maximum dose tolerated. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human fibroblastic cells HE2144	0, 83, 166 µg/ml	Negative[2]	(Sasaki et al., 1980)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mutation assay	Silk worms	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl 4-hydroxybenzoate [09.754])	Ames test	*S. typhimurium* TA98; TA100	1000 µg/plate	Negative[1]	(Haresaku et al., 1985)	
	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 1000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster fibroblast cells	60 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (plate incorporation assay)	*S. typhimurium* TA100	500 µg/plate	Negative[2]	(Ball et al., 1984)	
(Veratraldehyde [05.017])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA15378	8000 µg/plate	Negative[1]	(Nestmann et al., 1980)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	8000 µg/plate	Negative[1]	(Douglas et al., 1979)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TTA1537	6666 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA100; TA102; TA104; TA982; TA1538	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (preincubation protocol)	*S. typhimurium* TA100; TA102; TA104	33 - 3333 µg/plate	Negative[1]	(Dillon et al., 1998)	
	Mutation assay	*S. cerevisiae* D7; XV185-14C	Not reported	Negative[2]	(Nestmann and Lee, 1983)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	250 to 1800 µg/ml	Positive[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (250, 1400 - 1600, 1400 - 1800 µg/ml) were used in three independent experiments within which positive responses were observed. A 2.3 to 6.2 fold increase in the mutation frequency was observed both with and without S9.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
	Unscheduled DNA synthesis test	Rat hepatocytes	100 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(4-Methoxybenzaldehyde [05.015])	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA1537	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Engelhardt, 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	408 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	22 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Müller et al., 1993)	
	Ames test	*S. typhimurium* TA 100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Mutation assay	Phage PM2	1362 µg/ml	Negative	(Becker et al., 1996)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	500 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0068 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, ring and dicentric aberrations, chromatic exchanges).
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0 -3.0 mM (0 - 408 µg/ml) 3.6 - 5.1 mM (484 - 691 µg/ml)	Negative[2] Positive[2]	(Wangenheim and Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD Guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Jung et al., 1992)	Results confirmed at three separate contract laboratories.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	14 µg/ml	Negative	(Sasaki et al., 1987)	
	DNA alkaline unwinding assay	Mouse lymphoma L5178Y TK+/- cells	0, 4, 5, 6 mole/l (0, 544, 680, 816 µg/ml) 7, 8 mole/l (953, 1089 µg/ml)	Negative[2] Positive[2]	(Garberg et al., 1988)	Published study on 78 compounds not in accordance with standard guidelines. Test suitable for rapid screening only. Strand breaks or mutations observed only at cytotoxic concentrations.
2-Methoxybenzaldehyde [05.129]	Mutation assay	*E. coli* WP2*uvr*A, *trpE*	5000 µg/plate	Negative[2]	(Watanabe et al., 1989)	Published non-GLP study with limited report of experimental details and results. Study evaluating the enhancing effect on *N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced mutagenesis in pretreated cells and not on the mutagenicity of the substance itself. Absence of an enhancing effect reported.
	Sister chromatid exchange assay	Human lymphocytes	0 - 0.25 mM (0-34 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
3-Methoxybenzaldehyde [05.158]	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0 - 2.5 mM (0 - 340 µg/ml) 3 mM (408 µg/ml)	Negative[2] Positive[2]	(Wangenheim and Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD Guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
(4-Ethoxybenzaldehyde [05.056])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[2]	(Wild et al., 1983)	
(Methyl 4-methoxybenzoate [09.713])	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Gallic acid [08.080]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	3000 µg/plate	Negative[1]	(Chen and Chung, 2000)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate (solvent DMSO) 0, 100, 333, 1000, 3333, 10,000 µg/plate (solvent acetone)	Negative[1] Equivocal[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD Guideline 471. Results on gallic acid from two different laboratories using different solvent. A negative response was observed in both laboratories with TA98, TA1535, TA1537. A negative result was also reported with TA100 in the laboratory using DMSO as solvent. With acetone, a low-level response with a dose-related trend was found with TA100 both in the absence and in the presence of metabolic activation. The effect was reproducible in a second, not reproducible in a third experiment.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535	5000 µg/plate	Negative[1]	(Rashid et al., 1985)	Inhibition was noted at the 5000-µg/plate dose-level; however, this may have been due to toxicity. No mutagenicity was observed at the 1000-µg/plat dose-level.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	15 µM/plate	Negative[1]	(Wang and Klemencic,	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
					1979)	
	Ames test	*S. typhimurium* TA100	100 µg/plate	Positive[2] Positive[3]	(Yamaguchi, 1981)	Published non-GLP. Insufficient report of important details of method and results, thus the validity of the result cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sugimura et al., 1976)	
	Chromosomal aberration test	Chinese hamster ovary cells	50 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Results are reported for one concentration only which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be reduced by the addition of S9.
	Chromosomal aberration test	Chinese hamster ovary K1 cells	Up to 2 mM (up to 340 µg/ml)	Negative[1]	(Tayama and Nakagawa, 2001)	Published non-GLP study. Part of the study with insufficient report of important details of method and results. The validity of the results cannot be evaluated.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	0, 0.25, 0.5, 1.0, 1.5, 2.0 mM (0, 42.5, 85, 170, 255, 340 µg/ml)	Positive[2]	(Tayama and Nakagawa, 2001)	Published non-GLP study. Well conducted part of the study, however with insufficient report of some important details of method and results (results with metabolic activation not reported).
	Mitotic gene conversion assay	*S. cerevisiae* D7	0, 100, 1000 µg/ml	Negative[2] Positive[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Gallic acid did not induce a significant extent of gene conversions under acidic conditions. At neutral pH no convertogenic activity was reported at 100 µg/ml, however, gallic acid was considerably convertogenic at 1000 µg/ml. The presence of catalase completely inhibited the convertogenic activity gene conversions. Under alkaline conditions (pH 10), the concentration of 100 µg/ml was reported to induce a significant ($p < 0.01$) increase of Trp^+ convertants.
(Vanillin [05.018])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA 1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(Pool and Lin, 1982)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Ames test (preincubation assay)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 1000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Not reported	Negative[1]	(Nagabhushan and Bhide, 1985)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	2500 µg/plate	Negative[2]	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
	Mutation assay	*E. coli* CSH26/pYM3; CSH26/pSK 1002	15,215 µg/ml	Negative	(Takahashi et al., 1990)	
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Chromosomal aberration test	Chinese hamster cell line B241	5, 20, 40 nM (0.0008, 0.003, 0.006 µg/ml)	Negative	(Kasamaki and Urasawa, 1985)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster V79 lung cells	15,215 - 152,150 µg	Negative[2]	(Tamai et al., 1992)	
	Chromosomal aberration test	Human lymphocytes	0, 1, 2, 4 mM (0, 152, 304, 608 µg/ml)	Negative	(Jansson and Zech, 1987)	Published non-GLP study not in accordance with OECD Guideline 473 (no metabolic activation). Insufficient report of important details of method and results. No information on cytotoxicity. This study is not considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	20 nM (0.003 µg/ml)	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are only reported for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. No significant increase of single types of aberrations and of total aberrations.
	Sister chromatid exchange assay	Human lymphocyte cells	0 - 1.0 mM (0 - 152 µg/ml)	Positive[2]	(Jansson et al., 1986)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). This study is not considered valid. Dose-dependent effect reported. Insufficient report of important details of method and results.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	15 µg/ml	Negative	(Sasaki et al., 1987)	
	Sister chromatid exchange assay	Human lymphocytes	0, 1, 2 mM (0, 152, 304 µg/ml)	Positive[2]	(Jansson and Zech, 1987)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. Dose-dependent effect reported This study is not considered valid.
	Mutation assay	Mouse lymphoma L5178Y cells	1000 µg/ml (-S9), 1500 µg/ml (+S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	500 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Micronucleus assay	Human hepatoma (Hep-G2) cells	5, 50 µg/ml 500 µg/ml	Negative [2] Positive[2]	(Sanyal et al., 1997)	Published non-GLP study carried out only in the absence of metabolic activation. Thus, the study is not considered valid. A statistically significant increase of spontaneus micronucleus frequency was reported at the highest concentration. Low concentrations of vanillin (0.25 - 5 µg/ml) but not higher (50, 500 µg/ml) showed an inhibitory effect on micronuclei induced by heterocyclic amines.
(Vanillic acid [08.043])	Chromosomal aberration test	Chinese hamster ovary cells	25,000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be increased by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
4-Hydroxy-3,5-dimethoxybenzaldehyde [05.153]	Ames test	*S. typhimurium* TA100	10,000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
4-Hydroxy-3,5-dimethoxybenzoic acid [08.087]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Chromosomal aberration test	Chinese hamster ovary cells	3000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be reduced by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid.
(Salicylaldehyde [05.055])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki and Endo, 1978)	
	Ames test	*S. typhimurium* TA98; TA100	16 µg/ml	Negative[1]	(Kono et al., 1995)	
	Mutation assay	*S. typhimurium* TA1535/ pSK1002	111 µg/ml	Negative[1]	(Nakamura et al., 1987)	
	Chromosomal aberration test	CHL/IU cells	Not reported (max. 5 mg/ml)	Positive[1]	(Kusakabe et al., 2002)	Published study in accordance with OECD Guideline 473. However, some details on method and results are insufficiently reported. Thus, the validity of the study cannot be evaluated. Positive result with minimum effective dose manifesting over 50 % cytotoxicity at short-

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						term treatment (6 hours, less than 50 % cells with chromosomal aberrations without S9, less than 20 % cells with chromosomal aberrations with S9). Reduced effect at continuous treatment without S9 (24 hours less than 10 % cells with chromosomal aberrations). No chromosomal aberrations after 48 hours treatment without S9. After 48 hours treatment without S9 18 % polyploid cells.
	Sister chromatid exchange assay	Human lymphocyte cells	0-0.5 mM (0-61 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Methyl salicylate [09.749])	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	333.3 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblasts	250 µg/ml[4] (three concentrations, max. concentration inducing 50 % cell-growth inhibition)	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Positive[1]	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation. At 100 µg/plate, a positive response was observed in strain TA98 in the presence of S9 mix obtained from hamsters, a negative response was observed in TA98 in the presence of S9 mix obtained from rat, mouse and guinea pig.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	23 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Negative	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation.
	Mutation assay	Silkworm	Not reported	Negative	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						be evaluated.
	Chromosomal aberration test	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl vanillyl ether [04.093])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative[1]	(Watanabe and Morimoto, 1989c)	
	Mutation assay	*E. coli* WP2 *uvr*A	5000 µg/plate	Negative[1]	(Watanabe and Morimoto, 1989c)	
(Ethyl vanillin [05.019])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	8000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (six concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita and Sasaki, 1987)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblast cells	250 µg/ml (three concentrations, maximal concentration inducing 50 % cell-growth inhibition)[4]	Positive[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Polyploidization in 48 % of cells reported at 48 hours. Negative response for chromosomal aberrations.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	125-800 µg/ml	Negative[2] Weak positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (125-500 µg/ml, 600 µg/ml, 800 µg/ml) were used in three independent experiments within which positive responses were observed. In the presence of S9 a 2.1 to 3-fold increase in the mutant frequency was reported.
	Unscheduled DNA synthesis test	Rat hepatocytes	199 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Sister chromatid exchange	Human lymphocytes	0-2.0 mM (0-332 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay					Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	17 µg/ml	Negative	(Sasaki et al., 1987)	
(Ethyl vanillin isobutyrate [09.933])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(King and Harnasch, 1997)	
(Piperonyl acetate [09.220])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
(Piperonal [05.016])	Ames test					
	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 *E. coli* WP2*uvr*Atrp$^-$	0, 300, 600, 1200, 2400 µg/plate	Negative[1]	(Sekizawa and Shibamoto, 1982)	Valid study in accordance with OECD Guideline 471. The plate incorporation method was used -S9; the preincubation method +S9.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 5000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1537; TA1538	5000 µg/plate	Negative[1]	(White et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD Guideline 471.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	20 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	5000 µg/disc	Positive[2]	(Sekizawa and Shibamoto, 1982)	Well designed and reported study, however with some limitations with respect to results. DNA-repair tests in the presence of S9 were not successful (no data reported).
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0075 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Data are only reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Chromosomal aberration test	Chinese hamster cell line B241	0.15 µg/ml	Negative	(Kasamaki and Urasawa, 1985)	
	Mammalian cell gene	Mouse lymphoma L5178Y cells	1000 µg/ml[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	mutation test					design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	10 to 502 µg/ml	Positive	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(Vanillin 3-(l-menthoxy)propane-1,2-diol acetal [02.248])[5]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative[1]	(Kajiura, 1996b)	
	Mutation assay	*E. coli* WP2 uvrA	Up to 5000 µg/plate	Negative[1]	(Kajiura, 1996b)	
6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde [05.221]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; Ta102; TA1535; TA1537	0, 50, 150, 500, 1500, 5000 µg/plate	Negative[1]	(King and Harnasch, 2002c)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; Ta102; TA1535; TA1537	0, 15, 50, 150, 500, 1500, 5000 µg/plate	Negative[1]	(King and Harnasch, 2002c)	
3,4-Dihydroxybenzoic acid [08.133]	Sister chromatid exchange assay	Chinese hamster ovary cells		Negative[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be increased by the addition of S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0, 33, 100, 333, 1000, 3333 µg/ml	Negative[2]	(McGregor et al., 1988c)	

NR = not reported.

[1] With and without S9 metabolic activation.

[2] Without S9 metabolic activation.

[3] With S9 metabolic activation.

[4] Concentration listed is either the highest tested if the result was negative or the concentration at which the maximum effect was observed for positive results.

[5] Related substance.

In vivo mutagenicity/genotoxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 23 and for ten supporting substances evaluated by JECFA at the 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a). Supporting substances are listed in brackets.

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Benzyl alcohol [02.010])	*In vivo* Sex- linked recessive lethal mutations(SLRL)	*D. melanogaster*	Diet	5000 ppm	Negative	(Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	8000 ppm	Negative	(Foureman et al., 1994)	
	In vivo Micronucleus test	Mouse bone marrow cells	IP injection	200 mg/kg bw	Negative	(Hayashi et al., 1988)	
	In vivo Replicative DNA synthesis test	Mouse and rat hepatocytes	Not reported	Not reported	Negative	(Yoshikawa, 1996)	Screening test for the detection of non-genotoxic hepatocarcinogens. The substance was administered once at the maximum tolerated dose or at half the maximum tolerated dose to male mice and rats. Hepatocytes were prepared after 24, 39 and 48 hours.
	In vivo Replicative DNA synthesis test	Mouse hepatocytes	Oral gavage	800 mg/kg	Negative	(Miyagawa et al., 1995)	
	In vivo Replicative DNA synthesis test	Rat hepatocytes	Oral or SC injection	600 mg/kg	Negative	(Uno et al., 1994)	
(Benzyl acetate [09.014])	*In vivo* SLRL	*D. melanogaster*	Diet	300 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	20,000 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection	1700 mg/kg bw	Negative	(NTP, 1993d)	
	In vivo Chromosomal aberration test	Mouse bone marrow cells	IP injection	0 to 1700 mg/kg bw	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. The highest dose caused toxicity and cell cycle delay. Test not fully in compliance with the OECD guideline (insufficient cells per animal studied). GLP status not stated. The study is considered of limited validity.
	In vivo Micronucleus test	Mouse bone marrow cells	3 IP injection with 24 h intervals	0, 312, 625 and 1250 mg/kg bw	Negative	(NTP, 1993d; Shelby et al., 1993)	Test substance same batch as NTP chronic bioassays. Study in compliance with OECD guideline. GLP not stated. Micronuclei were determiend at 24 hours after the last dose. A dose-related decrease in PCE/NCE ratio was observed. The study is considered valid.
	In vivo Micronucleus test	Mouse erythrocytes	Dietary exposure for 13 weeks.	0 to 50,000 ppm (equal to 0 to 7900 mg/kg bw/day for males and 0 to 9400 mg/kg bw/day for females)	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. In life phase under GLP; for determination of genotoxic effects. GLP not specified. Test in compliance with OECD guideline. The test is considered valid, but of limited relevance because no change in PCE/NCE ratio was observed.
	In vivo Unscheduled DNA synthesis test	Rat hepatocytes	Oral gavage	0, 50, 200 and 1000 mg/kg bw	Negative	(Mirsalis et al., 1989)	Test substance same batch as NTP chronic bioassays. Test in compliance with OECD guidelines. GLP not stated. The test is considered valid.
	In vivo Unscheduled DNA synthesis test	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz and Mirsalis, 1984)	Only abstract available. Non guideline test. Validity cannot be assesed.
	In vivo DNA damage	Rat pancreatic cells	IP injection	0, 150, 500 and	Negative	(Longnecker et al., 1990)	Alkaline elution assay. GLP status not specified.

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
				1500 mg/kg bw			Limited number of animals/group; DNA damage monitored at 1 hour post dosing. The study is of limited validity.
	In vivo Comet assay	Mouse/ Rat	Oral	1600 mg/kg (mouse); 1200 mg/kg (rat)	Positive	(Sekihashi et al., 2002)	Non-GLP and non-guideline test; but in compliance with recommended protocols. Some important details of method and results insufficiently reported. No toxicity data reported. The administered dose was 0.5 x LD_{50}. Sampling time was 3, 8 and 24 hours after dosing. Positive result reported in mice for stomach, colon, kidney, urinary bladder and brain, in rats for stomach, colon, liver, kidney, urinary bladder, lung. After 24 hours no significant effect in mice, significant effects in rat only in lung and kidney. The study is of limited validity.
(Benzaldehyde [05.013])	*In vivo* SLRL	*D. melanogaster*	Diet	1150 ppm	Negative	(Woodruff et al., 1985)	
	In vivo SLRL	*D. melanogaster*	Injection	2500 ppm	Negative	(Woodruff et al., 1985)	
(Salicylic acid [08.112])	*In vivo* Chromosomal aberration assay	Mouse bone marrow cells	IP injection gavage	0, 50, 100, 200 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Published study widely in accordance with OECD Guideline 475 and well reported (except that only males were tested, only one sampling time was chosen and signs of toxicity were not reported). Oral and i.p. dose were selected to be 1/3 and 1/5 of the reported oral LD_{50}.
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection gavage	0, 25, 50, 100 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Well described published study of good quality. Oral and i.p. dose were selected to be 1/3 and 1/10 of the reported oral LD_{50}.
Ethyl 4-hydroxybenzoate [09.367]	*In vivo* Chromosomal aberration assay	Rat bone marrow cells	Not reported	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
(4-Ethoxybenzaldehyde [05.056])	*In vivo* Basc test Micronucleus test	*D. melanogaster*	NR	751 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	1005 mg/ kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
Gallic acid [08.080]	*In vivo* Medium-term rat liver bioassay	Male rats initiated with IP injection of diethylnitrosamine	Not reported.	Not reported	Negative	(Shirai, 1997)	Published non-GLP study. Unusual study protocol not following OECD guidelines. Some important details of method missing and only summarised results of a large screening study reported. Thus, the validity of the study cannot be evaluated.
(Vanillin [05.018])	*In vivo* Micronucleus test	Male BDF_1 mice	Oral gavage	500 mg/kg bw	Negative	(Inouye et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 474 (smaller group size, only males tested, no toxicity data reported, single dose level used, no negative control, effect on PCE/NCE ratio not reported). Induction of micronuclei in mitomycin-treated mice was suppressed by post-treatment with vanillin due to an anticlastogenic

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							effect. Vanillin itself did not induce micronucleated PCEs (vanillin control group without mitomycin-treatment, six sampling times from 5 to 65 hours).
(Salicylaldehyde [05.055])	*In vivo* Spot test	*D. melanogaster* BINSC *D. melanogaster* Oregon-R	NR	1.05 to 1.40 ppm 0.09 to 0.35 ppm	Negative Negative	(Kono et al., 1995)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Ethyl vanillin [05.019])	*In vivo* Basc test	*D. melanogaster*	NR	8309 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	Male BDF_1 mice	IP injection	Not reported	Negative	(Furukawa et al., 1989)	Only abstract available. Insufficient report of experimental details and result to evaluate the validity of the study.
	In vivo Micronucleus test	NMRI mice	NR	1000 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonyl acetate [09.220])	*In vivo* Basc test	*D. melanogaster*	NR	4855 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	970 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonal [05.016])	*In vivo* Dominant lethal assay	ICR/Ha Swiss mice	IP injection	0, 124, 620 mg/kg bw	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD Guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.
	In vivo Dominant lethal assay	ICR/Ha Swiss mice	Oral gavage	0, 1000 mg/kg bw (repeated doses on 5 successive days)	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD Guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Additives, Flavourings and Food Contact Materials
ANS	Food Additives and Nutrient Sources
AUC	Area Under Curve
BW	Body Weigt
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoA	Coenzyme A
CoE	Council of Europe
DMSO	Dimethyl sulphoxide
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
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
FSH	Follicle-stimulating hormone
GI	Gastroinstinal
GLP	Good Laboratory Practice
GSH	Glutathione
HPLC	High Performance Liquid Chromatography
ID	Identity
IP	Intraperitoneal
IV	Intravenous
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
LH	Luteinizing hormone
MDP	Methylenedioxyphenyl

MNNG	*N'*-nitro-*N*-nitrosoguanidine
MPD	4-Methyl-2-phenyl-1,3-dioxolane
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
OECD	Organisation for Economic Co-operation and Development
PAFA	Priority-based assessment of food additives
RACB	Reproductive assessment by continuous breeding
SC	Subcutaneous
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
TLC	Thin Layer Chromatography
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation

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World Health Organization
Geneva

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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organization and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Rome from 5 to 14 June 2001. The meeting was opened by Mr W. Clay, Chief, Nutrition Programmes Service, Food and Nutrition Division, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr Clay reminded the Committee that one of its tasks was to provide scientific advice to Member States of the two organizations with respect to food regulations and control. He noted that dioxins and some related compounds were to be discussed by the Committee for the first time, almost 25 years after the accident in Seveso, Italy, in which large quantities of dioxins had been released into the environment. That event had raised awareness and concern both in the general population and among regulators, leading to a greater demand for global assessment, management and communication of risks relating to environmental contamination and food. The Committee's deliberations on the topic would therefore be important and should help to improve communication between those responsible for risk assessment and risk management. Mr Clay informed the Committee that its activities would be part of a wider effort by FAO and WHO to improve food safety. The two organizations were planning to establish a Global Forum for Food Safety Regulators, in order to promote the exchange of information about ways of dealing with issues of potential importance to public health and international food trade among those responsible for regulating food safety.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been fifty-six previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the fifty-fifth meeting (Annex 1, reference *149*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
— to undertake toxicological evaluations of certain food additives, flavouring agents and contaminants (sections 3–5 and Annex 2);
— to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 4 and Annex 2).

2.1 Modification of the agenda

Annatto extracts were scheduled for evaluation at a future meeting, when the results of toxicological studies that were being performed would become available to the Committee for consideration. Amyloglucosidase from *Aspergillus oryzae*, var. had been included in the call for data erroneously.

Sodium ethyl *p*-hydroxybenzoate, sodium propyl *p*-hydroxybenzoate, sodium methyl *p*-hydroxybenzoate, calcium sulfite, sodium formate, calcium formate, synthetic γ-tocopherol, synthetic δ-tocopherol, calcium tartrate, sorbitan trioleate, dipotassium diphosphate and dimagnesium diphosphate have been removed from the draft Codex General Standard for Food Additives and were referred to the Committee for evaluation. There was no indication, however, that any of these substances are used as food additives, and consequently little information was provided that would permit the establishment of Acceptable Daily Intakes (ADIs) or the preparation of specifications.

Phenyl salicylate was removed from the agenda because no data were available.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), as well as the principles elaborated subsequently at a number of its meetings (Annex 1, references *77*, *83*, *88*, *94*, *101*, *107*, *116*, *122*, *131*, *137*, *143* and *149*), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference *76*) contains the most important observations, comments and recommendations made, up to the time of its publication, by the Committee and associated bodies in their reports on the safety assessment of food additives and contaminants. At its present meeting, the Committee noted that the publication included recommendations that are still appropriate and indicated potential problems associated with those that are no longer valid in the light of technological changes.

2.3 Principles for the safety assessment of chemicals in food

The Committee was informed that FAO and WHO are intending to update and consolidate principles and methods for the safety assessment of chemicals in food, including food additives, contaminants, and residues of veterinary drugs and pesticides. The project was

initiated on the basis of a recommendation of the Conference on International Food Trade Beyond 2000 that was held in Melbourne, Australia, in October 1999 (*2*), and in view of the scientific advances, changes in procedures and the increasing complexity of assessments of chemicals in food that have occurred since the publication of Environmental Health Criteria, No. 70 (Annex 1, reference *76*) and Environmental Health Criteria, No. 104, *Principles for the toxicological assessment of pesticide residues in food* (*3*). The project would include consideration of all those aspects of the assessment of chemicals in food that are addressed by the Committee and by the Joint FAO/WHO Meeting on Pesticide Residues.

The Committee recognized the importance of this initiative and recommended that it be undertaken as soon as possible.

2.4 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

The Committee questioned whether some of the substances included in the lists of flavouring agents that it had been asked to evaluate at its present meeting were in fact used as flavouring agents. The Committee noted that some of the substances were used extensively in food processing as solvents, emulsifiers or preservatives.

The Committee stressed that the Procedure for the Safety Evaluation of Flavouring Agents is intended for application to flavouring agents used to impart flavour to foods and not to other uses of these substances or to other chemicals that may be used in flavouring formulations. Consequently, the Committee was unable to finalize the evaluations of certain substances listed on the agenda,[1] pending confirmation of their use and intake as flavouring agents.

A clear definition of "flavouring agent" has not been elaborated by the Committee. Although Environmental Health Criteria, No. 70 (Annex 1, reference *76*) provides some guidance, the Committee recommended that this issue be addressed at a future meeting.

2.5 α,β-Unsaturated carbonyl compounds and aldehydes

The α,β-unsaturated carbonyl group is a reactive moiety that represents a potential structural alert for toxicity. Five flavouring agents containing such a group were considered by the Committee at its forty-ninth meeting (Annex 1, reference *131*), but their evaluation was postponed, pending consideration of other α,β-unsaturated carbonyl compounds. The safety of these five agents was reconsidered by

[1] See sections 4.1.3–4.1.5.

the Committee at its fifty-fifth meeting (Annex 1, reference *149*), when it also evaluated furfural, cinnamaldehyde, structural analogues of cinnamaldehyde, pulegone and esters of the corresponding alcohols, which are predicted to be metabolized by formation of α,β-unsaturated carbonyls. The available data on the toxicity of these compounds in experimental animals showed a number of adverse effects at high doses, and no-observed-effect-levels (NOELs) for these effects were identified. The presence of protective processes in cells, such as conjugation with glutathione, provides adequate capacity for detoxification at the low doses associated with the use of such compounds as flavouring agents. In consequence, the Committee concluded that the presence of an α,β-unsaturated carbonyl group in a flavouring agent, or its formation during metabolism, would not preclude assessment of that substance by the Procedure for the Safety Evaluation of Flavouring Agents. This conclusion was supported by data on the toxicokinetics of 4-phenyl-3-buten-2-one (No. 820), which was considered by the Committee at its present meeting. This α,β-unsaturated carbonyl compound undergoes complete first-pass metabolism in rats and mice after oral administration and is rapidly eliminated (with a half-life of 20 min in rats and 10 min in mice) after intravenous administration. A number of other α,β-unsaturated carbonyl compounds were also evaluated by the Committee at its present meeting (Nos 821, 826 and 829), as were several compounds predicted to be metabolized to an α,β-unsaturated carbonyl compound (Nos 819, 944, 946 and 948).

Aldehyde groups are also chemically reactive and can bind to soluble proteins and protein components of membranes. Several aldehydes were evaluated previously by the Committee, and the potential genotoxicity of furfural was considered in detail at the fifty-first meeting (Annex 1, reference *137*). Furfural was reported to be genotoxic in three of 16 assays for reverse mutation in *Salmonella typhimurium* and in one of three assays for *rec* gene mutation in *Bacillus subtilis*. A few chromosomal aberrations were seen in Chinese hamster ovary cells in culture when furfural was added at relatively high concentrations (Annex 1, reference *138*). Sister chromatid exchanges and forward mutations were induced in mouse lymphoma cells. The Committee concluded that the weak activity seen in vitro in some tests for genotoxicity might be explained by the reactivity of the aldehyde group. Various metabolic processes (i.e. oxidation, conjugation and condensation) effectively eliminate the reactive aldehyde functional group, when the metabolic pathways are not saturated by high, non-physiological doses. The flavouring agents evaluated at the present meeting included a number of aldehydes (Nos 22, 865, 866,

868, 869, 877–879, 889–893, 896–898 and 937) and compounds that are predicted to be metabolized to aldehydes, such as acetals (Nos 837–840, 867, 940–949 and 954). Metabolism of these flavouring agents is predicted to result in gradual formation of aldehydes, which undergo extensive biotransformation, resulting in only low concentrations of the aldehydes per se. The results of tests for reverse mutation in bacteria were positive for pyruvaldehyde (No. 937), but consistently negative for Nos 22, 80, 95, 98, 867, 868, 877–879, 889, 893, 896, 897 and 953; the results of assays for *rec* gene mutation in *B. subtilis* were negative (Nos 878, 889 and 893) or equivocal (Nos 22 and 896). Chromosomal aberrations were reported in vitro in some studies with Nos 22, 878, 889, 893, 896 and 937, but not with No. 80. Similarly, sister chromatid exchanges were reported in some studies with Nos 22, 80, 878, 889 and 937, but not with Nos 868, 893 and 897. Mutations were reported in mouse lymphoma cells exposed to some aldehydes (Nos 80, 877, 878 and 893), but not other aldehydes or acetate (Nos 867, 889 and 896). The results of studies in vivo did not indicate genotoxicity after oral administration in a variety of test systems: in *Drosophila melanogaster* (with Nos 22, 80, 879 and 893), in assays for micronucleus formation in mice (with Nos 879, 889 and 893) and in assays for dominant lethal mutations in mice (No. 896). Sister chromatid exchange was induced in mice and hamsters by intraperitoneal injection of acetaldehyde (No. 80), and weakly positive results were obtained in several tests in vivo with pyruvaldehyde (No. 937) at very high doses (>200 mg/kg of body weight). Pyruvaldehyde is a natural component of some foods, and the amount ingested due to its use as a flavouring agent would be much less than the estimated intake from natural sources. The Committee concluded that metabolic processes such as oxidation and conjugation effectively eliminate reactive aldehyde functional groups from such substances when they are consumed in the amounts that would arise from their use as flavouring agents.

2.6 Minimum assay values for flavouring agents

At its fifty-third meeting, the Committee developed criteria for establishing specifications for flavouring agents (Annex 1, reference *143*). The Committee noted that these criteria — chemical formula and relative molecular mass, identity test and the minimum amount that can be determined (minimum assay value) — constitute the core information required to establish acceptable specifications. At its present meeting, the Committee considered that a minimum assay value of 95% for an individual flavouring agent would apply to both the flavouring agent itself and to the agent plus its known secondary components. The minimum assay values of about 90% of the flavouring agents evaluated to date meet or exceed 95%, and the Committee received

information about the nature of the secondary components of the others. The Committee noted that 95% is not a fixed value for the acceptability of specifications for flavouring agents and that flexibility can be used in establishing an acceptable level of secondary components, taking into account the probable levels of intake and other considerations.

Many of the secondary components are structurally related to the named flavouring agents. They typically comprise small amounts of starting materials, isomers and other flavouring agents. As these secondary components share many of the properties of the named flavouring agents, and in some cases are metabolites, their safety would not be expected to present a concern or can be evaluated from appropriate data on metabolism and toxicity.

The Committee noted that, in applying the Procedure for the Safety Evaluation of Flavouring Agents, information on secondary components included in the specifications should be considered with data on intake and on the potential toxicity of the flavouring agent and its structural analogues. The Committee therefore recommended that data on specifications be submitted before or at the same time as all other information necessary for evaluating the safety of a flavouring agent.

2.7 Requests for data relating to intake assessments

The Committee recognized that it is unnecessary to request data for assessing intake for all the substances on its agenda, as it had done recently, and developed criteria for determining when such information would be needed. These are described below. In general, calls for data should specify the information required for each substance on the agenda, as the data required for evaluating food additives are different from those required for evaluating contaminants.

2.7.1 *Food additives*

Data for assessing intake should be requested in the case of food additives that are being evaluated for the first time or are being re-evaluated, except for food additives:

— for which only specifications are to be considered;
— for which the Committee has recently deferred an evaluation, pending the provision of a specific toxicological study or information on specifications, provided it has evaluated intake of the additive during the preceding 3–5 years.

Information on proposed maximum levels should be provided in the call for data for food additives included in the draft Codex General

Standard for Food Additives, so that national assessments of intake based on such maximum levels, national maximum levels and/or actual levels of use can be submitted. The Committee has formulated data sheets for submission of national data, which are included in the guidelines for the preparation of working papers on the intake of food additives.

2.7.2 *Contaminants*

For contaminants, an intake assessment is required in all cases. The call for data should request data on:

— the occurrence and concentrations of the contaminant (both individual and summary data) from all available sources, preferably submitted on data sheets, with information on sampling and analytical techniques, data quality and reliability, reporting conventions and appropriate processing factors;
— national intake of the contaminant derived from national surveys of food consumption and concentrations.

2.8 Principles governing the establishment and revision of specifications

2.8.1 *Inclusion of raw materials and manufacturing methods in specifications*

Principles for the safety assessment of food additives and contaminants in food (Annex 1, reference *76*) states that "to establish the chemical identities of additives, it is necessary to know the nature of the raw materials, methods of manufacture and impurities. This information is used to assess the completeness of analytical data on the composition of additives, and to assess the similarity of materials used in biological testing with those commercially produced."

As there are increasing volumes of food additives in international trade, specifications must include brief descriptions of the raw materials and methods of manufacture used, excluding proprietary details, in order to provide a full account of the product being evaluated. If this information is not available, the Committee cannot know whether the products being evaluated were produced from materials or methods that are different from those in the specifications; consequently, impurities might have arisen that were not considered during the toxicological evaluation. The level of detail of the descriptions should be similar to that in specifications elaborated by the Committee for additives made by fermentation or from plant materials.

2.8.2 *General specifications and considerations for enzyme preparations used in food processing*

The Committee has, on many occasions, addressed issues related to specifications for enzyme preparations used in food processing. The general specifications currently in use for enzymes were first elaborated by the Committee at its twenty-sixth meeting (Annex 1, reference *59*). Several revisions have been made, including the following:

— an addendum to address issues arising from use of enzymes from genetically modified microorganisms (Annex 1, references *94*, *96*, *137* and *139*);
— addition of an appendix to describe the method for determining antimicrobial activity (Annex 1, reference *58*);
— an amendment to address inclusion of microbial strain numbers in the specifications for enzyme preparations (Annex 1, reference *139*);
— addition of the general requirement that source microorganisms be non-pathogenic and non-toxigenic (Annex 1, reference *145*).

At its fifty-fifth meeting (Annex 1, reference *149*), the Committee reiterated its view, expressed at its fifty-third meeting (Annex 1, reference *145*), that Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*) required updating in the light of technological developments and to ensure consistency and coherence with the appendices, including Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms).

At its present meeting, the Committee noted that the revised general specifications require that all new enzyme preparations undergo a general safety assessment. Many of the requirements previously outlined for enzyme preparations from genetically modified microorganisms are appropriate for all preparations, regardless of source, and the Committee revised the general specifications to reflect those requirements. For enzymes from genetically modified sources, information is now required on the microbial strain used as the source organism and the genetic material introduced into and remaining in the final microbial strain used in production.

At its present meeting, the Committee noted that the list of mycotoxins contained in the existing general specifications was not relevant to all food enzyme preparations from fungal sources. It agreed that an attempt to list all known mycotoxins of potential concern was impractical and unwarranted. The Committee further agreed that enzyme preparations derived from fungal sources be evaluated for those mycotoxins that are known to be synthesized by strains of the species or related species used in the production of the enzyme preparation.

With regard to limits on heavy metals, the Committee agreed that the specifications for lead contained in the existing general specifications should be lowered from 10 mg/kg to 5 mg/kg. The Committee recognized that arsenic is not a concern in enzyme preparations, and therefore deleted the limit for this metal. Moreover, as there is no traceable source of cadmium or mercury in enzyme preparations, the Committee saw no need to establish limits for those metals. Such changes are consistent with the Committee's current policy on heavy metals (Annex 1, reference *145*).

In considering microbiological contamination of enzyme preparations, the Committee agreed that the existing microbiological criteria (for *Salmonella* spp., *Escherichia coli* and total coliforms) and the requirement that use of preparations should not increase the total microbial count in treated food above the threshold considered to be acceptable for the respective foods are sufficient to ensure microbial safety. The criteria and the requirement were thus retained. The Committee noted that the specification for a total viable count of 5×10^4 organisms per gram included in the existing general specifications did not provide an indication of the safety of an enzyme preparation. It was therefore deleted.

In considering allergenic potential, the Committee emphasized that, when the source organism of an enzyme preparation is a genetically modified microorganism, the necessity for evaluating the allergenic potential of the gene products encoded by the inserted DNA should be assessed. The Committee agreed that, when the DNA sequence of an enzyme from a genetically modified production microorganism is comparable to that coding for an enzyme already known to have a history of safe use in food, there would be no need to assess the allergenic potential of that enzyme further.

Finally, the Committee recognized that the revised specifications include many criteria for safety evaluation that would be more appropriately listed elsewhere. The Committee strongly recommended that *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*) be revised to include the safety assessment of enzymes intended for use in food and that such guidelines should subsequently be removed from the general specifications.

3. Specific food additives (other than flavouring agents)

The Committee evaluated two food additives for the first time and re-evaluated a number of others. Information on the safety evaluations

and on specifications is summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 Safety evaluations

3.1.1 *Emulsifiers*

3.1.1.1 Diacetyltartaric and fatty acid esters of glycerol

Diacetyltartaric and fatty acid esters of glycerol were reviewed by the Committee at its tenth and seventeenth meetings (Annex 1, references *13* and *32*). At its seventeenth meeting, the Committee allocated an ADI of 0–50 mg/kg of body weight on the basis of the results of biochemical and metabolic studies and feeding tests in animals. At the same meeting, the Committee also reviewed mixed tartaric, acetic and fatty acid esters of glycerol and allocated an ADI "not limited", with the provision that the total intake of tartaric acid from food additives should not exceed 30 mg/kg of body weight per day.

Specifications established by the Committee at its fifty-first meeting (Annex 1, reference *137*) covered both the above-mentioned products under the name "diacetyltartaric and fatty acid esters of glycerol", as the Committee was aware that the two products could not be distinguished analytically. At that meeting, the Committee recommended that the material defined in the specifications be evaluated toxicologically. At its present meeting, the Committee considered the data that were available previously as well as newly submitted information.

The diacetyltartaric and fatty acid esters of glycerol consist of mixed glycerol esters of mono- and diacetyltartaric acid and fatty acids of food fats. They can be manufactured either by the interaction of diacetyltartaric anhydride and mono- and diglycerides of fatty acids in the presence of acetic acid, or by the interaction of acetic anhydride and mono- and diglycerides of fatty acids in the presence of tartaric acid.

Owing to inter- and intramolecular exchange of acyl groups, the two methods of production result in essentially the same components, the distribution of which depends on the relative proportions of the basic raw materials, on temperature and on reaction time. Diacetyltartaric and fatty acid esters of glycerol may contain small amounts of free glycerol, free fatty acids and free tartaric and acetic acids. They may be further specified as to the acid value, total tartaric acid content, free acetic acid content, saponification value, iodine value, free fatty acid content and the solidification point of the free fatty acids.

The draft Codex General Standard for Food Additives includes use of diacetyltartaric and fatty acid esters of glycerol as an emulsifier, sequestrant and stabilizing agent in a wide range of foods at a maximum concentration of 10 g/kg.

Biological data. Biochemical studies suggest that diacetyltartaric and fatty acid esters of glycerol are hydrolysed in the gastrointestinal tract to yield mono- and diglycerides and acetylated tartaric acid. As mono- and diglycerides are natural dietary constituents, they would be subjected to natural digestion and absorption processes. Diacetyltartaric acid is not a natural constituent of the diet, and there is evidence that it may be further hydrolysed to yield acetic and tartaric acids. When labelled diacetyltartaric and fatty acid esters of glycerol were administered to rats, only about one-third of a ^{14}C label on tartaric acid was absorbed; slightly more was excreted in expired air than in urine.

The studies reviewed previously indicated little toxicity after administration of a single oral dose of diacetyltartaric and fatty acid esters of glycerol. Three studies of the potential long-term toxicity of this product when given in the diet to small numbers of rats showed no adverse effects of dietary concentrations of up to 200 g/kg on mortality rate, physical appearance, body weight, food consumption, reproduction or the histological appearance of the main organs. Dogs also showed no adverse effects when fed diets containing concentrations of up to 200 g/kg for more than 2 years.

The information reviewed for the first time at the present meeting consisted of a 2-week study of palatability, a long-term study of toxicity and carcinogenicity, a two-generation study of reproductive toxicity and a study of developmental toxicity, all conducted in rats, plus two studies of genotoxicity, for point mutations in bacteria and for clastogenicity in isolated human lymphocytes. In addition, a 6-month study was conducted in male rats to elucidate some of the effects seen in the long-term study of toxicity and carcinogenicity.

In the short- and long-term studies in rats, diacetyltartaric and fatty acid esters of glycerol at a concentration of 100 g/kg of diet caused a transient occurrence of soft stools, particularly in males. Food consumption was frequently depressed at this concentration, most consistently during the first weeks of treatment. The palatability of the diet was not improved by incorporating diacetyltartaric and fatty acid esters of glycerol into breadcrumbs or by volatilizing the fatty acids in the substance by freeze–drying the diet before administration. Bodyweight gain tended to be reduced, but this effect was not observed consistently in the short-term study. In the long-term study, consumption of a diet containing diacetyltartaric and fatty acid esters of glycerol at 100 g/kg was associated with decreased body weight. This effect was transient in male rats, but body weights more than 10% lower than those of controls persisted in females into the second year of the study. Supplementing the diet with additional protein,

magnesium, pyridoxine (vitamin B_6) and cyanocobalamin (vitamin B_{12}) reduced the decrease in weight observed with the compound at 100 g/kg of diet in the 6-month study.

Administration of diacetyltartaric and fatty acid esters of glycerol was associated with a decrease in the proportion of lymphocytes and an increase in the proportion of neutrophils in the total leukocyte count during the 6-month study and during the first year of the long-term study. However, these effects were transient and dependent on the type of diet.

In the 6-month study, inclusion of diacetyltartaric and fatty acid esters of glycerol at a concentration of 60 or 100 g/kg of diet was associated with an increase in the urinary excretion of calcium. In the long-term study, differences in the weights of the adrenal glands, kidneys and spleen were observed after 1 year of treatment but were no longer evident after 2 years. Males fed a diet containing 100 g/kg showed an increase in both the incidence and the severity of mineralization in the kidney papilla and pelvis after 1 and 2 years of treatment. Administration at 100 g/kg of diet for 2 years resulted in an increased prevalence of microscopic abscesses in the kidneys of males and an increased severity of nephrocalcinosis in females.

After 2 years of treatment, a dose-related increase in the incidence of adrenal medullary adenomas was seen in males, affecting 4/50, 6/50, 11/50 and 15/50 (statistically significant) animals at 0, 30, 60 and 100 g/kg of diet, respectively, and 1/50 and 4/50 females at 0 and 100 g/kg of diet, respectively. Focal medullary hyperplasia was observed in 3/50, 10/48 (statistically significant), 15/50 (statistically significant) and 15/50 (statistically significant) males at 0, 30, 60 and 100 g/kg of diet, respectively, and in 0/50 and 9/50 (statistically significant) females at 0 and 100 g/kg of diet, respectively.

Statistically significantly higher incidences of haemangioma and haemorrhage in the mesenteric lymph nodes were observed in males fed diets containing 100 g/kg of diacetyltartaric and fatty acid esters of glycerol, while the incidence of sinus histiocytosis of the mesenteric lymph nodes was statistically significantly increased in all treated males.

Myocardial fibrosis was observed more frequently in males at the highest dietary concentration (13/50) than in the control group (3/50). Females at the highest concentration had higher incidences of endometrial hyperplasia (7/50 vs 0/50) and cystic endometrial hyperplasia (14/50 vs 7/50) than controls at the end of the study. Histopathological examinations were carried out on the hearts of only

some of the males and on the uteri of only some of the females at the two lower doses.

In a two-generation study of reproductive toxicity, parental males of the F_0 generation ate less of the diet containing 100 g/kg of diacetyltartaric and fatty acid esters of glycerol and gained less weight during the pre-mating period. Although F_0 females at this dietary concentration also ate less food during the first few weeks of the study, their body-weight gains were not affected. The body weights, body-weight gains and food consumption of the F_1 adults were unchanged. The survival of the F_1 and F_2 litters was not affected by treatment. The weight gains during lactation of the F_1 generation litters of dams given diacetyltartaric and fatty acid esters of glycerol at concentrations of 60 or 100 g/kg of diet and of the F_2 generation litters of dams at 100 g/kg of diet were significantly reduced. The reproductive organs were not assessed histologically. The NOEL for reproductive toxicity was 30 g/kg of diet, equivalent to 1500 g/kg of body weight per day.

Evaluation. High dietary concentrations of diacetyltartaric and fatty acid esters of glycerol were associated with decreased body weights in adult rats and their offspring, but it could not be ascertained from the available data whether these decreases were secondary to or independent of decreased food consumption.

In the 2-year study in rats, the groups treated with diacetyltartaric and fatty acid esters of glycerol were apparently compared with controls fed diets containing monoglyceride. In order to assess whether some of the adverse effects were treatment-related, it would be necessary to compare the effects in treated groups with those in both untreated and monoglyceride-treated control groups, and to compare the control groups with one another. In the absence of additional data on the incidence of myocardial fibrosis and adrenal medullary hyperplasia in animals at the lowest and intermediate doses, no NOEL could be identified in the long-term study. The previous ADI of 0–50 mg/kg of body weight was made temporary until 2003, pending submission of all the necessary additional information.

The specifications for diacetyltartaric and fatty acid esters of glycerol were revised. As specifications no longer exist for tartaric, acetic and fatty acid esters of glycerol, mixed, the previous ADI is no longer applicable and was withdrawn.

A toxicological monograph was prepared, incorporating information from the earlier monographs and summaries of the studies reviewed for the first time at the present meeting.

3.1.1.2 Quillaia extracts

Quillaia extracts (synonyms: bois de Panama, Panama bark extracts, quillai extracts, Quillay bark extracts, soapbark extracts) are obtained by aqueous extraction of the milled inner bark or wood of pruned stems and branches of *Quillaja saponaria* Molina (family Rosaceae), which is a large evergreen with shiny, leathery leaves and a thick bark, native to China and several South American countries, principally Bolivia, Chile and Peru. The term "quillaia" refers to the dried inner bark of the tree.

Unpurified extracts contain over 60 triterpenoid saponins, consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are major components. Some simple sugars and calcium oxalate are also present. The saponin concentration of freshly prepared, unpurified extracts is 190–200 g/kg of solids (about 20%). The extracts are treated with "stabilizing agents" such as egg albumin and polyvinylpyrrolidone and then filtered through diatomaceous earth. The stabilizing agents remove substances that would probably precipitate during storage, such as protein–polyphenol complexes. After filtration, the liquid is concentrated, and the concentrate may be sold as such (solids constituting about 550 g/l) or be spray–dried and sold as a powder containing carriers such as lactose and maltodextrin. The unpurified extracts are used in food applications, primarily for their foaming properties.

Semi-purified powdered extracts are produced by subjecting unpurified extracts to ultra-filtration or affinity chromatography to remove most solids other than saponins, such as polyphenols. These semi-purified extracts have higher saponin concentrations (750–800 g/kg of solids; about 80%) and better emulsifying properties than unpurified extracts.

Highly purified extracts are produced for use as adjuvants in the production of animal and human vaccines and not for food use. These products generally contain more than 90% saponins.

In previous evaluations, the Committee considered data on unpurified quillaia extracts. Quillaia extracts were reviewed toxicologically by the Committee at its twenty-sixth meeting (Annex 1, reference *59*). The available toxicological data included adequate lifetime studies in mice and rats, from which a NOEL was identified. However, in the absence of data, no specifications were prepared, and, hence, no ADI could be allocated. At its twenty-ninth meeting (Annex 1, reference *70*), the Committee prepared new tentative specifications and established an ADI of 0–5 mg/kg of body weight. The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-

second Session (*4*) that the Expert Committee re-evaluate all relevant information on the toxicity and, in particular, the intake of quillaia extracts. No new data were submitted to the Committee at its present meeting. The Committee evaluated published reports on quillaia extracts or specific saponins that provided information relevant to a toxicological assessment of quillaia extracts.

Biological data. Quillaia extracts are mixtures of biologically active compounds that include saponins, tannins, polyphenols and calcium oxalate. The saponins present in quillaia extracts have a variety of biological activities: they are haemolytic, cytotoxic, enhance immune reactions, cause mucosal irritation and inflammation and are anti-hypercholesterolaemic. The biological activities and the potency of individual saponins vary widely and depend primarily on the route of administration.

Studies of acute toxicity showed that quillaia extracts are less toxic when administered orally than when administered subcutaneously or intravenously. Fractions isolated from *Q. saponaria* differed widely in acute toxicity as well as in adjuvant activity and cholesterol-binding capacity. QS-18, the major saponin of quillaia extracts, was more acutely toxic to mice than two other saponins that were isolated and was more toxic than the extract itself when administered intradermally.

In a 90-day study, rats were fed diets containing 40 g/kg quillaia extract (equivalent to 2000 mg/kg of body weight per day). The specifications of the preparation conformed to the Emulsifiers and Stabilisers in Food Regulations 1975 of the United Kingdom, but information on the actual composition of the material tested was not available. The animals showed decreased body-weight gain, decreased weight of the liver relative to body weight and increased stomach weight, with no treatment-related histological changes. The NOEL was 6 g/kg of diet, equivalent to 400 mg/kg of body weight per day.

In a more recent 90-day study, rats were given quillaia saponins in deionized water by gavage at a dose of 1200 mg/kg of body weight. Severe and lethal toxic effects were observed during the study. In the surviving animals, the weights of several organs were increased, and several haematological and clinical parameters were changed. Histopathological examination showed inflammatory changes in the forestomach, larynx, trachea and lungs.

Minor changes in body-weight gain and in the relative weights of some organs were reported in lifetime studies in mice and rats given

quillaia extracts (with specifications conforming to the Emulsifiers and Stabilisers in Food Regulations 1975 of the United Kingdom), at dietary concentrations of up to 30 g/kg for mice and 15 g/kg for rats. No compound-related histopathological changes were reported. The NOELs for quillaia extracts in the diet were 5 g/kg (equivalent to 700 mg/kg of body weight per day) for mice and 10 g/kg (equivalent to 500 mg/kg of body weight per day) for rats.

The Committee noted that the differences in toxicity observed in the 90-day studies in rats treated orally, outlined above, might have been due to differences in the concentrations and types of saponins present in the test material and/or differences in the method of administration, i.e. in the diet and by gavage in water.

Evaluation. The existing specifications for quillaia extracts were revised in order to clarify the differences between unpurified and semi-purified extracts. As additional information on composition was determined to be necessary, the specifications were designated as tentative. Once the requested information has been received, the Committee will consider whether separate specifications for unpurified and semi-purified extracts are required.

The Committee decided that the previously established ADI of 0–5 mg/kg of body weight for unpurified extracts should be made temporary and extended it until 2003, pending clarification of the specifications for quillaia extracts; further studies of toxicity with specified quillaia products similar to the product consumed by humans may be required. The Committee emphasized that the temporary ADI is not applicable to the semi-purified extract or to any other product derived from *Q. saponaria* or from other species of *Quillaia*.

Assessment of intake. Quillaia extracts can be used as foaming agents in soft drinks and cocktail mixes and as emulsifiers in foods such as baked goods, sweets, frozen dairy products, gelatine and puddings. Their major food use is in soft drinks such as ginger beer, root beer and cream soda.

Calculations based on the temporary ADI of 0–5 mg/kg of body weight and the assumption that quillaia extracts are used in soft drinks at a concentration of 500 mg/kg indicated that a person weighing 60 kg could drink up to 600 g/day of a soft drink before exceeding the ADI, while a child weighing 15 kg could drink only 150 g/day of a soft drink before exceeding the ADI. Data on food consumption submitted to the Committee indicated that consumers in Australia who are at the 95th percentile of the distribution of consumption of soft drinks that are likely to contain the additive and children aged 1.5–4 years in the United Kingdom who consume soft drinks at the

97.5th percentile could exceed these amounts. However, these calculations may have overestimated long-term consumption because the data were derived from short-term surveys.

Estimates of intake based on consumption of soft drinks likely to contain this food additive and the levels of use of quillaia extracts permitted in the draft Codex General Standard for Food Additives were submitted by Australia and the USA. Estimates of the mean intake in the United Kingdom were also available, which were based on consumption of all water-based flavoured drinks and are therefore conservative. In Australia, the mean intakes were 3 mg/kg of body weight per day (60% of the ADI) for consumers of drinks containing the additive at the level permitted in the draft Codex General Standard for Food Additives (500 mg/kg) and 7.2 mg/kg of body weight per day (145% of the ADI) for persons at the 95th percentile of consumption. In the USA, the estimated mean intakes of quillaia extracts were 1.5 mg/kg of body weight per day (30% of the ADI) for consumers of drinks containing the additive at the level permitted in the draft Codex General Standard for Food Additives and 2.7 mg/kg of body weight per day (54% of the ADI) for consumers at the 90th percentile.

Estimates of intake based only on consumption of soft drinks likely to contain the food additive and national levels of use were submitted by the USA. The maximum level of use of quillaia extracts by manufacturers in the USA is 100 mg/kg. The estimated mean intake by consumers was 0.3 mg/kg of body weight per day (6% of the ADI), and that by consumers at the 90th percentile was 0.54 mg/kg of body weight per day (11% of the ADI). Data from the United Kingdom, based on a level of use by manufacturers of 95 mg/kg, indicated that children who consume soft drinks at the 97.5th percentile level would consume quillaia extracts at 5.2 mg/kg of body weight per day (105% of the ADI), but this value may be an overestimate of intake as it is based on consumption of all water-based flavoured drinks.

Use at a maximum level of 95–100 mg/day (that reported by the manufacturers), as in the United Kingdom and the USA, appeared to be adequate for the technological functioning of quillaia extracts as foaming agents in soft drinks and did not appear to result in intakes that exceed the ADI. Young children are a possible exception, but, as the results of a short-term nutritional survey were used, the frequency or duration of their potential excursion above the ADI could not be determined.

The Committee recommended that the Codex Committee on Food Additives and Contaminants review the use of quillaia extracts at

500 mg/kg proposed in the draft Codex General Standard for Food Additives.

A toxicological monograph was prepared.

3.1.2 *Enzyme preparation*

3.1.2.1 Invertase from Saccharomyces cerevisiae

Invertase from *Saccharomyces cerevisiae*, or "bakers' yeast", hydrolyses sucrose to a mixture of glucose and fructose (invert sugar). This substance was reviewed by the Committee at its fifteenth meeting (Annex 1, reference *26*) as one of the active principles of carbohydrase from *Saccharomyces* species. It is produced by controlled, submerged fermentation of a pure culture of *S. cerevisiae*. At the end of fermentation, the yeast cells are collected, washed and subjected to autolysis. The lysate is centrifuged and/or filtered to remove cell debris. The resulting enzyme preparation may be dried or ultra-filtered to a desired enzyme concentration. Liquid ultra-filtered products can be treated further with activated charcoal to remove colour and then filtered under sterile conditions. The invertase product is standardized with food-grade diluents.

At its fifteenth meeting (Annex 1, reference *26*), the Committee concluded that enzymes derived from microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods should themselves be regarded as foods. Invertase from *S. cerevisiae* was evaluated at the present meeting because it was being considered for inclusion in the draft Codex General Standard for Food Additives.

Invertase is fundamental to the manufacture of soft-filled chocolates and liquid-centre confectionery, there being no additive that fulfils the same technological function. In the filling for chocolates, invertase is used at a concentration of 1 g/kg of sucrose, resulting in a concentration of 0.6 g/kg (600 mg/kg) in the finished product.

The intake of invertase predicted by the Scientific Committee on Food of the Commission of the European Union was 15 mg/day, assuming consumption of 25 g of filled chocolates per day out of a total of 50 g of chocolate of all types and a concentration of invertase of 600 mg/kg of chocolate.

The potential intake of invertase from its use in chocolates was also predicted for the Australian population from individual dietary records obtained in a survey in 1995. Assuming consumption of 600 mg/kg of filled chocolate, the mean invertase intake by consumers was 20 mg/day (33 g/day of filled chocolate), and that of persons at the 95th percentile of consumption was 61 mg/day (100 g/day of filled choco-

late). These estimates were based on 24-h recalls of food consumption, which tend to result in overestimates of consumption on a long-term basis. The intake of invertase by young children and adolescents was similar to that of adults but would be relatively higher than that of the general population if expressed per kilogram of body weight.

No biological data were available. *S. cerevisiae* has a well-established history of use in fermented foods, including bread, alcoholic beverages, some milk products and cocoa. In line with the general principles outlined in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), invertase from *S. cerevisiae* that meets the specifications developed at the present meeting was considered to be acceptable, as *S. cerevisiae* is commonly used in the preparation of food. Its use should be limited by good manufacturing practice.

A toxicological monograph was not prepared. New specifications were prepared.

3.1.3 *Food colours*

3.1.3.1 β-Carotene from Blakeslea trispora

The Committee did not undertake a general re-evaluation of β-carotene for use as a colouring agent but focused its assessment on the production and analytical characteristics of β-carotene produced from *Blakeslea trispora*.

β-Carotene is obtained from *B. trispora* by co-fermentation of the two sexual types of the fungus in specific proportions. Both types are stable in cultures maintained under conditions consistent with good manufacturing practice. These source organisms are neither pathogenic nor toxinogenic. The compound is isolated from the fungal biomass by solvent extraction and crystallized. The main articles of commerce are suspensions in food-grade vegetable or plant oil and water-dispersible powders, which are easy to use and improve stability, as carotenes readily undergo oxidation.

As in synthetic β-carotene, the colouring principle of β-carotene from *B. trispora* consists predominantly of the all-*trans* isomer of β-carotene. The content of total colouring matter is not less than 96% (expressed as β-carotene). β-Carotene from *B. trispora* may also contain other carotenoids, of which γ-carotene accounts for the major part, at concentrations up to 3%. These molecules occur naturally in carotenoid-containing vegetables.

The Committee considered that the source organisms, the production process and the composition of β-carotene from *B. trispora* do not

raise specific concerns and that the material should be considered toxicologically equivalent to chemically synthesized β-carotene, for which an ADI of 0–5 mg/kg of body weight was established by the Committee at its eighteenth meeting (Annex 1, reference *35*). This opinion was given further credence by the negative results obtained in two tests for genotoxicity (mutagenicity and chromosomal aberration) considered at the present meeting. Therefore, the Committee established a group ADI of 0–5 mg/kg of body weight for synthetic β-carotene and β-carotene derived from *B. trispora*. The ADI relates strictly to use of β-carotene as a food colouring agent and not to its use as a food supplement.

Use of this preparation is unlikely to result in increased use of β-carotene as a food colour because the material is expected to be substituted for synthetic β-carotene.

A toxicological monograph was prepared. New specifications were prepared and designated as tentative, pending information on a suitable method for determination of residual ethyl acetate and isobutyl acetate used as solvents. This information is required by 2003.

3.1.3.2 Curcumin

Curcumin is obtained by solvent extraction of turmeric, which is in turn derived from ground rhizomes of *Curcuma longa* L. (*C. domestica* Valeton). In order to obtain concentrated curcumin powder, the extract is purified by crystallization. The commercial product consists predominantly of curcumins: the colouring principle (1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) and its desmethoxy and bisdesmethoxy derivatives, in varying proportions. Minor amounts of oils and resins that occur naturally in turmeric may be present.

Turmeric oleoresin and curcumin, the main colouring component of turmeric oleoresin, were evaluated by the Committee at its thirteenth, eighteenth, twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth, thirty-ninth, forty-fourth and fifty-first meetings (Annex 1, references *19*, *35*, *47*, *53*, *59*, *73*, *88*, *101*, *116* and *137*). At its eighteenth meeting, the Committee established a temporary ADI of 0–0.1 mg/kg of body weight for curcumin on the basis of the ADI for turmeric oleoresin (0–2.5 mg/kg of body weight) and an assumed average concentration of 3% curcumin in turmeric. The temporary ADI for curcumin was extended at the twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth and thirty-ninth meetings of the Committee. At its thirty-ninth meeting, the Committee requested the results of studies of carcinogenicity in mice and rats fed turmeric

oleoresin and the results of a study of reproductive and developmental toxicity with curcumin.

At its forty-fourth meeting, the Committee evaluated the results of the studies of carcinogenicity in rats and mice given turmeric oleoresin containing 79–85% curcumin and new data on the biochemistry and genotoxicity of the compound. The Committee concluded that data on the developmental toxicity of curcumin were no longer required, but reiterated its request for a study of reproductive toxicity. On the basis of a NOEL of 220 mg/kg of body weight per day in the study of carcinogenicity in mice and a safety factor of 200, the Committee increased the temporary ADI to 0–1 mg/kg of body weight and extended it, pending submission of the results of a study of reproductive toxicity with curcumin.

At its fifty-first meeting, the Committee evaluated the results of studies of fertility in rats and mice treated with turmeric oleoresin and concluded that they did not provide assurance that the potential reproductive effects of curcumin had been adequately investigated. The Committee again extended the temporary ADI, pending submission of the results of a study of reproductive toxicity with a substance that complied with the specifications for curcumin, for review in 2001.

The results of the requested study were not available to the Committee at its present meeting. Nevertheless, the Committee was informed that a multigeneration study of reproductive toxicity with a substance that complied with the specifications for curcumin was under way and would be completed within the next few months. In view of this information, the temporary ADI of 0–1 mg/kg of body weight for curcumin was extended until 2003, pending submission of the results of this study.

A toxicological monograph was not prepared. The existing specifications were revised, with minor changes.

3.1.4 *Food salts*

3.1.4.1 Phosphates, diphosphates and polyphosphates

Phosphates, diphosphates and polyphosphates were evaluated by the Committee at its sixth, seventh, eighth, ninth, thirteenth, fourteenth, seventeenth and twenty-sixth meetings (Annex 1, references *6–8*, *11*, *19*, *22*, *32* and *59*). A maximum tolerable daily intake (MTDI) of 70 mg/kg of body weight was established at the twenty-sixth meeting on the basis of the lowest concentration of phosphorus (6600 mg/day) that caused nephrocalcinosis in rats. It was considered inappropriate to establish an ADI, as phosphorus (as phosphates) is an essential nutrient and an unavoidable constituent of food. The MTDI is

expressed as phosphorus and applies to the sum of phosphates naturally present in food and the phosphates derived from use of these food additives.

This MTDI was considered to cover a number of phosphate salts, according to the principle established by the Committee at its ninth, twenty-third and twenty-ninth meetings (Annex 1, references *11*, *50* and *70*) that the ADI (or MTDI) established for ionizable salts should be based on previously accepted recommendations for the constituent cations and anions. However, in this case, while an MTDI has been established for the class of phosphate salts, certain specific salts were not included because specifications were lacking and because information was not available to indicate whether they were being used as food-grade materials.

At its present meeting, the Committee established specifications for certain specific phosphate salts, pending further information, as indicated below.

- Calcium dihydrogen diphosphate is manufactured by calcination of calcium orthophosphate at a temperature of about 270 °C, with a molar ratio of calcium : phosphorus of about 1 : 2. It is used in fine bakery wares at concentrations of up to 20 g/kg.
- Monomagnesium orthophosphate is manufactured by partial neutralization of phosphoric acid with magnesium oxide and drying the resultant product. It is used in fine bakery wares at concentrations of up to 20 g/kg. The Committee noted that this substance is more concisely denoted as monomagnesium phosphate. It therefore deleted the prefix "ortho" for the substance in the specifications. The specifications were made tentative, pending further information on loss on drying, loss on ignition, the test method for loss on ignition and the assay method for the dihydrate.
- Sodium calcium polyphosphate is manufactured by the fusion of sodium phosphate and calcium carbonate at temperatures greater than 1000 °C. Phosphoric acid is neutralized with sodium and calcium hydroxides in a molar ratio of 5 : 1. The resulting mixture undergoes calcination and is cooled, milled and sieved. It is used in processed cheese and processed cheese analogues at concentrations of up to 20 g/kg.
- Trisodium diphosphate is manufactured by hydration and drying of mixtures of sodium diphosphate. It is used in meat products at concentrations of up to 5 g/kg. The specifications were made tentative, pending further information on loss on drying, loss on ignition, the test method for loss on ignition and the assay method for the monohydrate.

The Committee included these salts in the group MTDI for phosphates, diphosphates and polyphosphates.

A toxicological monograph was not prepared.

3.1.5 *Glazing agent*

3.1.5.1 Hydrogenated poly-1-decene

Hydrogenated poly-1-decene is obtained by catalytic hydrogenation of mixtures of trimers, tetramers, pentamers and hexamers of 1-decenes, produced by oligomerization of 1-decene in the presence of a catalyst. The product is purified by filtration through activated clay. Hydrogenated poly-1-decene consists of a mixture of branched isomeric hydrocarbons, predominantly with more than 30 carbon atoms. Minor amounts of molecules with fewer carbons may be present.

Hydrogenated poly-1-decene was first evaluated by the Committee at its forty-ninth meeting (Annex 1, reference *131*) for use as a glazing and releasing agent. A 28-day range-finding study and a 90-day study in rats that were available at that time were considered inadequate to support use of this product as a food additive. Data were requested to demonstrate that the oily coats observed in rats fed hydrogenated poly-1-decene in the 90-day study were not the result of systemic absorption of the material. The Committee also requested that the results of a study demonstrating lack of absorption in humans be provided. In the absence of such data, the results of long-term studies of toxicity and reproductive toxicity and information on the metabolism, distribution and excretion of hydrogenated poly-1-decene would be required.

At its fifty-third meeting (Annex 1, reference *143*), the Committee reviewed a study of the distribution and excretion of [^{3}H]hydrogenated poly-1-decene conducted in rats. This study established that the oiliness of the fur observed within 1–6 h of administration of a bolus dose was associated with radiolabelled material originating from the anal region, which was spread by grooming. However, while the study indicated that very little hydrogenated poly-1-decene was absorbed after oral administration, it did not allow clear definition of the fate or disposition of any absorbed material. The Committee was therefore unable to establish an ADI and requested an adequate study of the absorption and deposition of hydrogenated poly-1-decene in order to determine whether further studies were required.

At its present meeting, the Committee re-evaluated the results of the study of the distribution and excretion of hydrogenated poly-1-decene. Although no additional studies on distribution and excretion were submitted, the Committee's attention was drawn to arguments

that supported the validity of the previous study. In addition, the Committee evaluated a study of the effect of hydrogenated poly-1-decene on the absorption, distribution and excretion of linoleic acid and glycerol trioleate that had been submitted for consideration.

The Committee also revised the existing specifications for hydrogenated poly-1-decene in order to take into account the decrease from 3% to 1.5% in the concentration of molecules with fewer than 30 carbon atoms in products on the market for food additive use.

In its re-evaluation, the Committee accepted that equivalent information can be obtained with ^{3}H and ^{14}C, provided that the label is located in a metabolically stable position, as was the case for [^{3}H]hydrogenated poly-1-decene. It also accepted that, for technical reasons, use of ^{14}C-labelled hydrogenated poly-1-decene might be less appropriate, as the synthetic ^{14}C-labelled compound might be different from the substance used in the studies of toxicity. The results of the study indicated that <1% of the dose of [^{3}H]hydrogenated poly-1-decene was absorbed from the gut. The absorbed radiolabel was present largely as $^{3}H_2O$, probably arising from tritium exchange between the labelled substance and body water. The Committee concluded that absorption of hydrogenated poly-1-decene was negligible. This conclusion was corroborated by the results of the 90-day study in rats, which provided no evidence of its accumulation in tissues. Furthermore, the revised specifications for the substance, which require that it contains a maximum of 1.5% of compounds with fewer than 30 carbon atoms should ensure that absorption of components of low relative molecular mass is kept to a minimum.

The Committee concluded that the available studies were adequate to assess the toxicity and safety of hydrogenated poly-1-decene. An ADI of 0–6 mg/kg of body weight was established on the basis of the NOEL of 550 mg/kg of body weight per day in the 90-day study in rats for effects on the condition of the fur, liver weight and histological appearance, and a safety factor of 100.

An additional study in rats submitted for consideration by the Committee suggested that hydrogenated poly-1-decene may decrease the bioavailability of linoleic acid, an essential fatty acid. However, the Committee concluded that a nutritionally relevant decrease in bioavailability would not occur if hydrogenated poly-1-decene was consumed at the level of the ADI, i.e. a maximum of 360 mg/person per day.

Hydrogenated poly-1-decene can be used as a release agent in bread prepared in commercial baking operations at concentrations of up to 300–500 mg/kg and in glazed fruit at concentrations of up to 2000 mg/kg. Bread is expected to be the major source of total intake of

this compound. If use only in bread is assumed, it can be calculated that up to 720 g of bread containing hydrogenated poly-1-decene at a concentration of 500 mg/kg could be consumed by a 60-kg person before the ADI of 0–6 mg/kg of body weight was exceeded. However, it was considered highly unlikely that a person would consume this amount of bread containing hydrogenated poly-1-decene at the maximum level of use each day.

An addendum to the toxicological monograph was prepared.

3.1.6 *Preservative*

3.1.6.1 Natamycin (pimaricin)

Natamycin (pimaricin) is a polyene macrolide antibiotic produced by submerged aerobic fermentation of *Streptomyces natalensis* and related species. The fermentation process takes several days, after which the antibiotic is isolated by extraction from broth or by extraction of the mycelium.

Natamycin is used as a food additive to control the growth of yeasts and moulds on the surface of cheese and other non-sterile products, such as meat and sausages.

Natamycin was evaluated by the Committee at its twelfth and twentieth meetings (Annex 1, references *17* and *41*). At its twentieth meeting, the Committee established an ADI of 0–0.3 mg/kg of body weight. The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (*4*).

The Committee considered information on the current uses of natamycin, data on its intake and biological data that had not been evaluated previously.

Uses. Because natamycin is active against yeasts and moulds, but not bacteria, it is used in foods that undergo a ripening period after processing. Its low solubility in water and most organic solvents makes it appropriate for the surface treatment of foods.

Natamycin is used topically in veterinary medicine to treat mycotic infections, such as ringworm in cattle and horses. Previously, it was used topically against fungal infections of the skin and mucous membranes in humans. Its medical use is now confined to topical treatment of fungal infections of the cornea and the prevention of such infections in contact lens wearers.

Assessment of intake. The Committee noted that as the draft Codex General Standard for Food Additives proposes restricted use of natamycin only in cheese and in dried, non-heat-treated meats, intake would not be expected to exceed the ADI.

Data submitted by Australia, Germany, New Zealand, the United Kingdom and the USA indicated that the intakes at mean and high percentiles of consumption were well below the ADI, although the estimates for the United Kingdom and the USA covered cheese consumption only. The estimated mean intakes by consumers ranged from 0.01 to 0.03 mg/kg of body weight per day (representing 3% and 9% of the ADI in Germany and the United Kingdom, respectively), and those by consumers at high percentiles were 0.03–0.08 mg/kg of body weight per day (representing 9% and 27% of the ADI in Australia and the United Kingdom, respectively), if it is assumed that natamycin was used at 40 mg/kg in all cheese products and 20 mg/kg in all cured meat products, as proposed in the draft Codex General Standard for Food Additives. The estimated intakes of natamycin were lower when national levels of use were assumed.

Toxicological studies. The Committee considered eight studies that had not been evaluated previously and had been conducted before the 1980s. A study of single intraperitoneal administration was considered to be irrelevant to the safety assessment of an ingested substance. The results of two studies of genotoxicity in three bacterial systems (*Bacillus subtilis*, *Salmonella typhimurium* and *Escherichia coli*) were negative.

Two studies in rats and one in dogs given radiolabelled material for investigation of the distribution and elimination of the compound supported the previous conclusion that natamycin is excreted primarily in the faeces, with minimal absorption. The only adverse effect reported in a short-term study of toxicity in dogs was diarrhoea, which occurred most frequently in animals given the highest dose (equivalent to 25 mg/kg of body weight per day); however, the usefulness of this study was limited, as only two dogs were tested.

In a study of developmental toxicity, an aqueous suspension of natamycin at 500 mg/l was given to groups of 20–26 rabbits at a dose of 0, 5, 15 or 50 mg/kg of body weight per day by gavage on days 6–18 of gestation. The maternal mortality rate was 0%, 5%, 9% and 19% at the four doses, respectively. No clinical signs of toxicity were observed in the does, and the cause of death was unknown. The mean maternal body weight, pregnancy rate, number of implantation sites, number of resorption sites, numbers of live and dead fetuses, proportion of viable fetuses and incidence of soft-tissue anomalies were comparable in the treated groups and a control group given the vehicle only. The fetal body weight in the group dosed at 15 mg/kg of body weight by gavage was lower than that of fetuses in the control group given the vehicle only. The incidence of extra sternebrae was increased at the two highest doses in comparison with the control

group, but not in a dose-related manner. However, in view of the unusual sensitivity of the gastrointestinal tract of rabbits to poorly absorbed substances and to compounds with antimicrobial activity, this study was considered unsuitable for deriving the ADI.

Microbiological studies. The antifungal activities of natamycin and other polyenes depend on their binding to cell membrane sterols, primarily ergosterol, the principal sterol in fungal membranes. Oomycetes fungi and bacteria are insensitive to these antibiotics because their membranes lack ergosterol.

Use of natamycin as an antifungal agent in food may result in exposure of the indigenous microflora to trace quantities of antimicrobial residues. The human intestinal microflora is a complex mixture of more than 400 bacterial species, consisting primarily of bacterial cells at a concentration of approximately 10^{11}–10^{12} colony-forming units per gram (CFU/g). Fungi are much less abundant than bacteria in the human gastrointestinal tract, the concentration of yeast in stool samples from healthy subjects being up to 10^5 CFU/g. As bacteria are not affected by polyenes, natamycin residues should not harm them; as yeasts are found in low quantities, the consequences of exposure to traces of natamycin would be minimal.

Several studies in experimental animals indicated a lack of antimicrobial activity in the colon, suggesting that natamycin was degraded into microbiologically inactive compounds by bacterial flora. However, no data were available on the degradation of natamycin by human intestinal microflora. In one study, natamycin was present in faecal specimens of volunteers who ingested 500 mg of the compound, indicating that it is incompletely absorbed or degraded.

As emergence of resistance to antimicrobials is a concern, the Committee evaluated the possible development of resistance among microflora as a consequence of exposure to natamycin. A preparation containing 50% natamycin has been used since the 1980s to preserve cheese and sausages. Surveys in cheese warehouses and in dry-sausage factories where the preparation has been used showed no change in the composition or the sensitivity of the contaminating fungal flora. All but one of the species of yeasts and moulds isolated in cheese warehouses where natamycin was used were inhibited by similarly low concentrations (0.5–8 µg/ml). In another study, 26 strains of fungi were isolated in eight warehouses where natamycin was used and two warehouses where it had never been used, and were tested for sensitivity to the compound; no insensitive yeasts or moulds were found. The results of laboratory experiments to induce resistance to natamycin in strains of fungi isolated from cheese

warehouses indicated that, after 25–30 transfers to media with increasing concentrations of natamycin, none of the strains had become less sensitive. When the sensitivity of yeasts and moulds isolated from dry-sausage factories where natamycin had been used for several years was compared with that of isolates from factories where natamycin had never been used, no significant differences were demonstrated.

It has been found difficult to induce resistance to polyenes, especially natamycin, in fungi in vitro. Resistant isolates invariably show reduced metabolic and growth rates and, in the absence of polyenes, readily revert to normal metabolism, growth and sensitivity to natamycin. One means of obtaining isolates resistant to natamycin is successive subculturing in vitro in the presence of gradually increasing concentrations of the polyene. Typically, such isolates are resistant only up to the highest concentration to which they have been exposed. After 25 passages, the concentration that inhibited *Candida albicans* was minimally increased, from 2.5–12 µg/ml to 12–50 µg/ml.

Evaluation. Natamycin is a polyene macrolide antibiotic that is effective against yeasts and moulds but not against bacteria or oomycetes fungi. The antifungal activities of natamycin depend on its binding to cell membrane sterols, primarily ergosterol, the principal sterol in fungal membranes, which is absent in bacteria. The use of natamycin as an antifungal agent in food may result in exposure of the indigenous flora to trace quantities of antimicrobial residues. As bacteria in the human gastrointestinal tract are not affected by polyenes, the Committee concluded that natamycin would not have an effect and that disruption of the barrier to colonization of the intestinal tract was therefore not a concern. Fungi are much less prevalent than bacteria in the human gastrointestinal tract, and, in light of the negative results of the studies of acquired resistance, selection of natamycin-resistant fungi was not considered an issue.

The Committee noted the finding of extra sternebrae in the study of developmental toxicity in rabbits, in which a dose-related increase in the mortality rate was also reported. It considered, however, that administration of an antimicrobial agent to rabbits by gavage was an inappropriate way of testing for developmental toxicity. In addition, extra sternebrae have been described as a skeletal variation rather than a frank sign of teratogenicity. Thus, the Committee did not consider the finding of extra sternebrae to be evidence that natamycin is teratogenic.

The Committee confirmed the previously established ADI of 0–0.3 mg/kg of body weight for natamycin, which was based on observations of gastrointestinal effects in humans. The Committee noted that the estimated intakes of natamycin, based on maximum levels of use

in cheese and processed meats proposed in the draft Codex General Standard for Food Additives, do not exceed this ADI.

A toxicological monograph was prepared and the existing specifications were revised. The title of the specifications was changed from pimaricin to natamycin, the commonly used designation. The specifications were made tentative, pending the receipt of information on the level and determination of water content, limit for lead, specific rotation, assay value and method of assay for the commercial product. This information was required for evaluation in 2003.

3.1.7 *Sweetening agent*

3.1.7.1 D-Tagatose

D-Tagatose is a keto-hexose, an epimer of D-fructose inverted at C-4, with a sweet taste. It is obtained from D-galactose by isomerization under alkaline conditions in the presence of calcium.

D-Tagatose was evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference *149*), when it concluded that the available data indicated that D-tagatose is not genotoxic, embryotoxic or teratogenic. It noted that the increased liver weights and hepatocellular hypertrophy seen in Sprague-Dawley rats occurred concurrently with increased glycogen deposition; however, the reversal of increased glycogen storage after removal of D-tagatose from the feed was more rapid than regression of the liver hypertrophy. Although the gastrointestinal symptoms seen in adult humans with the expected daily intake of D-tagatose were minor, the Committee was concerned about the increased serum uric acid concentrations observed in a number of studies in humans after administration of either single or repeated doses of D-tagatose. Similar increases were observed with other sugars, such as fructose, but D-tagatose appeared to be a more potent inducer of this effect. The Committee also noted that this effect of D-tagatose had not been studied in persons prone to high serum uric acid concentrations. The Committee concluded that an ADI could not be allocated for D-tagatose because of concern about its potential to induce glycogen deposition in the liver and liver hypertrophy and to increase the serum concentration of uric acid.

Two studies of up to 7 days' duration in Wistar and Sprague-Dawley rats given repeated doses of D-tagatose were submitted to the Committee at its fifty-fifth meeting, but the reports were received only in draft form and were not suitable for consideration at that time. The Committee therefore asked for the final reports and for further data to clarify the extent, mechanism and toxicological consequences of the increased serum uric acid concentrations observed in humans exposed to D-tagatose. At its present meeting, the Committee reviewed the

reports of the two studies in rats, the results of a study in volunteers (on the relevance of the glycogen deposition and liver hypertrophy) and some published studies on the increased uric acid concentrations in serum after intake of D-tagatose, other sugars and other food components.

Biological data. Review of the results of the studies considered by the Committee at its fifty-fifth meeting and comparisons with the data reviewed at the present meeting revealed a difference in sensitivity between Wistar and Sprague-Dawley rats. Sprague-Dawley rats given D-tagatose at a concentration of 50 g/kg of diet for 28 days showed increased hepatic glycogen only when they had not been fasted the night before necropsy, and this effect was not associated with any microscopic changes in the liver. In a 90-day study in which Sprague-Dawley rats were killed after fasting overnight, administration of D-tagatose at a concentration of 50 g/kg of diet had no adverse effect on the liver. In a 6-month study in Wistar rats in which the animals were killed after fasting 3, 7, 14 and 28 days and 3 and 5 months after treatment, administration of D-tagatose at concentrations of up to 100 g/kg of diet had no adverse effects. Wistar rats are therefore less susceptible to the hepatic effects of D-tagatose than Sprague-Dawley rats. As D-tagatose stimulated glycogen deposition to a similar degree in the two rat strains in short-term studies, the difference is likely to occur at a later stage, during glycogen-induced or other stimulation of liver growth.

The authors suggested that the increase in normal liver mass seen in fasted rats fed diets containing 100 or 200 g/kg D-tagatose is triggered by increased postprandial storage of liver glycogen resulting from simultaneous feeding of D-tagatose and glucose equivalents. In order to test this hypothesis, the effects of separate and simultaneous administration of D-tagatose and glycogen precursors on liver weight and glycogen level were investigated in Wistar and Sprague-Dawley rats. The results neither supported nor invalidated the hypothesis.

As several studies have been performed in healthy volunteers and in patients with diabetes, the number of persons varying from 4 to 73, the Committee based its toxicological evaluation on the data from these studies. The length of these studies varied from several days to several weeks; one study of 12 months' duration included only a limited number of patients with type 2 diabetes. The toxicological aspects investigated included gastrointestinal effects, increased serum uric acid concentrations and hepatic effects.

Mild gastrointestinal symptoms were reported in only one study, in 3 of 10 patients with type 2 diabetes receiving D-tagatose at 10 g/day for several days, whereas in other studies diarrhoea was observed only in patients receiving 25 g three times daily for 8 weeks. In healthy individuals, administration of a single dose of 30 g induced diarrhoea in

some persons only, whereas other studies showed no laxative effect of single doses of D-tagatose as high as 75 g.

The serum or plasma concentration of uric acid was increased transiently in some studies, but the increased uric acid concentration was above the normal range for a number of days in only one study of persons receiving 75 g/day. The other studies showed either no increase or a transient increase in serum uric acid concentrations within the normal range.

In a 28-day study in which 15 g of D-tagatose or 15 g of sucrose were given three times daily to volunteers, magnetic resonance imaging was used to determine liver volume, and glycogen concentrations and several clinical chemical parameters were measured. The results did not reveal any relevant effect on the liver. In addition, no diarrhoea and no increase in serum uric acid concentration were observed. Therefore, the NOEL was 45 g/person per day, equivalent to 0.75 g/kg of body weight per day (for a person weighing 60 kg).

Evaluation. The Committee considered the 28-day study in which humans received a daily dose of 45 g of D-tagatose or sucrose in three divided doses as most representative of human dietary intake and therefore most relevant for assessing the acceptable intake of D-tagatose accurately. While effects were observed after administration of a single dose of 75 g, no effects were seen following administration of three daily doses of 15 g of D-tagatose, equivalent to 0.75 g/kg of body weight per day. The Committee established an ADI of 0–80 mg/kg of body weight on the basis of this NOEL and a safety factor of 10.

Assessment of intake. D-Tagatose is proposed for use as a bulk sweetener in low-energy foods, such as edible ices (at a concentration of 3 g/kg), chewing-gum and confectionery (at 15 g/kg), breakfast cereals (at 15 g/kg) and soft drinks (at 1 g/kg). At its present meeting, the Committee considered that the predicted intakes of D-tagatose determined at the fifty-fifth meeting, which were based on the manufacturers' proposed levels of use and individual dietary records in several countries, were conservative. This was because use had been assumed in the entire food category rather than only in the low-energy food component. The mean consumer intakes of D-tagatose from all proposed uses (except chewing-gum, dietary supplements and meal replacements) predicted for Australia, the Member States of the European Union and the USA ranged from 3 to 9 g/day (63–190% of the ADI), and the predicted intakes by persons at high percentiles of consumption were up to 18 g/day (375% of the ADI). On the basis of the information on possible uses, the Committee concluded that the ADI for D-tagatose may be exceeded by some groups of the population.

A toxicological monograph was prepared. The specifications prepared by the Committee at its fifty-fifth meeting were maintained.

3.1.8 *Thickening agents*

3.1.8.1 Carrageenan and processed Eucheuma seaweed

Carrageenan, a substance with hydrocolloid properties owing to the presence of sulfated polyglycans with average relative molecular masses well above 100000, is derived from a number of seaweeds of the family Rhodophyceae. It has no nutritional value and is used in food preparation for its gelling, thickening and emulsifying properties. Three main types of carrageenan, known as ι-, κ- and λ-carrageenan, are used commercially in the food industry. These names do not reflect definitive chemical structures but only general differences in the composition and degree of sulfation at specific locations in the polymer. Processed *Eucheuma* seaweed is derived from either *E. cottonii* (κ-carrageenan) or *E. spinosum* (λ-carrageenan), which are also Rhodophyceae.

Carrageenan is obtained by extraction of the seaweed into water or aqueous dilute alkali and may be recovered by precipitation with alcohol, by drying in a rotary drum or by precipitation with aqueous potassium chloride and subsequent freezing. In contrast, processed *Eucheuma* seaweed is prepared by soaking the cleaned seaweed in alkaline solution for a short time at elevated temperatures. The treated material is then thoroughly washed with water to remove residual salts and further washed with alcohol, dried and milled to a powder. For both carrageenan and processed *Eucheuma* seaweed, the alcohols that may be used during purification are restricted to methanol, ethanol and isopropanol. The articles of commerce may contain sugars added for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from the drum-drying process.

Carrageenan was reviewed by the Committee at its thirteenth, seventeenth, twenty-eighth and fifty-first meetings (Annex 1, references *19*, *32*, *66* and *137*). At its twenty-eighth meeting, the Committee established an ADI "not specified"[1] on the basis of the results of a number

[1] ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in the individual evaluation, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

of toxicological studies on carrageenans obtained from various sources.

Processed *Eucheuma* seaweed was considered by the Committee at its thirtieth, thirty-ninth, forty-first, forty-fourth and fifty-first meetings (Annex 1, references *73*, *101*, *107*, *116* and *137*). At its forty-fourth meeting, the Committee concluded that, because of the chemical relationship between processed *Eucheuma* seaweed and traditionally refined carrageenan, the toxicological data on carrageenan were relevant to the safety assessment of the carrageenan polysaccharide constituents of processed *Eucheuma* seaweed, but could not replace adequate toxicological studies on processed *Eucheuma* seaweed itself. At its fifty-first meeting, the Committee reviewed the results of a 90-day study on toxicity in rats fed processed *Eucheuma* seaweed from *E. cottonii* and *E. spinosum*. The Committee concluded that the toxicity of this material was sufficiently similar to that of carrageenan to allow extension of the previous ADI "not specified" for carrageenan to a group ADI that covered processed *Eucheuma* seaweed. The Committee also considered all studies on carrageenan that had been published since its twenty-eighth meeting and, for the earlier studies, noted the identity of the source material and the type of carrageenan, when these could be identified. It expressed concern about the potential promotion of colon carcinogenesis by carrageenans and processed *Eucheuma* seaweed and therefore made the group ADI "not specified" temporary, pending clarification of the significance of the promotion of colon cancer observed in studies in rats. At its present meeting, the Committee reviewed the available evidence for the tumour-promoting and related effects of these compounds in rat colon.

Assessment of intake. Carrageenan and processed *Eucheuma* seaweed are used as thickeners, gelling agents, stabilizers or emulsifiers in a wide range of foods at concentrations of up to 1500 mg/kg. Per capita intakes in 1995 derived from "poundage" (disappearance) data in Europe and the USA ranged from 28 to 51 mg/day. These estimates corresponded to those reported for 1993 by the Seaweed Industry Association of the Philippines on the basis of sales of 44 mg/person per day for the populations of Canada and the USA and 33 mg/person per day for European populations.

The estimates derived from poundage data were also consistent with those derived for the population of the USA from model diets, with reported mean intakes of carrageenan of 20 mg/day for all consumers and 40 mg/day for persons at the 90th percentile of consumption (derived by multiplying the mean by a factor of 2). The intakes were derived from data on the food consumption of individuals aged

2 years and over that were available in 1976 from nutrition surveys in the USA, combined with the results of a 2-week study by the Marketing Research Corporation of America on the frequency of food consumption.

Biological data. Two studies showed that carrageenan administered before, during and after administration of known carcinogens (dimethylhydrazine, azoxymethane, *N*-methyl-*N*-nitrosourea) enhanced the tumorigenicity of these carcinogens. One of the studies involved administration of carrageenan at 150 g/kg of diet, which resulted in decreased body-weight gain. In the second study, involving administration of carrageenan at 60 g/kg of diet, the body-weight gain of treated animals was comparable to that of controls. The increased incidence of tumours seen under these circumstances may have resulted from promotion but may also have resulted from altered toxicokinetics or biotransformation of the carcinogen. In addition, there were indications that the bacterial flora had been altered as a result of administration of carrageenan. In a separate study conducted according to a classical tumour initiation–promotion protocol, in which rats were given dimethylhydrazine, subsequent administration of carrageenan at dietary concentrations of up to 50 g/kg did not result in a statistically significant increase in the incidence of colon tumours over that seen with dimethylhydrazine alone.

Two further studies in rats involved use of a conventional tumour initiation–promotion protocol but in which formation of aberrant crypt foci was the end-point, instead of tumour formation. Rats were given azoxymethane with or without subsequent administration of carrageenan in their drinking-water. The higher concentration of carrageenan, 25 g/kg, was given in the form of a solid gel, which may have altered the food and water consumption patterns of the animals. The first study demonstrated that dietary administration of carrageenan after the carcinogen decreased the number of aberrant crypt foci seen relative to the number observed with the carcinogen alone, but significantly increased their size. A subsequent study in rats injected with human faecal microflora showed no effect of carrageenan on either the number or size of aberrant crypt foci. As the relationship between aberrant crypt foci and tumorigenesis is still unclear, it is difficult to interpret the biological significance of these results.

Increased cell proliferation has frequently been postulated as a mechanism of non-genotoxic carcinogenicity or tumour promotion. The preferred methods of assessing cell proliferation are based on histological techniques, which allow identification of the nature and location of proliferating cells. There was no consistent pattern of colon damage in rats treated with carrageenan for prolonged periods.

Some studies showed caecal enlargement, but most did not show histological damage. In one study in which rats underwent autoradiographic examination, no significant difference from controls in the number of cells per crypt or in the proportion of labelled cells was seen in rats fed a diet containing carrageenan at 74 g/kg for 28 days.

Methods for measuring cell proliferation that are based on measurement of cell cycle-dependent enzyme activities, such as thymidine kinase activity, are cruder means of measuring overall cell proliferation in an entire tissue specimen. A significant increase in thymidine kinase activity, expressed relative to protein content, was found in homogenized mucosal scrapings from the colon of rats fed diets containing carrageenan at 26 or 50 g/kg for 4 weeks; no significant effects were observed in the animals fed 0, 6.5 or 13 g/kg carrageenan in the diet for 4 weeks. Histological examination revealed no evidence of infiltration by inflammatory cells in any of the treated groups. In another study, the increased thymidine kinase activity observed in rats fed diets containing carrageenan at 50 g/kg returned to the basal level within 28 days when the animals were returned to a diet with no carrageenan. No increase in thymidine kinase activity was seen in animals receiving diets containing 2 or 15 g/kg carrageenan for 28 days. Staining for proliferating cell nuclear antigen (PCNA) revealed a significant increase in PCNA-positive cells in the upper third of the crypts of rats receiving a diet containing carrageenan at 50 g/kg for 91 days, but not after 28 or 64 days followed by a 28-day recovery period on a normal diet. No PCNA-positive cells were observed at the luminal surface. The pattern of staining for PCNA seen with carrageenan was considered indicative of an adaptive response, which would not contribute to an increased risk for colonic neoplasia.

In one study, carrageenan inhibited gap-junctional intercellular communication in vitro. However, the mechanism of action was different from that of a known tumour-promoting agent, phorbol ester, and the relevance of this observation is unclear for a substance that is not absorbed in vivo.

Evaluation. In a recent study with a classical tumour initiation–promotion protocol, administration of carrageenan at concentrations of up to 50 g/kg of diet did not promote colon carcinogenesis in rats given dimethylhydrazine. The Committee noted, however, that, in two studies that showed enhancement of colon carcinogenesis in rats, higher dietary concentrations of carrageenan were used and carrageenan was administered before, during and after the carcinogens. The enhanced carcinogenicity seen under these circumstances may have resulted from promotion or from altered toxicokinetics or biotransformation of the carcinogen. Therefore, the mechanism of the

enhancement of colon carcinogenesis in these studies remains unresolved. Continuous feeding of high doses of carrageenan caused a generalized proliferative response, measured as increased thymidine kinase activity, in the mucosal tissue of the colon of male rats. This effect might play a role in the observed enhancement of the tumorigenicity of known colon carcinogens by high dietary concentrations of carrageenan. However, a proliferative effect of carrageenan on the mucosa of the colon was seen only at a dietary concentration of 26 g/kg or more. No effect was seen at a concentration of 15 g/kg in the diet, corresponding to 750 mg/kg of body weight per day, which greatly exceeded the estimated human intake of carrageenan and processed *Eucheuma* seaweed of 30–50 g/person per day from their use as food additives. Bearing in mind that the enhancement of colon carcinogenesis in rats was seen at much higher concentrations and that carrageenan at 50 g/kg of diet did not promote tumours in rat colon in a classical initiation–promotion study, the Committee considered that the intake of carrageenan and processed *Eucheuma* seaweed from their use as food additives was of no concern. It therefore allocated a group ADI "not specified"[1] to the sum of carrageenan and processed *Eucheuma* seaweed.

An addendum to the toxicological monograph was prepared. The existing specifications for both carrageenan and processed *Eucheuma* seaweed were revised by incorporating more complete descriptions of the analytical procedures for the determination of lead, cadmium and mercury and by raising the acceptable limit for lead from 2 mg/kg to 5 mg/kg and the acceptable limit for cadmium from 1 mg/kg to 2 mg/kg. These limits were raised to take into account new information on inadequacies of the analytical methods for determination of these elements, which are due to the high salt content of the polysaccharides of both processed *Eucheuma* seaweed and carrageenan. The changes were not made because of information about higher concentrations of lead and cadmium than those previously considered by the Committee. The Committee also observed that the new limits are consistent with the limits established for these heavy metals in specifications for other hydrocolloids, such as alginic acid.

3.1.8.2 Curdlan

Curdlan (synonym, β-1,3-glucan) is a linear polymer of high relative molecular mass, consisting of β-1,3-linked glucose units. Curdlan is produced by fermentation of pure cultures from a non-pathogenic, non-toxinogenic strain of *Agrobacterium* Biovar1 (identified as *Alcaligens faecalis* var. *myxogenes* at the time of its isolation) or

[1] See footnote on page 32.

Alcaligens radiobacter. Curdlan is recovered from the fermentation medium by addition of acid and alkali to disrupt the cells, which releases the curdlan into the medium, followed by separation by centrifugation. It is then washed with copious amounts of water to eliminate mineral salts and other water-soluble substances that may have been carried over from the fermentation broth. The commercial product is an odourless or nearly odourless, tasteless, white to nearly-white spray-dried powder.

The use of curdlan in a wide variety of foods is based on its ability to form an elastic gel upon heating in an aqueous suspension. Thus, it can be used in processed meat, fish and poultry products and in gelatins, puddings and fillings as a firming or gelling agent or as a stabilizer or thickener.

The Committee reviewed curdlan at its fifty-third meeting (Annex 1, reference *143*), when it allocated a temporary ADI "not specified",[1] pending information on the use of curdlan, including the maximum and typical expected levels in the food categories in which it is proposed for use in the draft Codex General Standard for Food Additives, and on the consumption in various regions of the world of foods that might contain curdlan.

Use of curdlan is based on its physical properties, which imply a self-limiting level of use in solid foods. A submission from the USA described a model constructed to predict the intakes of curdlan by a long-term consumer on the basis of a study of the frequency of consumption of foods in 1982–1988 from the Market Research Corporation of America, and average portion sizes from a 3-day national food consumption survey conducted in 1987–1988 by the United States Department of Agriculture. Intake was assessed on the basis of the self-limiting levels of use (20 mg/kg of processed meat, 15 mg/kg of processed poultry and fish, 10 mg/kg of dairy products, 35 mg/kg of egg products, 15 mg/kg of grain products and pasta, 30 mg/kg of cereals and starch desserts, 20 mg/kg of gravies and sauces and 40 mg/kg of gelatins). The resulting mean intake by consumers was estimated to be 3.6 g/person per day, corresponding to 60 mg/kg of body weight per day.

The sponsor submitted an estimate based on daily food intake per capita and typical levels of use in Japan (15 mg/kg of processed meat, 10 mg/kg of processed poultry and fish, 5 mg/kg of dairy products, 30 mg/kg of egg products, 10 mg/kg of grain products and pasta, 10 mg/kg of cereals and starch desserts, 10 mg/kg of gravies and sauces and 30 mg/kg of gelatins). The mean intakes were estimated to be

[1] See footnote on page 32.

0.77 g/person per day, corresponding to 13 mg/kg of body weight per day, for typical levels of use and 1.7 g/person per day, corresponding to 28 mg/kg of body weight per day, for maximum levels of use.

Estimates of the intake of curdlan based on individual dietary records were submitted by the USA on the basis of a survey by the Department of Agriculture and the Continuing Survey of Food Intakes by Individuals (1989–1992). When intake was estimated on the basis of the upper limit of the range of recommended use, the intake of curdlan by consumers was 20 mg/kg of body weight per day for consumption at the mean and 47 mg/kg of body weight per day for consumption at the 90th percentile. When intake was estimated on the basis of self-limiting levels of use, the intake of consumers was 30 mg/kg of body weight per day for consumption at the mean and 68 mg/kg of body weight per day for consumption at the 90th percentile.

The data on uses and intake requested by the Committee at its fifty-third meeting were provided and raised no safety concern. The Committee therefore established an ADI "not specified"[1] for use of curdlan as a food additive.

The existing specifications were revised, with minor changes.

3.1.9 *Miscellaneous substances*

3.1.9.1 *Acetylated oxidized starch*

Acetylated oxidized starch is a chemically modified root or grain starch. It is produced by oxidation of a slurry of starch granules in alkaline hypochlorite at low temperatures (21–38 °C). The alkaline medium is neutralized with sodium bisulfite, and the resulting organic salts are removed by washing with water. The oxidized starch is then esterified with acetic anhydride under mildly alkaline conditions. The product is neutralized with hydrochloric acid, washed and dried.

Acetylated oxidized starch had not been evaluated previously by the Committee. At the present meeting, it was proposed for use as a binding agent in soft confectionery at a concentration of about 300 mg/kg — it is mixed with water, sugars and flavours in a batch process until a clear solution with a dry-solid content of 70% is obtained. The characteristics of the end-product important for confectionery use are gel strength and clarity. Acid hydrolysis results in starch products that are relatively unclear, and oxidized starch products result in overly soft confectionery. Acetylation of oxidized starch enhances the desired properties, resulting in a gummy, clear jelly. It

[1] See footnote on page 32.

can be used as a substitute for gelatin or gum arabic and would replace a large amount of sugar.

Acetylated oxidized starch has a stable configuration under normal conditions in food. It is hydrolysed slowly in the presence of strong acids, yielding glucose, gluconic acid and acetic acid. No degradation products are expected or known to result from storage or use of this substance in the preparation of foods at neutral pH. The substance is not known to sequester minerals, nor does it interact with proteins or vitamins. It has no known effect on other nutrients.

In a 14-day range-finding study in rats, administration of a diet containing acetylated oxidized starch at a concentration of 300 or 500 mg/kg increased the weights of full and empty caeca, and dilated caeca were found at autopsy. At the higher concentration, soft faeces also occurred. The NOEL was 100 mg/kg of diet.

In a 90-day study in rats given a diet containing acetylated oxidized starch, increased full and empty caecal weights were seen at the highest concentration of 300 mg/kg of diet. Macroscopic examination showed a dilated caecum in one male rat. Histological examination did not reveal changes in the caecal wall or other parts of the digestive tract. Increased caecal weights are a known response to high dietary concentrations of poorly digested carbohydrates in rats, due perhaps to an increased osmotic load of short-chain fatty acids produced by microbial degradation and the associated water retention. Focal hyperplasia of the urinary bladder epithelium was seen in 4 out of 10 male rats that received the highest dietary concentration but not in males given lower concentrations, in controls or in females. The change was probably treatment-related and a consequence of irritation of the urinary bladder by calculi. The NOEL was 100 mg/kg of diet, equivalent to 5900 mg/kg of body weight per day.

If acetylated oxidized starch was to be used only in jelly confectionery at a concentration of 300 g/kg and if the maximum consumption by consumers was 200 g of jelly confectionery per day, the maximum intake of acetylated oxidized starch would be 60 g/day.

The effects seen in the 14-day and 90-day studies in rats were similar to those observed with high dietary concentrations of other slowly digested carbohydrates and are commonly seen in rats given other modified starches in the diet. Because of the nature of acetylated oxidized starch and its similarity to other modified starches with non-systemic effects, the Committee established an ADI "not specified",[1] on the basis of the known uses of acetylated oxidized starch as an ingredient in confectionery products.

[1] See footnote on page 32.

A toxicological monograph was prepared. New specifications for acetylated oxidized starch were prepared and incorporated into the specifications for modified starches.

3.1.9.2 α-Cyclodextrin

α-Cyclodextrin is a non-reducing cyclic saccharide composed of six glucose units linked by α-1,4 bonds. It is produced by the action of cyclodextrin glucosyltransferase (CGTase, EC 2.4.1.19) on hydrolysed starch syrups at neutral pH (6.0–7.0) and moderate temperatures (35–40 °C). The annular structure of α-cyclodextrin provides a hydrophobic cavity that allows formation of inclusion complexes with a variety of non-polar organic molecules of appropriate size. The hydrophilic nature of the outer surface of the cyclic structure makes α-cyclodextrin water-soluble.

The principal method for the isolation and purification of α-cyclodextrin takes advantage of its complex-forming ability. At the end of the reaction, 1-decanol is added to the reaction mixture to form an insoluble 1:1 inclusion complex of α-cyclodextrin:1-decanol. The complex is continuously mixed with water and separated from the reaction mixture by centrifugation. The recovered complex is resuspended in water and dissolved by heating. Subsequent cooling leads to precipitation of the complex. The precipitate is recovered by centrifugation, and 1-decanol is removed by steam distillation. Upon cooling, α-cyclodextrin crystallizes from the solution. The crystals are removed by filtration and dried, yielding a white crystalline powder with a water content of less than 11%. The purity on a dried basis is at least 98%.

The hydrophobic cavity and the hydrophilic outer surface of α-cyclodextrin form the basis for its use in the food industry. α-Cyclodextrin, like its homologues β- and γ-cyclodextrin, can function as a carrier and stabilizer for flavours, colours and sweeteners; as an absorbent for suppression of undesirable flavours and odours in foods; as an absorbent for suppression of halitosis (in breath-freshening preparations); and as a water-solubilizer for fatty acids and vitamins.

α-Cyclodextrin had not been evaluated previously by the Committee, but the structurally related compound β-cyclodextrin was evaluated at the forty-first and forty-fourth meetings (Annex 1, references *107* and *116*), and γ-cyclodextrin was evaluated at the fifty-first and fifty-third meetings (Annex 1, references *137* and *143*). At its present meeting, the Committee noted the close structural similarity between α- and β-cyclodextrin (seven glucose units) and γ-cyclodextrin (eight glucose units), which permitted comparisons of the metabolism and toxicity of these compounds.

Biological data. α-Cyclodextrin, like β-cyclodextrin, is not digested in the gastrointestinal tract but is fermented by the intestinal microflora. In germ-free rats, α-cyclodextrin is almost completely excreted in the faeces, whereas γ-cyclodextrin is readily digested to glucose by the luminal and/or epithelial enzymes of the gastrointestinal tract. At low concentrations in the diet (about 20 g/kg), α-cyclodextrin is absorbed intact from the small intestine and is then excreted rapidly in the urine. The majority of the absorption takes place after metabolism of the substance by the microflora in the caecum. Although no studies of metabolism in humans in vivo were available, in vitro studies indicated that α- and β-cyclodextrin, unlike γ-cyclodextrin, cannot be hydrolysed by human salivary and pancreatic amylases.

The acute toxicity of α-cyclodextrin was studied in mice and rats that received the substance by intraperitoneal or intravenous injection. It caused osmotic nephrosis, probably because it was not degraded by lysosomal amylases. At high doses, this led to renal failure.

The results of short-term (28-day and 90-day) studies of the toxicity of α-cyclodextrin indicated that it had little effect when given orally to rats or dogs. After administration of a very high dietary concentration (200 g/kg), caecal enlargement and associated changes were seen in both species. This effect was probably the consequence of the presence of a high concentration of an osmotically active substance in the large intestine. No studies of intravenous administration were available to permit a comparison of the systemic toxicity of this compound with that of β- and γ-cyclodextrin.

Studies conducted in mice, rats and rabbits given α-cyclodextrin in the diet at concentrations of up to 200 g/kg did not indicate any teratogenic effects. Similarly, the results of assays for genotoxicity were negative. No long-term studies of toxicity, carcinogenicity or reproductive toxicity have been conducted with α-cyclodextrin, but the Committee concluded that, given the known fate of this compound in the gut, such studies were not required for an evaluation.

In vitro, α-cyclodextrin, like β-cyclodextrin, sequestered components of the membranes of erythrocytes, causing haemolysis. The threshold concentration for this effect was, however, higher than that observed with β-cyclodextrin.

While the potential interaction of α-cyclodextrin with lipophilic vitamins, which might impair their bioavailability, has not been studied directly, such an effect was considered unlikely, by analogy with the results of studies with β-cyclodextrin. Complexes between fat-soluble vitamins and β-cyclodextrin have been shown to have greater bioavailability than uncomplexed forms.

The enzyme cyclodextrin-glycosyltransferase, which is used in the production of α-cyclodextrin, is derived from a non-genotoxic, non-toxinogenic source and is completely removed from α-cyclodextrin during purification.

Assessment of intake. The predicted mean intake of α-cyclodextrin by consumers, based on individual dietary records for 1994–1998 in the USA and the proposed maximum levels of use in a variety of foods, would be 1.7 g/day (28 mg/kg of body weight per day) for the whole population and 1.6 g/day (87 mg/kg of body weight per day) for children aged 2–6 years. The main contributors to the total intake of α-cyclodextrin are likely to be soya milk and sweets. For persons at the 90th percentile of consumption, the predicted intake of α-cyclodextrin would be 3 g/day (50 mg/kg of body weight per day) for the whole population and 2.6 g/day (140 mg/kg of body weight per day) for children aged 2–6 years.

Evaluation. No studies of human tolerance to α-cyclodextrin were submitted to the Committee, despite the potentially high dietary intake. Nevertheless, the Committee was reassured by the relatively low toxicity of this compound in animals and the fact that it was less toxic than β-cyclodextrin, for which studies of human tolerance were available. Furthermore, the fact that it is fermented in the gastrointestinal tract in an analogous manner to β-cyclodextrin supported the conclusion that, as in laboratory animals, it would be fermented to innocuous metabolites before its absorption in the human gastrointestinal tract.

The Committee concluded that, on the basis of the available studies on α-cyclodextrin and studies on the related compounds β-cyclodextrin and γ-cyclodextrin, for which ADIs have been allocated, there was sufficient information to allocate an ADI "not specified".[1] This ADI was based on the known current uses of α-cyclodextrin within good manufacturing practice as a carrier and stabilizer for flavours, colours and sweeteners; as a water-solubilizer for fatty acids and certain vitamins; as a flavour modifier in soya milk; and as an absorbent in confectionery.

A toxicological monograph and new specifications for α-cyclodextrin were prepared.

3.1.9.3 Sodium sulfate

Sodium sulfate was evaluated by the Committee at its fifty-third meeting (Annex 1, reference *143*), when a temporary ADI "not specified"[1] was established. The ADI was made temporary because information was required on the functional effect and actual uses of

[1] See footnote on page 32.

sodium sulfate in food. This information was provided to the Committee at its fifty-fifth meeting (Annex 1, reference *149*), and the "tentative" designation was removed from the specifications. At that time, the temporary ADI was not reconsidered.

Sodium sulfate is used as a colour adjuvant. Worldwide consumption from its use in food is approximately 100 tonnes per year.

At its present meeting, the Committee noted that the results of the few published studies conducted in experimental animals do not raise concern about the toxicity of sodium sulfate. Little is absorbed from the gut, and it is therefore used clinically as a laxative. The small amount absorbed remains in the extracellular fluid space and is rapidly excreted via the kidneys. Minor adverse effects have been reported in a small number of clinical trials and in case reports. All of the effects were seen with preparations containing sodium sulfate and may have resulted from other components of the preparations.

In the absence of evidence of toxicity and given the current uses of this substance, the Committee allocated an ADI "not specified"[1] for sodium sulfate.

A toxicological monograph was not prepared. The specifications prepared by the Committee at its fifty-fifth meeting were maintained.

3.2 Revision of specifications

3.2.1 *Acesulfame K*

Acesulfame K is prepared in a three-step process in which sulfamic acid and diketene are reacted to produce an adduct, which undergoes cyclization to the acid form of acesulfame. This product is neutralized with potassium hydroxide to form the potassium salt.

The specifications for acesulfame K were revised. In addition to editorial revisions, a new criterion for purity with regard to the pH value of the aqueous solution was introduced, and the limit for lead was lowered from 10 mg/kg to 1 mg/kg.

3.2.2 *Blackcurrant extract*

Blackcurrant extract is obtained from blackcurrant pomace by aqueous extraction. The main colouring principles are four anthocyanins (cyanidin 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-glucoside and delphinidin 3-glucoside). Most of the extracted sugars are fermented to alcohol, and virtually all the alcohol is removed during concentration of the extract by vacuum evaporation. Sulfur dioxide is used during the extraction process, and residual sulfur dioxide may

[1] See footnote on page 32.

be present in the final product. The commercial products are concentrated liquids, pastes or spray-dried powders. Spray-dried powder may contain an added carrier such as maltodextrin or glucose syrup.

At its present meeting, the Committee revised the specifications to include a chromatographic identification test which distinguishes blackcurrant extract from other anthocyanin colours and removed the "tentative" designation.

3.2.3 *L-Malic acid*

The Committee received no information about the uses of L-malic acid, other than its well-established use as a flavouring agent. As DL- and L-malic acid are different compounds made by different manufacturing processes, the specifications for DL-malic acid were corrected by removing the reference to the specifications for L-malic acid.

3.2.4 *Oxystearin*

The specifications for oxystearin were considered by the Committee at its fifty-fifth meeting (Annex 1, reference *149*). At that meeting, the Committee maintained the "tentative" designation, with the stipulation that the specifications would be withdrawn if information on the levels of, and a suitable analytical method for, epoxides was not provided by 1 May 2001. The Committee noted that oxystearin was no longer in commercial use as a food additive.

At its present meeting, the Committee withdrew the specifications, as the requested information had not been received. The Committee also withdrew the ADI of 0–25 mg/kg of body weight for oxystearin established at its seventeenth meeting (Annex 1, reference *32*), as it considered that there could not be an ADI for a substance for which there were no specifications.

3.2.5 *Pectins*

Pectins consist mainly of the partially methylated esters of polygalacturonic acid and its ammonium, sodium, potassium and calcium salts. Amidated pectins also contain amides of polygalacturonic acid. Pectins are obtained by extraction in an aqueous medium of an appropriate edible plant material, usually citrus fruits or apples. Amidated pectins are obtained by treating the extract with ammonia under alkaline conditions.

The specifications for pectins were revised. The four separate tests for the identification of pectins contained in the specifications prepared at the thirty-ninth meeting (Annex 1, reference *101*) were replaced by a new test involving enzymatic degradation, which is specific for pectins, as the Committee had been informed that the previous tests were

not adequate for all commercial samples of pectins. The Committee was also informed that new separation techniques were used which could result in contamination of pectins with insoluble organic compounds. Therefore, a new criterion for purity, the percentage of "total insolubles", was introduced. In addition, the limits for copper, zinc and arsenic were deleted, and the limit for lead was lowered from 10 mg/kg to 5 mg/kg.

3.2.6 *Smoke flavourings*

Smoke flavourings are complex mixtures of components of smoke obtained by subjecting untreated hardwoods to pyrolysis in a limited, controlled amount of air, dry distillation at 200–800 °C or exposure to superheated steam at 300–500 °C. The major flavouring principles are carboxylic acids, compounds with carbonyl groups and phenolic compounds.

During manufacture of smoke flavourings, hazardous constituents such as polycyclic aromatic hydrocarbons are removed by subjecting wood smoke to aqueous extraction or to distillation, condensation and separation for collection of the aqueous phase. The aqueous smoke fraction, containing water-soluble constituents, can be diluted with water or extracted with an edible vegetable oil to produce a smoke flavouring with a higher concentration of non-polar constituents, which may be further extracted with food-grade substances, such as propylene glycol or aqueous solutions of polysorbates.

The commercial products may also contain additives such as emulsifiers, antifoaming agents and gums. Smoke flavourings may also be prepared in dry form by the addition of carriers such as yeasts, flours, salt, phosphates, carbohydrates and anticaking agents.

The specifications for smoke flavourings were considered by the Committee at its fifty-fifth meeting (Annex 1, reference *149*) and were maintained as "tentative", pending the receipt of information on an alternative solvent to benzene for use in the analysis of the carbonyl content. At its present meeting, the Committee revised the existing tentative specifications and removed the "tentative" designation. The revised specifications apply only to water-soluble distillates of condensed wood smoke, to their aqueous, vegetable oil or polysorbate extracts and to concentrates of these products. They do not apply to products derived from the water-insoluble tars, to certain commercial products or to pyroligneous acid, a by-product of the manufacture of charcoal by carbonation of wood in the absence of air.

3.2.7 *Tagetes extract*

Tagetes extract is obtained by hexane extraction of dried petals of *Tagetes erecta* L., with subsequent removal of the solvent. The major

colouring principles are the xanthophyll lutein and its dipalmitate (helenien). Other hydroxy derivatives of carotenes may be present, together with other oxy derivatives, such as epoxides. The product may contain fats, oils and waxes that occur naturally in the plant material. The articles of commerce are usually further formulated, e.g. in order to standardize the colour content or to obtain water-soluble or dispersible products.

The specifications for tagetes extract were considered by the Committee at its fifty-fifth meeting (Annex 1, reference *149*) and were designated as "tentative", pending the receipt of information on the composition of the commercial products, a test for the identification of xanthophylls and a method of assay. As the Committee had received the requested information, it revised the existing tentative specifications and removed the "tentative" designation.

3.3 Revision of limits for metals in food additives

At its fifty-fifth meeting (Annex 1, reference *149*), the Committee began to implement a systematic 5-year programme to replace the outdated test for heavy metals (as lead) in all existing food additive specifications with appropriate limits for individual metals of concern. Limits for lead and arsenic in 43 emulsifiers were proposed. As no alternative proposals were received by the Secretariat before the deadline for submission of data for the present meeting, the new published limits (Annex 1, reference *151*) were adopted, replacing those published in the *Compendium of food additive specifications* and its addenda (Annex 1, references *103*, *109*, *118*, *124*, *133*, *139, 145* and *151*).

The second group of substances, considered at the present meeting, comprised 10 anticaking agents, 17 flavour enhancers, 10 sweetening agents and 13 thickening agents. In response to the call for data, proposed limits and data to support the proposals were received for sodium ferrocyanide. Comments and proposals only were received for calcium silicate, magnesium silicates (synthetic), silicon dioxide (amorphous), sodium aluminosilicate, monosodium L-glutamate, sorbitol, lactitol, xylitol, ammonium alginate, tara gum, methyl cellulose, ethyl cellulose, methylethyl cellulose, powdered cellulose, hydroxypropyl cellulose and hydroxypropylmethyl cellulose.

All the comments, proposals and supporting data were taken into account. Comments on the Committee's new proposed limits (see Table 1) are invited. When higher limits are requested, analytical data in support of such limits must be provided. If alternative values and supporting data are not received by the deadline for submission of data for the fifty-ninth meeting of the Committee, the proposed limits will supersede the existing ones, replacing those published in the

Table 1
Limits for metals in food additives

Category	Additive name	INS No.	Limits (mg/kg)				
			Arsenic	Lead	Mercury	Cadmium	Other elements
Anticaking agents	Aluminium silicate	0559	—	5	—	—	
	Calcium aluminium silicate	0556	—	5	—	—	Iron <50
	Calcium silicate	0552	—	5	—	—	Iron <50
	Ferrocyanides of calcium, potassium and sodium	0538	3	5	—	—	Copper <10, zinc <25
	Magnesium oxide	0530	—	2	—	—	
	Magnesium silicates (synthetic)	0553a	—	5	—	—	Iron <10
	Silicon dioxide (amorphous)	0551	—	5	—	—	
	Sodium aluminosilicate	0554	—	5	—	—	
	Tricalcium phosphate	0341(iii)	—	4	—	—	Iron <50
	Trimagnesium phosphate	0342(iii)	—	4	—	—	Iron <5
Flavour enhancers	Calcium 5′-guanylate	0629	—	1			
	Calcium 5′-inosinate	0633	—	1			
	Calcium 5′-ribonucleotides	0634	—	1	—	—	
	Calcium di-L-glutamate	0623	—	1	—	—	
	Dipotassium 5′-guanylate	0628	—	1	—	—	
	Dipotassium 5′-inosinate	0632	—	1	—	—	
	Disodium 5′-guanylate	0627	—	1	—	—	
	Disodium 5′-inosinate	0631	—	1	—	—	
	Disodium 5′-ribonucleotides	0635	—	1	—	—	
	Ethyl maltol	0637	—	1	—	—	
	L-Glutamic acid	0620	—	1	—	—	
	5′-Guanylic acid	0626	—	1	—	—	
	5′-Inosinic acid	0630	—	1	—	—	
	Magnesium di-L-glutamate	0625	—	1	—	—	
	Monoammonium L-glutamate	0624	—	1	—	—	
	Monopotassium L-glutamate	0622	—	1	—	—	
	Monosodium L-glutamate	0621	—	1	—	—	

Table 1 (*continued*)

Category	Additive name	INS No.	Limits (mg/kg)				
			Arsenic	Lead	Mercury	Cadmium	Other elements
Sweeteners	Alitame	0956	—	1	—	—	
	Aspartame	0951	—	1	—	—	
	Cyclohexylsulfamic acid	0952	—	1	—	—	Selenium <30
	Isomalt	0953	—	1	—	—	Nickel <2
	Lactitol	0966	—	1	—	—	Nickel <2
	Mannitol	0421	—	1	—	—	Nickel <2
	Saccharin and its sodium, potassium and calcium salts	0954	—	1	—	—	Selenium <30
	Sorbitol/sorbitol syrup	0420	—	1	—	—	Nickel <2
	Sucralose	0955	—	1	—	—	
	Xylitol	0967	—	1	—	—	Nickel <2
Thickeners	Ammonium alginate	0403	—	2	—	—	
	Ethyl cellulose	0462	—	2	—	—	
	Gum ghatti	0419	—	2	—	—	
	Hydroxypropyl cellulose	0463	—	2	—	—	
	Hydroxypropylmethyl cellulose	0464	—	2	—	—	
	Karaya gum	0416	—	2	—	—	
	Konjac flour	0425	—	2	—	—	
	Methylethyl cellulose	0465	—	2	—	—	
	Methyl cellulose	0461	—	2	—	—	
	Polyvinylpyrrollidone	1201	—	2	—	—	
	Powdered cellulose	0460(ii)	—	2	—	—	
	Tara gum	0417	—	2	—	—	
	Tragacanth gum	0413	—	2	—	—	

INS: International Numbering System.

Compendium of food additive specifications and its addenda (Annex 1, references *103*, *109*, *118*, *124*, *133*, *139, 145* and *151*).

In summary, the proposed changes to the current limits are as follows:

- The limits for arsenic are to be deleted, except in ferrocyanides of calcium, potassium and sodium, for which a limit of 3 mg/kg is proposed.
- The proposed limits for lead are 2 mg/kg in thickening agents and in the anticaking agent magnesium oxide, 1 mg/kg in flavour enhancers and sweeteners, 4 mg/kg in phosphates and 5 mg/kg in silicate and ferrocyanide anticaking agents.
- No limits were proposed for cadmium or mercury, as there was no concern that they are present in any of the substances under review.
- The limits for heavy metals (as lead) were deleted.

The Committee emphasized that the absence of a limit test for a particular metal from a specification which previously included the limit test for heavy metals (as lead) indicated that the level of contamination with that particular metal is so low as to be of no concern.

4. Flavouring agents

4.1 Substances evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

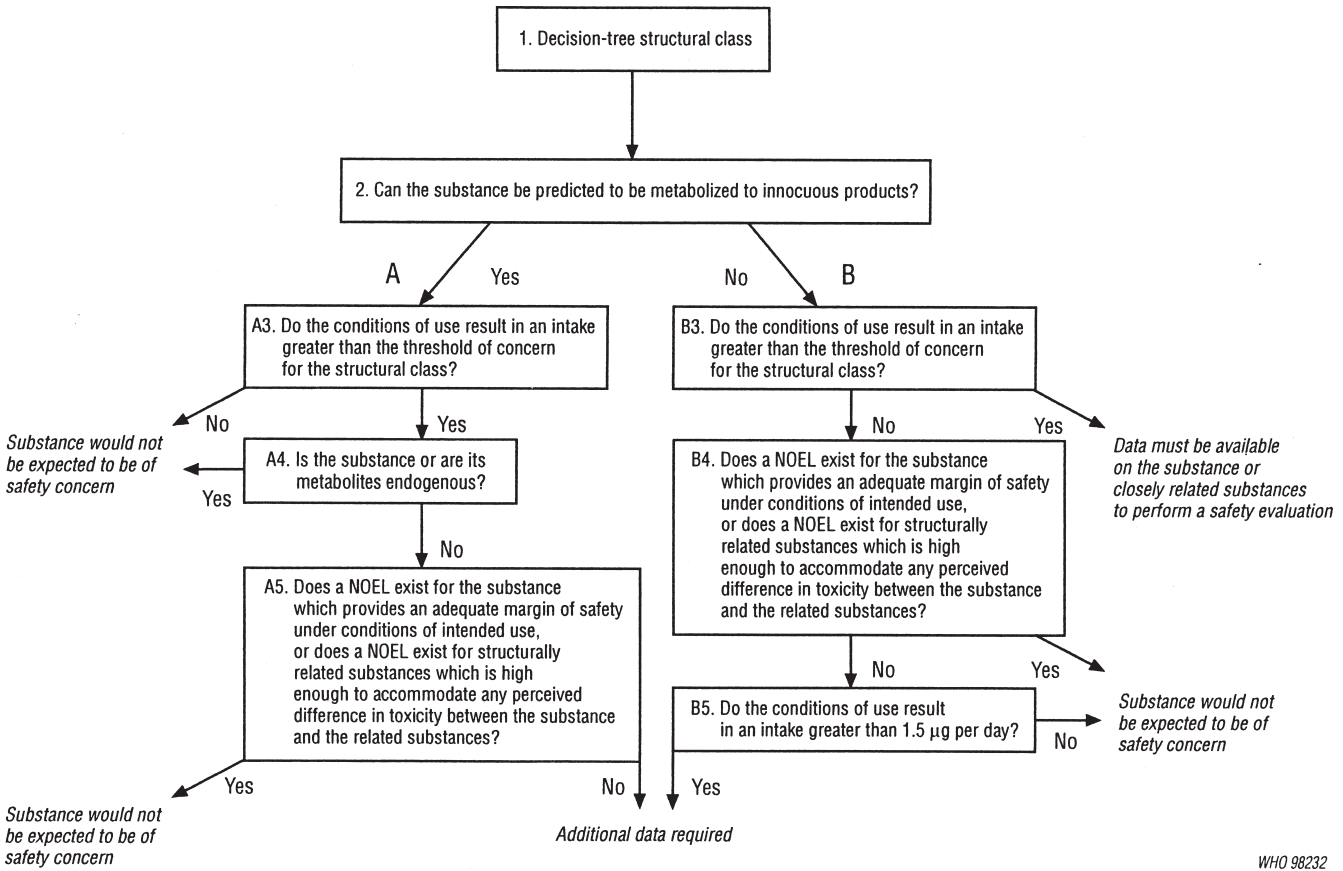
Six groups of flavouring agents were evaluated by the Procedure for the Safety Evaluation of Flavouring Agents, as outlined in Fig. 1 (Annex 1, references *116, 122, 131* and *137*).

The Committee noted that, in applying the Procedure, a flavouring agent is first assigned to a structural class, as identified at the forty-sixth meeting (Annex 1, reference *122*). The structural classes are as follows:

- Class I. Substances that have simple chemical structures and efficient modes of metabolism which would suggest a low order of toxicity when given by the oral route.
- Class II. Substances that have structural features that are less innocuous than those of substances in class I but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- Class III. Substances that have structural features that permit no strong initial presumption of safety or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations,

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents



the Committee used the following definitions, adapted from the report of its forty-sixth meeting (Annex 1, reference *122*):

Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

Endogenous substances are 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. The estimated intake of a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

Intake data

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data were derived from surveys in Europe and the USA. In Europe, a survey was conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring agent that had been incorporated into food sold in the European Union during the previous year. Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products.

In the USA, a series of surveys was conducted between 1970 and 1987 by the National Research Council of the National Academy of Sciences (under contract to the Food and Drug Administration), in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximal levels at which each substance was added to foods in a number of broad categories.

In using the data from these surveys to estimate intakes of flavouring agents, the Committee assumed that only 60% of the total amount used in Europe and 80% of that used in the USA is reported and that the total amount used in food is consumed by only 10% of the population. Intake was thus calculated from the following equation:

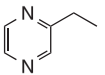
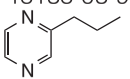
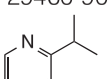
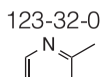
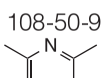
$$\text{Intake } (\mu g/\text{person per day}) = \frac{\text{Annual volume of production (kg)} \times 10^9 (\mu g/\text{kg})}{\text{Population of consumers} \times 0.6 \text{ (or } 0.8) \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe and 26×10^6 in the USA.

4.1.1 *Pyrazine derivatives*

The Committee evaluated a group of 41 flavouring agents consisting of pyrazine and pyrazine derivatives (see Table 2) by the Procedure

Table 2
Summary of results of the safety evaluations of pyrazine derivatives used as flavouring agents[a]

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
Structural class II					
2-Methylpyrazine	761	109-08-0 	No Europe: 20 USA: 7	See note 1	No safety concern
2-Ethylpyrazine	762	13925-00-3 	No Europe: 3 USA: 6	See note 1	
2-Propylpyrazine	763	18138-03-9 	No Europe: 0.1 USA: 0.1	See note 1	
2-Isopropylpyrazine	764	29460-90-0 	No Europe: 0.1 USA: 0.1	See note 1	
2,3-Dimethylpyrazine	765	5910-89-4 	No Europe: 16 USA: 4	See note 1	
2,5-Dimethylpyrazine	766	123-32-0 	No Europe: 22 USA: 8	See note 1	
2,6-Dimethylpyrazine	767	108-50-9 	No Europe: 2 USA: 2	See note 1	

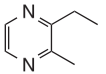
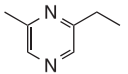
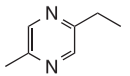
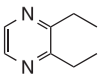
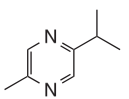
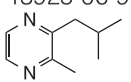
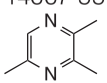
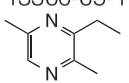
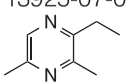
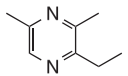
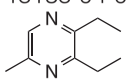
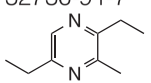
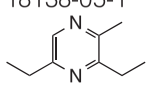
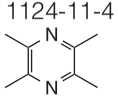
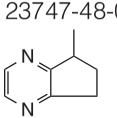
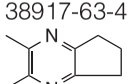
2-Ethyl-3-methylpyrazine	768	15707-23-0 	No Europe: 84 USA: 9	See note 1	No safety concern
2-Ethyl-6-methylpyrazine	769	13925-03-6 	No Europe: 0.4 USA: 0.4	See note 1	
2-Ethyl-5-methylpyrazine	770	13360-64-0 	No Europe: 5 USA: 1	See note 1	
2,3-Diethylpyrazine	771	15707-24-1 	No Europe: 2 USA: 1	See note 1	
2-Methyl-5-isopropylpyrazine	772	13925-05-8 	No Europe: ND USA: 0.4	See note 1	
2-Isobutyl-3-methylpyrazine	773	13925-06-9 	No Europe: 0.04 USA: 0.01	See note 1	
2,3,5-Trimethylpyrazine	774	14667-55-1 	No Europe: 120 USA: 46	See note 1	
2-Ethyl-3,(5 or 6)-dimethylpyrazine	775	13360-65-1  13925-07-0 	No Europe: 44 USA: 9	See note 1	

Table 2 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
3-Ethyl-2,6-dimethylpyrazine	776	13925-07-0 	No Europe: 2 USA: 0.3	See note 1	No safety concern
2,3-Diethyl-5-methylpyrazine	777	18138-04-0 	No Europe: 0.2 USA: 1	See note 1	
2,5-Diethyl-3-methylpyrazine	778	32736-91-7 	No Europe: 0.01 USA: 0.01	See note 1	
3,5-Diethyl-2-methylpyrazine	779	18138-05-1 	No Europe: 0.01 USA: 0.01	See note 1	
2,3,5,6-Tetramethylpyrazine	780	1124-11-4 	No Europe: 8 USA: 19	See note 1	
5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine	781	23747-48-0 	No Europe: 5 USA: 4	See note 1	
6,7-Dihydro-2,3-dimethyl-5*H*-cyclopentapyrazine	782	38917-63-4 	No Europe: 0.01 USA: 0.01	See note 1	

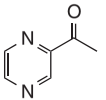
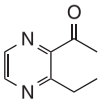
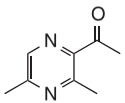
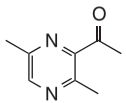
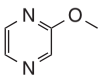
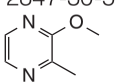
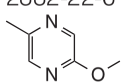
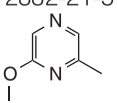
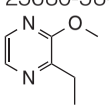
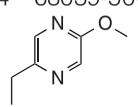
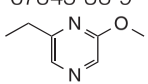
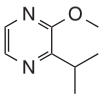
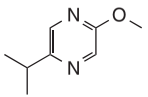
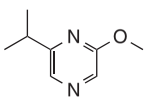
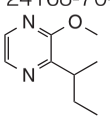
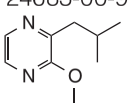
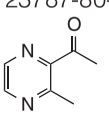
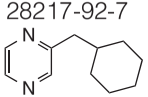
Acetylpyrazine	784	22047-25-2 	No Europe: 14 USA: 120	See note 2	No safety concern
2-Acetyl-3-ethylpyrazine	785	32974-92-8 	No Europe: 1 USA: 0.1	See note 2	
2-Acetyl-3,(5 or 6)-dimethylpyrazine	786	54300-08-2  54300-09-3 	No Europe: 1 USA: 1	See note 2	
Methoxypyrazine	787	3149-28-8 	No Europe: 4 USA: 1	See note 3	
(2 or 5 or 6)-Methoxy-3-methylpyrazine	788	2847-30-5  2882-22-6  2882-21-5 	No Europe: ND USA: 15	See note 3	
2-Ethyl-(3 or 5 or 6)-methoxypyrazine	789	25680-58-4  68039-50-9  67845-38-9 	No Europe: ND USA: 1	See note 3	

Table 2 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
2-Methoxy-(3 or 5 or 6)-isopropylpyrazine	790	25773-40-4  56891-99-7  68039-46-3 	No Europe: ND USA: 0.1	See note 3	
2-Methoxy-3-(1-methylpropyl)pyrazine	791	24168-70-5 	No Europe: 1 USA: 0.1	See note 3	No safety concern
2-Isobutyl-3-methoxypyrazine	792	24683-00-9 	No Europe: 2 USA: 1	See note 3	
2-Acetyl-3-methylpyrazine	950	23787-80-6 	No Europe: 0.1 USA: 0.1	See note 2	
Structural class III					
(Cyclohexylmethyl)pyrazine	783	28217-92-7 	No Europe: ND USA: 0.01	See note 1	No safety concern

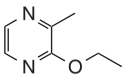
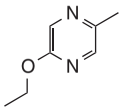
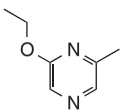
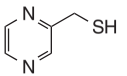
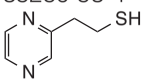
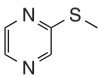
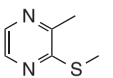
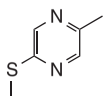
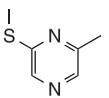
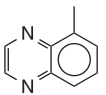
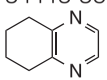
2-Methyl-(3 or 5 or 6)-ethoxypyrazine	793	32737-14-7  67845-34-5  53163-97-6 	No Europe: ND USA: 0.01	See note 3	No safety concern
2-(Mercaptomethyl)pyrazine	794	59021-02-2 	No Europe: 0.01 USA: 0.01	See note 4	
2-Pyrazinylethane thiol	795	35250-53-4 	No Europe: 0.2 USA: 1	See note 4	
Pyrazinylmethyl methyl sulfide	796	21948-70-9 	No Europe: ND USA: 0.01	See note 5	
(3 or 5 or 6)-(Methylthio)-2-methylpyrazine	797	2882-20-4  2884-14-2  2884-13-1 	No Europe: 7 USA: 13	See note 5	

Table 2 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
5-Methylquinoxaline	798	13708-12-8 	No Europe: 26 USA: 1	See note 1	No safety concern
Pyrazine	951	290-37-9 	No Europe: 0.2 USA: 0.2	See note 1	
5,6,7,8-Tetrahydroquinoxaline	952	34413-35-9 	No Europe: 8 USA: ND	See note 1	

CAS: Chemical Abstracts Service; ND: no data on intake reported.
[a] *Step 2*: All of the flavouring agents in this group are expected to be metabolized to innocuous products.
[b] The thresholds for human intake for structural classes II and III are 540 μg/day and 90 μg/day, respectively. All intake values are expressed in μg/day.

Notes to Table 1
1. Detoxication by excretion in the urine unchanged, side-chain oxidation followed by conjugation and excretion, or ring hydroxylation followed by conjugation and excretion.
2. Detoxication as given in note 1 plus reduction to the corresponding alcohol and conjugation with glucuronic acid.
3. Detoxication as given in note 1 plus *O*-dealkylation followed by conjugation and excretion.
4. Detoxication as given in note 1 plus thiol oxidation, methylation, formation of mixed disulfides and conjugation with glucuronic acid.
5. Detoxication as given in note 1 plus *S*-oxidation to sulfoxide and sulfone analogues.

for the Safety Evaluation of Flavouring Agents (see Fig. 1). None of these agents has previously been evaluated by the Committee.

Thirty-four of the flavouring agents in this group are naturally occurring components of food. Members of this group have been detected in asparagus, potato, kohlrabi and wheaten bread.

4.1.1.1 Estimated daily per capita intake

The total annual volume of production of pyrazine and the 40 pyrazine derivatives in this group is approximately 2700 kg in Europe and 2100 kg in the USA. About 64% of the total annual volume of production in Europe is accounted for by 2,3,5-trimethylpyrazine (No. 774), 2-ethyl-3-methylpyrazine (No. 768) and 2-ethyl-3,(5 or 6)-dimethylpyrazine (No. 775). In the USA, about 66% of the total annual volume of production is accounted for by acetylpyrazine (No. 784), 2,3,5-trimethylpyrazine (No. 774) and 2,3,5,6-tetramethylpyrazine (No. 780). The estimated daily per capita intake of 2,3,5-trimethylpyrazine (No. 774) in Europe and of acetylpyrazine (No. 784) in the USA is about 120 µg. The daily per capita intake of each agent in Europe and the USA is reported in Table 2.

4.1.1.2 Absorption, distribution, metabolism and elimination

Pyrazine is a weak base ($\log_{10}$ of the reciprocal of the dissociation constant, 13.4). Absorption of weak amine bases such as pyrazine derivatives is optimal at the pH of the intestine (5.0–7.0). In humans and laboratory rodents, orally administered substituted pyrazines are rapidly absorbed from the gut and excreted.

Alkyl-, alicyclic- and alkylaryl-substituted pyrazine derivatives. The biotransformation of alkyl-, alicyclic- and alkylaryl-substituted pyrazine derivatives (Nos 761–783 and 798) is expected to occur by oxidation of the alkyl side-chains. Methyl-substituted pyrazines are oxidized to yield the corresponding pyrazine-2-carboxylic acids. 5-Methylquinoxaline (No. 798) would be expected to be metabolized by the same pathway as the methyl- and ring-substituted pyrazine derivative 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), which is oxidized to yield the corresponding hydroxymethyl derivatives. An alternative pathway for the metabolism of pyrazine derivatives and the primary metabolic pathway for pyrazine (No. 951) itself involves hydroxylation of the pyrazine ring. Products of oxidative metabolism can be excreted unchanged or conjugated with glycine, glucuronic acid or sulfate before excretion.

Pyrazine derivatives containing an oxygenated functional group in the side-chain. In pyrazine derivatives containing a ring activator (e.g. a

methoxy substituent), significant ring hydroxylation may occur. Pyrazines with a methoxy side-chain, such as methoxypyrazine (No. 787), are more susceptible to nucleophilic attack, probably by molybdenum hydroxylases, and therefore primarily undergo ring hydroxylation. Additionally, the methoxy side-chain is *O*-demethylated. In rats, 3-acetylpyridine is reduced mainly to the secondary alcohol and excreted as the glucuronic acid conjugate. Therefore, acylated pyrazines (Nos 784–786 and 950) are expected to be metabolized mainly by reduction of the ketone functional group.

Pyrazine derivatives containing a thiol or sulfide functional group in the side-chain. Four pyrazine derivatives in this group contain either a thiol or a sulfide functional group in their side-chain. The possible metabolic pathways for the thiols, 2-(mercaptomethyl)pyrazine (No. 794) and 2-pyrazinylethane thiol (No. 795), include oxidation to form sulfinic acid (RSO_2H) and sulfonic acid (RSO_3H); methylation to yield methyl sulfides, which then form sulfoxides and sulfones; reaction with physiological thiols to form mixed disulfides and conjugation with glucuronic acid; or oxidation of the α-carbon, which results in desulfuration and formation of an aldehyde. Pyrazinylmethyl methyl sulfide (No. 796) and (3 or 5 or 6)-(methylthio)-2-methylpyrazine (No. 797) are predicted to be metabolized to sulfoxides and then to sulfones, which are the main urinary metabolites of simple sulfides. The Committee at its fifty-third meeting (Annex 1, reference *143*) considered the pathways of metabolism of sulfur centres in its evaluation of a group of 137 flavouring agents that included aliphatic and aromatic sulfides and thiols, with and without an additional oxygenated functional group.

4.1.1.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned 32 of the 41 flavouring agents with one aromatic ring to structural class II on the basis of structural considerations and because they occur naturally (Nos 761–782, 784–792 and 950). Six flavouring agents with one aromatic ring were assigned to structural class III (Nos 783 and 793–797), as was 5,6,7,8-tetrahydroquinoxaline (No. 952). Pyrazine (No. 951) is the only agent in the group that bears no ring substituent, and it was therefore also assigned to structural class III. 5-Methylquinoxaline (No. 798) was assigned to structural class III because it is a polyheteroaromatic substance that does not contain sodium, potassium or calcium sulfonate or sulfamate.

Step 2. At current levels of intake, all 41 flavouring agents can be predicted to be metabolized to innocuous products, and the pathways

involved would not be expected to be saturated. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of all 32 flavouring agents in structural class II and all nine substances in structural class III are below the thresholds of concern for these classes (540 μg and 90 μg, respectively). The Committee concluded that these substances would not be expected to be of safety concern at the currently estimated levels of use.

Table 2 summarizes the evaluations of pyrazine and 40 pyrazine derivatives used as flavouring agents.

4.1.1.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 32 pyrazine derivatives in structural class II were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for this class (540 μg/day). In the unlikely event that all nine flavouring agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for this class (90 μg/day). All the flavouring agents in this group are expected to be efficiently metabolized, and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

4.1.1.5 Conclusions

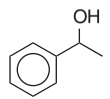
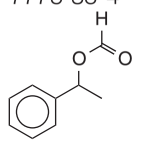
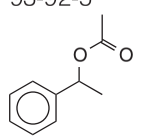
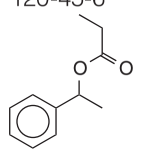
The Committee concluded that the safety of pyrazine and the 40 derivatives of pyrazine in this group would not raise concern at the currently estimated levels of intake.

A monograph summarizing the data on the safety of this group of flavouring agents and specifications were prepared.

4.1.2 *Aromatic substituted secondary alcohols, ketones and related esters*

The Committee evaluated a group of flavouring agents that included α-methylbenzyl alcohol (No. 799), acetophenone (No. 806) and 36 structurally related aromatic secondary alcohols, ketones and related esters (Table 3) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All the members of this group are considered to be aromatic secondary alcohols, ketones or related esters. The aromatic ring may contain additional alkyl substituents or a methoxy group, and the aliphatic side-chain may be unsaturated or

Table 3
Summary of results of the safety evaluations of aromatic secondary alcohols, ketones and related esters used as flavouring agents[a]

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for substance or related substance?	*Step B5* Does intake exceed 1.5μg/day?	Comments on predicted metabolism	Conclusions based on current intake
Structural class I							
α-Methylbenzyl alcohol[c]	799	98-85-1 	No Europe: 32 USA: 72	NR	NR	See note 1	No safety concern
α-Methylbenzyl formate	800	7775-38-4 	No Europe: 0.04 USA: 0.4	NR	NR	See note 2	
α-Methylbenzyl acetate	801	93-92-5 	No Europe: 200 USA: 650	NR	NR	See note 2	
α-Methylbenzyl propionate	802	120-45-6 	No Europe: 1 USA: 27	NR	NR	See note 2	

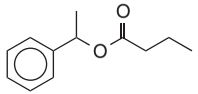
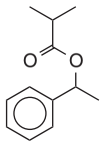
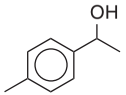
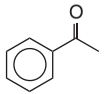
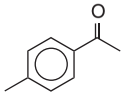
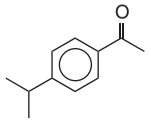
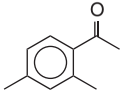
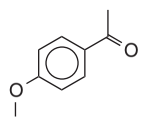
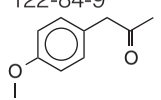
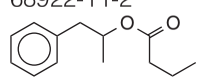
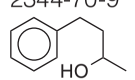
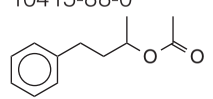
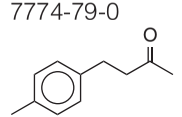
α-Methylbenzyl butyrate	803	3460-44-4 	No Europe: 1 USA: 0.01	NR	NR	See note 2	No safety concern
α-Methylbenzyl isobutyrate	804	7775-39-5 	No Europe: 29 USA: 1	NR	NR	See note 2	
p, α-Dimethylbenzyl alcohol	805	536-50-5 	No Europe: 0.2 USA: 1	NR	NR	See note 1	
Acetophenone	806	98-86-2 	No Europe: 18 USA: 170	NR	NR	See note 3	
4-Methylacetophenone	807	122-00-9 	No Europe: 26 USA: 37	NR	NR	See note 3	
p-Isopropylacetophenone	808	645-13-6 	No Europe: 0.01 USA: 0.4	NR	NR	See note 3	
2,4-Dimethylacetophenone	809	89-74-7 	No Europe: 0.3 USA: 0.01	NR	NR	See note 3	

Table 3 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for substance or related substance?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusions based on current intake
Acetanisole	810	100-06-1 	No Europe: 150 USA: 84	NR	NR	See notes 3 and 4	No safety concern
1-(*p*-Methoxyphenyl)-2-propanone	813	122-84-9 	No Europe: 0.2 USA: 0.1	NR	NR	See note 5	
α-Methylphenethyl butyrate	814	68922-11-2 	No Europe: 0.1 USA: 0.1	NR	NR	See note 6	
4-Phenyl-2-butanol	815	2344-70-9 	No Europe: 1 USA: 0.3	NR	NR	See note 7	
4-Phenyl-2-butyl acetate	816	10415-88-0 	No Europe: ND USA: 7	NR	NR	See note 7	
4-(*p*-Tolyl)-2-butanone	817	7774-79-0 	No Europe: 0.01 USA: 0.4	NR	NR	See notes 8 and 9	

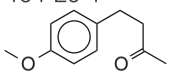
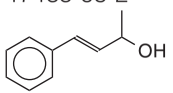
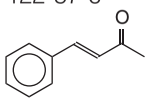
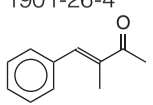
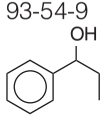
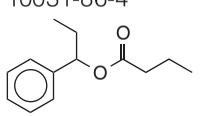
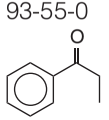
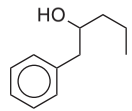
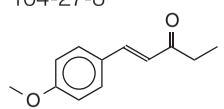
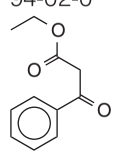
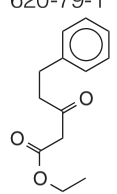
4-(*p*-Methoxyphenyl)-2-butanone	818	104-20-1 	No Europe: 5 USA: 840	NR	NR	See notes 8 and 9	No safety concern
4-Phenyl-3-buten-2-ol	819	17488-65-2 	No Europe: 2 USA: 0.1	NR	NR	See note 10	
4-Phenyl-3-buten-2-one	820	122-57-6 	No Europe: 3 USA: 7	NR	NR	See note 10	
3-Methyl-4-phenyl-3-buten-2-one	821	1901-26-4 	No Europe: 0.1 USA: 0.1	NR	NR	See note 10	
1-Phenyl-1-propanol	822	93-54-9 	No Europe: 0.3 USA: 0.1	NR	NR	See note 11	
α-Ethylbenzyl butyrate	823	10031-86-4 	No Europe: ND USA: 0.3	NR	NR	See note 2	
Propiophenone	824	93-55-0 	No Europe: 0.01 USA: 0.03	NR	NR	See note 11	

Table 3 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for substance or related substance?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusions based on current intake
α-Propylphenethyl alcohol	825	705-73-7 	No Europe: 0.1 USA: 1	NR	NR	See note 6	No safety concern
1-(*p*-Methoxyphenyl)-1-penten-3-one	826	104-27-8 	No Europe: 0.5 USA: 110	NR	NR	See note 10	
Ethyl benzoylacetate	834	94-02-0 	No Europe: 0.01 USA: 140	NR	NR	See note 12	
Ethyl 2-acetyl-3-phenyl-propionate	835	620-79-1 	No Europe: ND USA: 0.4	NR	NR	See note 12	

Structural class II

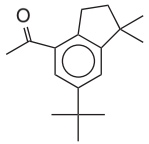
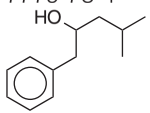
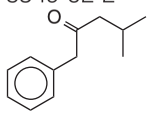
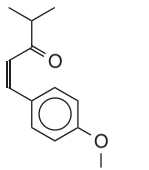
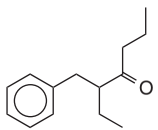
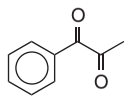
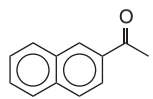
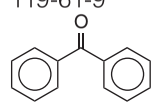
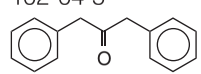
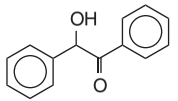
4-Acetyl-6-*tert*-butyl-1,1-dimethylindan	812	13171-00-1 	No Europe: 6 USA: 1	No	Yes		Additional data required
α-Isobutylphenethyl alcohol	827	7779-78-4 	No Europe: 29 USA: 3	NR	NR	See note 6	No safety concern
4-Methyl-1-phenyl-2-pentanone	828	5349-62-2 	No Europe: 10 USA: 0.3	NR	NR	See note 6	
1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one	829	103-13-9 	No Europe: 33 USA: 0.3	NR	NR	See note 10	
3-Benzyl-4-heptanone	830	7492-37-7 	No Europe: ND USA: 1	NR	NR	See note 13	
1-Phenyl-1,2-propanedione	833	579-07-7 	No Europe: 6 USA: 0.1	NR	NR	See note 14	

Table 3 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for substance or related substance?	*Step B5* Does intake exceed 1.5 μg/day?	Comments on predicted metabolism	Conclusions based on current intake
Structural class III							
Methyl β-naphthyl ketone	811	93-08-3 	No Europe: 7 USA: 48	Yes The NOEL of 33 mg/kg of body weight per day in a 90-day study in rats is >10 000 times the estimated intake of methyl β-naphthyl ketone when used as a flavouring agent	NR		No safety concern
Benzophenone	831	119-61-9 	No Europe: 27 USA: 11	NR	NR	See note 15	
1,3-Diphenyl-2-propanone	832	102-04-5 	No Europe: 0.1 USA: 0.1	NR	NR	See note 15	

Benzoin	836	119-53-9 	No Europe: 7 USA: 21	NR	NR	See note 16	No safety concern

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation.

[a] *Step 1*: Twenty-eight of the flavouring agents in this group are in structural class I, six are in structural class II and four are in structural class III.
Step 2: All the flavouring agents in this group, except for Nos 811 and 812, are predicted to be metabolized to innocuous products.

[b] The thresholds for human intake for structural classes I, II and III are 1800 μg/day, 540 μg/day and 90 μg/day, respectively. All intake values are expressed in μg/day.

[c] An ADI of 0–0.1 mg/kg of body weight was established for α-methylbenzyl alcohol by the Committee at its forty-first meeting (Annex 1, reference *107*), which was maintained at the present meeting.

Notes to Table 3

1. α-Methylbenzyl alcohol is conjugated with glucuronic acid and excreted in the urine. Oxidation of the methyl group to yield mandelic acid and hippuric acid may also occur.
2. α-Methylbenzyl esters are hydrolysed to simple aliphatic carboxylic acids and α-methylbenzyl alcohol. The acids are completely oxidized, and the alcohol is conjugated with glucuronic acid and excreted.
3. Acetophenone is reduced to α-methylbenzyl alcohol, conjugated with glucuronic acid and excreted primarily in the urine. The ketone may also undergo methyl group oxidation, eventually yielding mandelic acid and hippuric acid. These are also excreted in the urine.
4. Acetanisole may also undergo *O*-demethylation to yield the corresponding phenol. The phenol is excreted as the sulfate or glucuronic acid conjugate.
5. 1-(*p*-Methoxyphenyl)-2-propanone is reduced to the corresponding alcohol and excreted. *O*-Demethylation to yield the corresponding phenol may also occur.
6. α-Methylphenethyl butyrate is hydrolysed to butyric acid and α-methylphenethyl alcohol. The acid is completely metabolized and the alcohol is conjugated with glucuronic acid and excreted.
7. 4-Phenyl-2-butanol is conjugated with glucuronic acid and excreted. The corresponding acetate is predicted to be hydrolysed first to the alcohol and then to acetic acid.
8. 4-(*p*-Tolyl)-2-butanone is reduced to the corresponding alcohol. The alcohol may be conjugated with glucuronic acid and excreted primarily in the urine. The ketone may also undergo methyl group oxidation, eventually yielding a related carboxylic acid that is further oxidized to the phenylacetic acid derivative, which can be conjugated with glycine and excreted.
9. The alcohol may also be oxidized to the corresponding ketone and conjugated with glutathione.
10. 4-Phenyl-3-buten-2-one may be reduced to 4-phenyl-3-buten-2-ol. The alcohol is then further metabolized and excreted primarily as glycine conjugates. The ketone may also be conjugated with glutathione.
11. Propiophenone is reduced to the corresponding alcohol. The butyrate ester (No. 823) is predicted to be hydrolysed to the same alcohol (and butyric acid). The alcohol is conjugated with glucuronic acid and excreted primarily in the urine.
12. The esters are hydrolysed to ethanol and keto-carboxylic acids. The acids may be further oxidized and excreted as hippuric acid. The alcohol is completely metabolized.
13. Oxidation of side-chains is anticipated, primarily at the ω or ω-1 carbon atom. Carbonyl groups are reduced to alcohol, which can be conjugated with glucuronic acid. Oxidation of the side-chain can continue to benzoic or phenylacetic acid.
14. Reduced to the corresponding diol, which is conjugated with glucuronic acid.
15. Fifty per cent of a dose of 360 mg of benzophenone (No. 831) administered to rabbits by gavage was excreted as the glucuronide of the corresponding secondary alcohol within 48 h. 1,3-Diphenyl-2-propanone (No. 832), which is less sterically hindered than benzophenone, is also anticipated to be reduced to the alcohol and excreted as the glucuronide.
16. Benzoin is excreted as the glucuronic acid conjugate.

contain additional oxygenated functional groups. Of the 38 flavouring agents in this group, 34 are simple saturated or unsaturated methoxy- or alkyl-substituted benzene derivatives containing a secondary alcohol, corresponding ketone and/or related ester functional group.

The Committee had previously evaluated three members of this group. α-Methylbenzyl alcohol (No. 799) was evaluated at the forty-first meeting (Annex 1, reference *107*), when an ADI of 0–0.1 mg/kg of body weight was established. At its twenty-fourth meeting, the Committee reviewed data on α-isobutylphenethyl alcohol (No. 827) (Annex 1, reference *53*), and at its twenty-third and twenty-fifth meetings, it reviewed data on methyl β-naphthyl ketone (No. 811) (Annex 1, references *50* and *56*). No ADI was allocated to either of these flavouring agents.

Of the 38 aromatic substituted secondary alcohols, ketones and related esters considered, 16 have been reported to occur naturally in foods. For instance, α-methylbenzyl alcohol (No. 799) has been detected in cheese, fruit and tea, and the corresponding ketone acetophenone (No. 806) is a natural component of berries, seafood, beef and nuts.

4.1.2.1 Estimated daily per capita intake

The total annual volume of production of the 38 aromatic secondary alcohols, ketones and related esters considered here is approximately 4.2 tonnes in Europe and 17 tonnes in the USA. Approximately 58% of the total annual volume of production in Europe is accounted for by α-methylbenzyl acetate (No. 801) and acetanisole (No. 810). The estimated daily per capita intakes of these two flavouring agents in Europe are 200 µg and 150 µg, respectively. In the USA, approximately 80% of the total volume of production arises from use of α-methylbenzyl acetate (No. 801), acetophenone (No. 806), 4-(*p*-methoxyphenyl)-2-butanone (No. 818) and ethyl benzoylacetate (No. 834). The estimated daily per capita intakes of these agents are 650 µg, 170 µg, 840 µg and 140 µg, respectively.

The estimated daily intake of each flavouring agent in the group is reported in Table 3.

4.1.2.2 Absorption, distribution, metabolism and elimination

Generally, the flavouring agents in this group are rapidly absorbed from the gut. The aromatic secondary alcohols (and aromatic ketones after reduction to the corresponding secondary alcohols) are then either conjugated with glucuronic acid and excreted primarily in the urine, or are further oxidized and excreted mainly as glycine conjugates. As aromatic esters are generally hydrolysed in vivo by the

catalytic activity of carboxylesterases, which are found predominantly in hepatocytes, it is anticipated that the 10 esters in this group of flavouring agents will be hydrolysed to their parent aromatic or aliphatic alcohols and carboxylic acids. The eight aromatic secondary alcohols formed as a result of this process are excreted as their glucuronides or are further metabolized and excreted in the urine. The corresponding eight simple aliphatic carboxylic acids are metabolized completely by well-known pathways. The two remaining esters (Nos 834 and 835) are hydrolysed to ethanol and aromatic keto-carboxylic acids (3-oxo-3-phenylpropanoic acid and 3-oxo-5-phenylpentanoic acid, respectively), which are anticipated to be further metabolized and excreted in the urine, like other aromatic ketones.

Simple aromatic ring substitution with methyl, isopropyl or methoxy groups (Nos 805, 807–810, 813, 817, 818, 826 and 829) is predicted to have little effect on the principal metabolic pathways. It is more difficult to predict the metabolic fate of Nos 811 and 812 on the basis of the available data, as it is not known to what extent they are distributed in the tissues and eliminated. One of these substances, 4-acetal-6-*tert*-butyl-1,1-dimethylindan (No. 812), might accumulate in human adipose tissue.

4.1.2.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to these 38 aromatic flavouring agents, the Committee assigned 28 to structural class I (Nos 799–810, 813–826, 834 and 835). Six flavouring agents were assigned to structural class II, one (No. 833) because it is a vicinal diketone and the other five because they contain a fused non-aromatic carbocyclic ring (No. 812) or aliphatic substituent chains with more than five carbon atoms (Nos 827–830). Four of the agents (Nos 811, 831, 832 and 836) were assigned to structural class III because they contain more than one aromatic ring and cannot be hydrolysed to mononuclear residues.

Step 2. At current levels of estimated intake, 36 of the 38 flavouring agents in this group are predicted to be metabolized to innocuous products and the available metabolic pathways would not be expected to be saturated. Evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. The two remaining flavouring agents (Nos 811 and 812) cannot be predicted to be metabolized to innocuous products, and therefore their evaluation proceeded via the right-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of the 28 flavouring agents in structural class I, five of the six flavouring agents in

structural class II and three of the four agents in structural class III are below the thresholds of concern for these classes (1800 μg, 540 μg and 90 μg, respectively). The Committee concluded that these substances would not be expected to be of safety concern at their currently estimated levels of use as flavouring agents.

Step B3. The estimated daily per capita intakes of one agent in structural class II (No. 812) and one in structural class III (No. 811) are below the thresholds of concern for these classes (540 μg and 90 μg, respectively).

Step B4. The NOEL identified for methyl β-naphthyl ketone (No. 811) in a 90-day study in rats treated orally was the highest dose tested, 33 mg/kg of body weight per day. This dose provided safety margins >100 000 and >10 000 times the estimated daily per capita intakes in Europe and in the USA, respectively. The Committee concluded that methyl β-naphthyl ketone does not pose a safety concern at currently estimated levels of use as a flavouring agent.

No data were available on the toxicity of the remaining agent (No. 812) or of relevant structurally related substances. Accordingly, the evaluation of this substance proceeded to step B5.

Step B5. As the estimated daily per capita intake of 4-acetyl-6-*tert*-butyl-1,1-dimethylindan (No. 812) in Europe (6 μg) exceeds the threshold of 1.5 μg/person per day, further data are required for a safety evaluation. The Committee concluded that this flavouring agent cannot be classified as of "no safety concern at current level of intake".

Table 3 summarizes the evaluations of α-methylbenzyl alcohol and acetophenone and 36 structurally related flavouring agents.

4.1.2.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all foods containing all the flavouring agents in structural classes I and II were to be consumed simultaneously on a daily basis, the estimated combined intake would exceed the human intake threshold for class II (540 μg). However, the agents are expected to be metabolized efficiently and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

4.1.2.5 Conclusions

The Committee concluded that 37 of this group of 38 aromatic secondary alcohols, ketones and related esters would not pose a safety concern at currently estimated levels of use as flavouring agents.

The Committee noted that when data on toxicity were available, they were consistent with the results of the safety evaluation. Data on toxicity were required for two agents (Nos 811 and 812) in application of the Procedure. Relevant data were available for one of these substances (No. 811), which gave a large safety margin in relation to the estimated intake.

The Committee required additional data to evaluate the safety of 4-acetyl-6-*tert*-butyl-1,1-dimethylindan (No. 812), which could not be predicted to be metabolized to innocuous products, for which satisfactory data on toxicity were not available and of which the estimated daily intake, 6 µg/person in Europe, exceeded the threshold of 1.5 µg/person per day.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

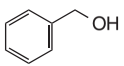
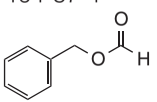
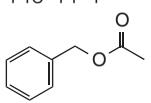
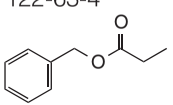
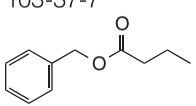
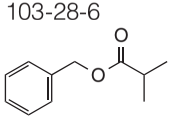
4.1.3 *Benzyl derivatives*

The Committee evaluated a group of 37 flavouring agents[1] that consisted of benzyl alcohol (No. 25), benzaldehyde (No. 22), benzoic acid (No. 850) and related substances (Table 4) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All members of this group are aromatic primary alcohols, aldehydes, carboxylic acids or related esters or acetals. The benzene ring may be ring-substituted with alkyl substituents (Nos 863–869).

The Committee had previously evaluated five members of the group. Benzyl alcohol (No. 25) was evaluated at the twenty-third and forty-sixth meetings (Annex 1, references *50* and *122*); benzyl acetate (No. 23) was evaluated at the eleventh, twenty-seventh, twenty-ninth, thirty-first, thirty-fifth, forty-first and forty-sixth meetings (Annex 1, references *14, 62, 70, 77, 88, 107* and *122*); benzyl benzoate (No. 24) was evaluated at the fifteenth and twenty-third meetings (Annex 1, references *26* and *50*); benzaldehyde (No. 22) was evaluated at the eleventh and forty-sixth meetings (Annex 1, references *14* and *122*; and benzoic acid (No. 850) was evaluated at the sixth, ninth, seventeenth, twenty-seventh and forty-sixth meetings (Annex 1, references *6, 11, 32, 62* and *122*). At its forty-sixth meeting, the Committee evaluated benzyl acetate, benzyl alcohol, benzaldehyde, benzoic acid and the benzoate salts (calcium, potassium and sodium) as a group and maintained the group ADI of 0–5 mg/kg of body weight as benzoic acid equivalents (Annex 1, reference *122*).

[1] During evaluation of these flavouring agents, the Committee questioned whether some substances in this group (Nos 850, 861 and 862) were used as flavouring agents and therefore appropriately evaluated by the Procedure. Information to address this question will be sought from the relevant manufacturers.

Table 4
Summary of results of the safety evaluations of benzyl derivatives[a]

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Benzyl alcohol[c]	25	100-51-6 	Yes Europe: 16000 USA: 17000	Yes		See note 1	No safety concern
Benzyl formate	841	104-57-4 	No Europe: 41 USA: 51	NR		See note 2	
Benzyl acetate[c]	23	140-11-4 	No Europe: 1400 USA: 850	NR		See note 2	
Benzyl propionate	842	122-63-4 	No Europe: 49 USA: 99	NR		See note 2	
Benzyl butyrate	843	103-37-7 	No Europe: 120 USA: 290	NR		See note 2	
Benzyl isobutyrate	844	103-28-6 	No Europe: 15 USA: 21	NR		See note 2	

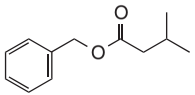
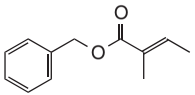
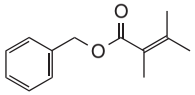
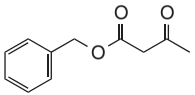
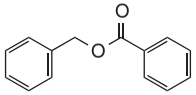
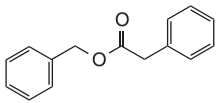
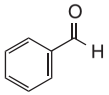
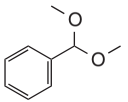
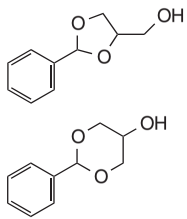
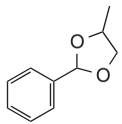
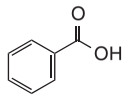
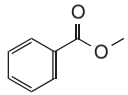
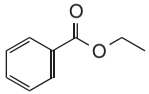
Benzyl isovalerate	845	103-38-8 	No Europe: 14 USA: 19	NR	See note 2	No safety concern
Benzyl *trans*-2-methyl-2-butenoate	846	37526-88-8 	No Europe: 0.01 USA: 0.03	NR	See note 2	
Benzyl 2,3-dimethyl-crotonate	847	7492-69-5 	No Europe: 0.01 USA: 1	NR	See note 2	
Benzyl acetoacetate	848	5396-89-4 	No Europe: 0.2 USA: 0.07	NR	See note 2	
Benzyl benzoate[c]	24	120-51-4 	Yes Europe: 1900 USA: 4200	Yes	See notes 2 and 6	
Benzyl phenylacetate	849	102-16-9 	No Europe: 5 USA: 57	NR	See note 2	
Benzaldehyde[c]	22	100-52-7 	Yes Europe: 9300 USA: 36000	Yes	See note 3	
Benzaldehyde dimethyl acetal	837	1125-88-8 	No Europe: 0.2 USA: 0.3	NR	See note 4	

Table 4 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Benzaldehyde glyceryl acetal	838	1319-88-6 	No Europe: 16 USA: 300	NR		See note 4	No safety concern
Benzaldehyde propylene glycol acetate	839	2568-25-4 	No Europe 0.04 USA: 110	NR		See note 4	
Benzoic acid[c,d]	850	65-85-0 	No Europe: 39 USA: 340	NR		See note 5	Evaluation not finalized
Methyl benzoate	851	93-58-3 	No Europe: 47 USA: 230	NR		See note 6	No safety concern
Ethyl benzoate	852	93-89-0 	No Europe: 110 USA: 110	NR		See note 6	

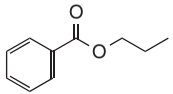
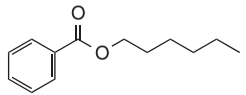
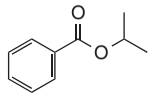
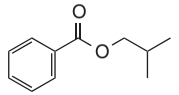
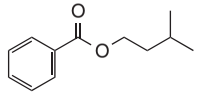
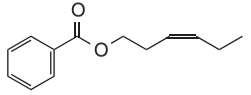
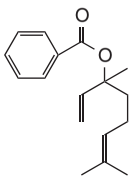
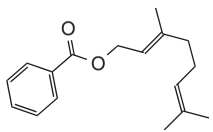
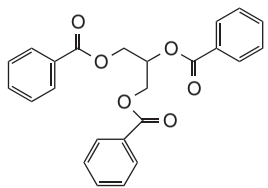
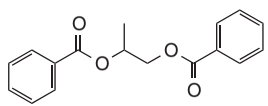
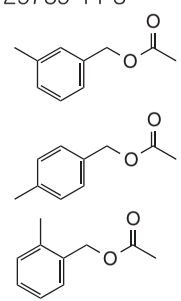
Propyl benzoate	853	2315-68-6 	No Europe: 0.01 USA: 0.3	NR	See note 6	No safety concern
Hexyl benzoate	854	6789-88-4 	No Europe: 380 USA: 1	NR	See note 6	
Isopropyl benzoate	855	939-48-0 	No Europe: 0.004 USA: 0.3	NR	See note 6	
Isobutyl benzoate	856	120-50-3 	No Europe: 0.4 USA: 1	NR	See note 6	
Isoamyl benzoate	857	94-46-2 	No Europe: 110 USA: 33	NR	See note 6	
cis-3-Hexenyl benzoate	858	25152-85-6 	No Europe: 8 USA: 0.1	NR	See note 6	
Linalyl benzoate	859	126-64-7 	No Europe: 10 USA: 2	NR	See note 6	

Table 4 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Geranyl benzoate	860	94-48-4 	No Europe: 4 USA: 0.03	NR		See note 6	No safety concern
Glyceryl tribenzoate[d]	861	614-33-5 	No Europe: ND USA: 49	NR		See note 6	Evaluation not finalized
Propylene glycol dibenzoate[d]	862	19224-26-1 	No Europe: ND USA: 14	NR		See note 6	
Methylbenzyl acetate (mixed *ortho*-, *meta*- and *para*-isomers)	863	29759-11-3 	No Europe: ND USA: 3	NR		See note 2	No safety concern

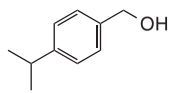
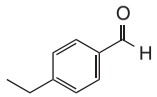
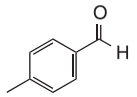
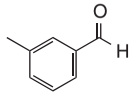
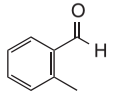
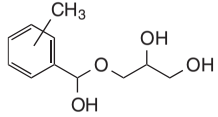
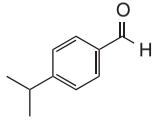
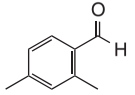
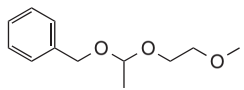
p-Isopropylbenzyl alcohol	864	536-60-7 	No Europe: 0.3 USA: 0.3	NR	See note 1	No safety concern
4-Ethylbenzaldehyde	865	4748-78-1 	No Europe: 0.4 USA: 6	NR	See note 3	
Tolualdehydes (mixed *ortho*-, *meta*- and *para*-isomers)	866	1334-78-7   	No Europe: 260 USA: 1100	NR	See note 3	
Tolualdehyde glyceryl acetal	867	1333-09-1 	No Europe: 0.01 USA: 1	NR	See note 4	
Cuminaldehyde	868	122-03-2 	No Europe: 130 USA: 1	NR	See note 3	
2,4-Dimethylbenzaldehyde	869	15764-16-6 	No Europe: 0.4 USA: 0.1	NR	See note 3	

Table 4 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Benzyl 2-methoxyethyl acetal	840	7492-39-9 	No Europe: ND USA: 1		Yes The NOEL of 6 mg/kg of body weight per day in a two-generation study of reproductive toxicity in rats is > 10 000 times the estimated intake of benzyl 2-methoxyethyl acetal when used as a flavouring agent	See note 7	No safety concern

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

[a] *Step 1*: All of the flavouring agents in this group are in structural class I.
Step 2: All of the flavouring agents in this group are expected to be metabolized to innocuous products, except for benzyl 2-methoxyethyl acetal (No. 840).

[b] The threshold for human intake for structural class I is 1800 μg/day. All intake values are expressed in μg/day.

[c] A group ADI of 0–5 mg/kg of body weight for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate and benzyl alcohol, expressed as benzoic acid equivalents, was confirmed by the Committee at its forty-sixth meeting (Annex 1, reference *122*) and extended to include benzyl benzoate at the present meeting.

[d] Further information is required to determine whether this substance is in current use as a flavouring agent.

Notes to Table 4

1. Benzyl alcohols are oxidized to the corresponding acids, which are conjugated with glycine and excreted as hippuric acid.
2. Benzyl esters are hydrolysed to the corresponding acids and alcohols.
3. Benzyl aldehydes are oxidized to the corresponding acids.
4. Benzaldehyde acetals are hydrolysed to yield the aldehyde.
5. Benzoic acid is conjugated with glycine and excreted as hippuric acid.
6. Benzoate esters are hydrolysed to yield the corresponding alcohols and acids.
7. Hydrolysed to acetaldehyde, benzyl alcohol and 2-methoxyethanol.

Of the 37 substances in this group, 29 have been reported to occur naturally in foods. They have been detected in a wide variety of fruits, vegetables, meats, cheeses and wine.

4.1.3.1 Estimated daily per capita intake

The total annual volume of production of the 37 benzyl derivatives in this group is approximately 210 tonnes in Europe and 460 tonnes in the USA. About 91% of the total annual volume of production in Europe and 94% of that in the USA is accounted for by benzyl alcohol (No. 25), benzaldehyde (No. 22) and benzyl benzoate (No. 24). About 31% of the total annual volume of production in Europe is accounted for by benzaldehyde, 54% by benzyl alcohol and 6% by benzyl benzoate. About 59% of the total annual volume of production in the USA is accounted for by benzaldehyde, 28% by benzyl alcohol and 7% by benzyl benzoate. The estimated daily intake per capita of these three agents in Europe is 9300 µg of benzaldehyde, 16 000 µg of benzyl alcohol and 1900 µg of benzyl benzoate. The estimated daily intake per capita in the USA is 36 000 µg of benzaldehyde, 17 000 µg of benzyl alcohol and 4200 µg of benzyl benzoate. The estimated daily per capita intake of each flavouring agent in Europe and the USA is reported in Table 4.

Benzoic acid is not only present in food and flavours but is also endogenous in the human body. Endogenous benzoic acid is formed through the phenylalanine–tyrosine pathway (Annex 1, reference *123*).

4.1.3.2 Absorption, distribution, metabolism and elimination

In general, aromatic esters are hydrolysed in vivo by the catalytic activity of carboxylesterases, which are found predominantly in hepatocytes. All the benzyl and benzoate esters and acetals of benzaldehyde (or acetaldehyde) are anticipated to be hydrolysed readily under acidic conditions to yield benzyl alcohol (and carboxylic acids) and to benzaldehyde (and alcohols), respectively, followed by oxidation to yield benzoic acid. Benzoate esters are hydrolysed to benzoic acid (and alcohols). The remaining alcohol or acid components formed by hydrolysis are simple aliphatic substances, which are either oxidized to polar metabolites and excreted or metabolized in the fatty acid pathway and tricarboxylic acid cycle.

Benzyl derivatives have been shown to be absorbed rapidly in the gut, metabolized primarily in the liver and excreted in the urine as glycine conjugates of benzoic acid derivatives. Once absorbed, benzyl derivatives are oxidized and excreted primarily as the glycine conjugate of benzoic acid (hippurate). When high doses of benzyl derivatives are

given, formation of the glycine conjugate is limited; when glycine is depleted, free benzoic acid may sequester acetyl coenzyme A or be excreted unchanged or as the glucuronic acid conjugate. Aromatic ring substitution is anticipated to have little effect on the principal pathway of metabolism.

Oxidation of the alcohol or aldehyde group may be accompanied by oxidation of the alkyl side-chain.

4.1.3.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to the 37 benzyl derivatives, the Committee assigned all of them to structural class I.

Step 2. At currently estimated levels of intake, 36 of the 37 substances in this group can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. One compound, benzyl 2-methoxyethyl acetal (No. 840), cannot be predicted to be metabolized to innocuous products, and its evaluation therefore proceeded via the right-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 33 of the flavouring agents in this group are below the threshold of concern for structural class I (1800 µg). The Committee concluded that these substances would not be expected to be of safety concern at current estimated levels of use as flavouring agents. The estimated daily per capita intakes of the remaining three substances are above the threshold of concern for this class, that of benzyl alcohol (No. 25) being 16 000 µg in Europe and 17 000 µg in the USA, that of benzyl benzoate (No. 24) being 1900 µg in Europe and 4200 µg in the USA, and that of benzaldehyde (No. 22) being 9300 µg in Europe and 36 000 µg in the USA. Accordingly, the evaluation of these three substances proceeded to step A4.

Step A4. Benzyl alcohol, benzyl benzoate and benzaldehyde are readily metabolized to benzoic acid, which is endogenous in humans. These agents would therefore not be expected to be of safety concern.

Step B3. For benzyl 2-methoxyethyl acetal (No. 840), no data on intake were reported for Europe and an intake of 1 µg/person per day was reported for the USA, which is below the threshold of concern for substances in structural class I (1800 µg/person per day). The evaluation of this substance therefore proceeded to step B4.

Step B4. The NOEL of 6 mg/kg of body weight per day for benzyl 2-methoxyethyl acetal (No. 840) in a two-generation study of reproductive toxicity in rats provides a margin of safety >10000 times the estimated daily per capita intake in the USA. The Committee concluded that this substance would not pose a safety concern at the currently estimated level of intake.

Table 4 summarizes the evaluations of the 37 benzyl derivatives used as flavouring agents.

4.1.3.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all the benzyl derivatives used as flavouring agents, except benzyl 2-methoxyethyl acetal (No. 840), were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for structural class I. However, these agents are expected to be efficiently detoxicated and the available detoxication pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

Furthermore, the total combined daily intake per kilogram of body weight of all benzyl derivatives (0.5 mg in Europe and 1 mg in the USA) is lower than the group ADI of 0–5 mg/kg of body weight for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate and benzyl alcohol, expressed as benzoic acid equivalents, which was maintained by the Committee at its forty-sixth meeting (Annex 1, reference *122*). The three benzyl derivatives that account for more than 90% of the total intake of this group of substances in Europe and the USA are benzyl benzoate (No. 24), which is rapidly hydrolysed to benzyl alcohol and benzoic acid, benzaldehyde (No. 22) and benzyl alcohol (No. 25). All these substances are readily metabolized to benzoic acid, which is endogenous in humans. The Committee considered that the endogenous concentration of this substance would not give rise to perturbations outside the physiological range. Therefore, these three substances were considered to be of no safety concern at currently estimated levels of intake.

4.1.3.5 Conclusions

The Committee concluded that the safety of the flavouring agents in the group of benzyl derivatives would not present concern at currently estimated levels of use as flavouring agents. No data on toxicity were required in application of the Procedure to 36 of the 37 benzyl derivatives in the group, and the Committee noted that the available information was consistent with the results of the safety evaluation.

The necessary data on toxicity were available for benzyl 2-methoxyethyl acetal (No. 840).

A monograph summarizing the safety data on this group of flavouring agents and specifications were prepared.

4.1.4 *Hydroxy- and alkoxy-substituted benzyl derivatives*

The Committee evaluated a group of flavouring agents[1] comprising 46 structurally related substances by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All members of this group are aromatic primary alcohols, aldehydes, carboxylic acids or their corresponding esters or acetals. The structural feature common to all members of the group is a primary oxygenated functional group bound directly to a benzene ring. The ring also contains hydroxy or alkoxy substituents (see Table 5).

The Committee had previously evaluated four members of this group. Ethyl vanillin (No. 893) was evaluated by the Committee at its eleventh meeting (Annex 1, reference *14*), when a conditional ADI of 0–10 mg/kg of body weight was established. At its thirty-fifth meeting, the Committee converted this ADI to a temporary ADI of 0–5 mg/kg of body weight (Annex 1, reference *88*). At its thirty-ninth meeting, the Committee extended the temporary ADI (Annex 1, reference *101*). At its forty-fourth meeting, the Committee allocated an ADI of 0–3 mg/kg of body weight to ethyl vanillin (Annex 1, reference *116*). Vanillin (No. 889) was evaluated by the Committee at its eleventh meeting (Annex 1, reference *14*), when an ADI of 0–10 mg/kg of body weight was established. Methyl salicylate (No. 899) was evaluated by the Committee at its eleventh meeting (Annex 1, reference *14*), and an ADI of 0–0.5 mg/kg of body weight was established. Piperonal (No. 896) was also evaluated at the eleventh meeting, and an ADI of 0–2.5 mg/kg of body weight was established (Annex 1, reference *14*).

Twenty-nine of the 46 substances in this group of flavouring agents have been reported to occur naturally in food. Vanillin (No. 889), a major constituent of natural vanilla, is also present in strawberries and milk. Methyl salicylate (No. 899), the predominant substituent of oil of wintergreen, is also found in tomatoes and grilled beef. Ethyl vanillin (No. 893) has been detected in raspberries and ginger, while piperonal (No. 896) is found in cooked chicken and pepper.

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[1] During evaluation of these flavouring agents, the Committee questioned whether one substance in this group (No. 870) was currently used as a flavouring agent and therefore appropriately evaluated by the Procedure. Information to address this question will be sought from relevant manufacturers.

Table 5
Summary of results of the safety evaluations of hydroxy- and alkoxy-substituted benzyl derivatives used as flavouring agents[a]

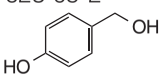
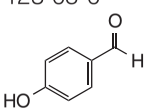
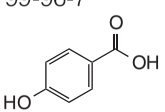
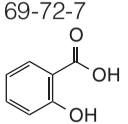
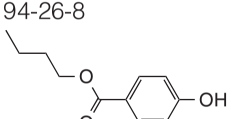
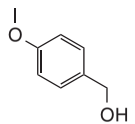
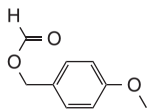
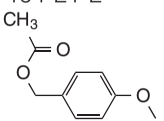
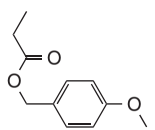
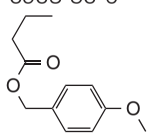
Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I							
4-Hydroxybenzyl alcohol	955	623-05-2 	No Europe: 6 USA: 0.06	NR	NR	See note 1	No safety concern
4-Hydroxybenzaldehyde	956	123-08-0 	No Europe: 64 USA: 59	NR	NR	See note 1	
4-Hydroxybenzoic acid	957	99-96-7 	No Europe: 19 USA: 17	NR	NR	See note 1	
2-Hydroxybenzoic acid	958	69-72-7 	No Europe: 0.03 USA: 0.03	NR	NR	See note 1	
Butyl *p*-hydroxybenzoate[c]	870	94-26-8 	No Europe: ND USA: 0.03	NR	NR	See note 2	Evaluation not finalized

Table 5 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Anisyl alcohol	871	105-13-5 	No Europe: 150 USA: 58	NR	NR	See note 1	No safety concern
Anisyl formate	872	122-91-8 	No Europe: 46 USA: 24	NR	NR	See note 2	
Anisyl acetate	873	104-21-2 	No Europe: 59 USA: 300	NR	NR	See note 2	
Anisyl propionate	874	7549-33-9 	No Europe: ND USA: 5	NR	NR	See note 2	
Anisyl butyrate	875	6963-56-0 	No Europe: 34 USA: 0.1	NR	NR	See note 2	

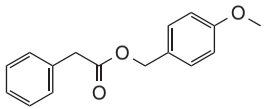
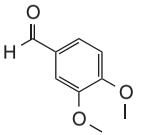
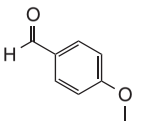
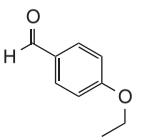
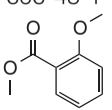
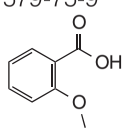
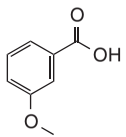
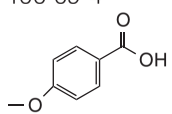
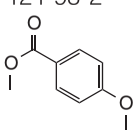
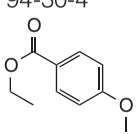
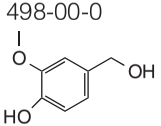
Anisyl phenylacetate	876	102-17-0 	No Europe: 0.003 USA: 0.1	NR	NR	See note 3	No safety concern
Veratraldehyde	877	120-14-9 	No Europe: 140 USA: 55	NR	NR	See note 1	
p-Methoxybenz-aldehyde	878	123-11-5 	No Europe: 440 USA: 580	NR	NR	See note 1	
p-Ethoxybenz-aldehyde	879	10031-82-0 	No Europe: 0.1 USA: 0.01	NR	NR	See note 1	
Methyl *o*-methoxy-benzoate	880	606-45-1 	No Europe: 57 USA: 8	NR	NR	See note 2	
2-Methoxybenzoic acid	881	579-75-9 	No Europe: ND USA: 0.01	NR	NR	See note 1	

Table 5 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
3-Methoxybenzoic acid	882	586-38-9 	No Europe: ND USA: 0.01	NR	NR	See note 1	No safety concern
4-Methoxybenzoic acid	883	100-09-4 	No Europe: ND USA: 0.1	NR	NR	See note 1	
Methyl anisate	884	121-98-2 	No Europe: 1 USA: 0.01	NR	NR	See note 2	
Ethyl *p*-anisate	885	94-30-4 	No Europe: 11 USA: 2	NR	NR	See note 2	
Vanillyl alcohol	886	498-00-0 	No Europe: 6 USA: 6	NR	NR	See note 1	

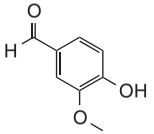
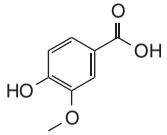
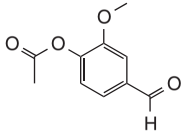
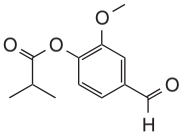
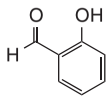
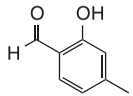
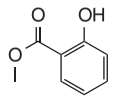
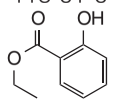
Vanillin[d]	889	121-33-5 	Yes Europe: 55000 USA: 150000	No	Yes The NOEL of 1000mg/kg of body weight per day in a 2-year study in rats is >100 times the estimated daily intake of vanillin when used as a flavouring agent	See note 1	No safety concern
4-Hydroxy-3-methoxy-benzoic acid	959	121-34-6 	No Europe: 29 USA: 26	NR	NR	See note 1	
Vanillin acetate	890	881-68-5 	No Europe: 2 USA: 1	NR	NR	See note 2	
Vanillin isobutyrate	891	20665-85-4 	No Europe: 64 USA: 0.04	NR	NR	See note 2	

Table 5 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Salicylaldehyde	897	90-02-8 	No Europe: 98 USA: 16	NR	NR	See note 1	No safety concern
2-Hydroxy-4-methyl-benzaldehyde	898	698-27-1 	No Europe: 1 USA: 0.3	NR	NR	See note 1	
Methyl salicylate[e]	899	119-36-8 	Yes Europe: 490 USA: 44000	No	Yes The NOEL of 50 mg/kg of body weight per day in a 2-year study in dogs is >100 times the estimated daily intake of methyl salicylate when used as a flavouring agent	See note 2	
Ethyl salicylate	900	118-61-6 	No Europe: 31 USA: 1700	NR	NR	See note 2	

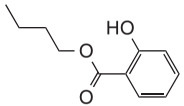
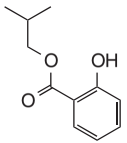
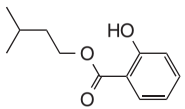
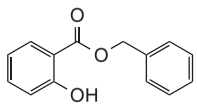
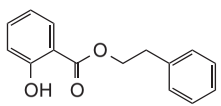
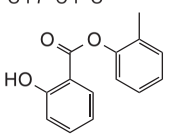
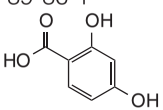
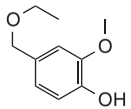
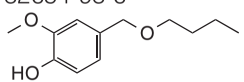
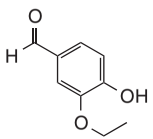
Butyl salicylate	901	2052-14-4 	No Europe: 0.01 USA: 0.0007	NR	NR	See note 2	No safety concern
Isobutyl salicylate	902	87-19-4 	No Europe: 1 USA: 6	NR	NR	See note 2	
Isoamyl salicylate	903	87-20-7 	No Europe: 49 USA: 7	NR	NR	See note 2	
Benzyl salicylate	904	118-58-1 	No Europe: 30 USA: 29	NR	NR	See note 3	
Phenethyl salicylate	905	87-22-9 	No Europe: 0.2 USA: 4	NR	NR	See note 3	
o-Tolyl salicylate	907	617-01-6 	No Europe: ND USA: 30	NR	NR	See note 3	
2,4-Dihydroxybenzoic acid	908	89-86-1 	No Europe: ND USA: 6	NR	NR	See note 1	

Table 5 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class II							
Vanillyl ethyl ether	887	13184-86-6 	No Europe: 22 USA: 22	NR	NR	See note 1	No safety concern
Vanillyl butyl ether	888	82654-98-6 	No Europe: ND USA: 0.1	NR	NR	See note 1	
Ethyl vanillin[f]	893	121-32-4 	Yes Europe: 6200 USA: 43000	No	Yes The NOEL of 500 mg/kg of body weight per day in a 14-week study in rats is >100 times the estimated daily intake of ethyl vanillin when used as a flavouring agent	See note 1	

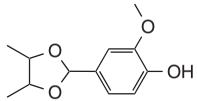
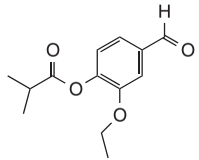
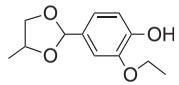
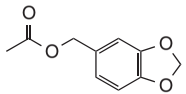
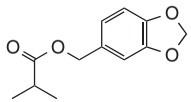
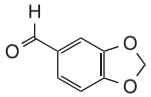
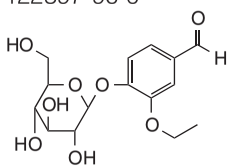
Vanillin *erythro*- and *threo*-butan-2,3-diol acetal	960	63253-24-7 	No Europe: 4 USA: 3	NR	NR	See note 2	No safety concern
Ethyl vanillin isobutyrate	953	188417-26-7 	No Europe: 64 USA: ND	NR	NR	See note 2	
Ethyl vanillin propylene glycol acetal	954	68527-76-4 	No Europe: 39 USA: 36	NR	NR	See note 2	
Piperonyl acetate	894	326-61-4 	No Europe: 41 USA: 11	NR	NR	See note 4	
Piperonyl isobutyrate	895	5461-08-5 	No Europe: 0.1 USA: 3	NR	NR	See note 4	
Piperonal[g]	896	120-57-0 	Yes Europe: 1700 USA: 3200	No	Yes The NOEL of 250mg/kg of body weight per day in a 2-year study in rats is >1000 times the estimated daily intake of piperonal when used as a flavouring agent	See note 4	

Table 5 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Ethyl vanillin β-D-glucopyranoside	892	122397-96-0 	No Europe: ND USA: 30	NR	NR	See note 2	No safety concen

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

[a] *Step 2*: All of the flavouring agents in this group are expected to be metabolized to innocuous products.

[b] The thresholds for human intake for structural classes I and II are 1800 μg/day and 540 μg/day, respectively. All intake values are expressed in μg/day.

[c] Further information is required to determine whether this substance is in current use as a flavouring agent.

[d] An ADI of 0–10 mg/kg of body weight was established for vanillin by the Committee at its eleventh meeting (Annex 1, reference *14*), which was maintained at the present meeting.

[e] An ADI of 0–0.5 mg/kg of body weight was established for methyl salicylate by the Committee at its eleventh meeting (Annex 1, reference *14*), which was maintained at the present meeting. The estimated daily per capita intake of methyl salicylate is 0.7 mg/kg of body weight when calculated on the basis of the usual 10% proportion of eaters; however, a survey of intake showed that >50% of the population would be expected to consume methyl salicylate. When this measured proportion of eaters was used, the calculated intake was 0.1 mg/kg of body weight.

[f] An ADI of 0–3 mg/kg of body weight was established for ethyl vanillin by the Committee at its forty-fourth meeting (Annex 1, reference *116*), which was maintained at the present meeting.

[g] An ADI of 0–2.5 mg/kg of body weight was established for piperonal by the Committee at its eleventh meeting (Annex 1, reference *14*), which was maintained at the present meeting.

Notes to Table 5

1. Detoxication by excretion in the urine unchanged or as glucuronic acid, glycine or sulfate conjugates; aldehyde groups will undergo oxidation or reduction to the corresponding carboxylic acid or alcohol, respectively, followed by conjugation and excretion; *O*-dealkylation followed by conjugation and excretion; other, minor metabolic routes, which probably occur in the intestinal microflora after biliary excretion of conjugates, include decarboxylation and reduction of benzyl groups to the methyl analogues.
2. Detoxication as described in note 1 plus hydrolysis of esters to the corresponding benzyl alcohol or benzoic acid derivatives, acetal hydrolysis to the parent benzaldehyde derivative and simple aliphatic alcohol, or glycosidic bond hydrolysis to the corresponding phenolic derivative.
3. Detoxication as described in note 1, preceded by hydrolysis to yield mononuclear residues, each of which would be detoxicated as described in note 1.
4. Detoxication as described in note 1 plus limited oxidation of the methylenedioxyphenyl group to a catechol, which would undergo conjugation.

4.1.4.1 Estimated daily per capita intake

The total annual volume of production of the 46 flavouring agents in this group is 450 tonnes in Europe and 1800 tonnes in the USA. Vanillin (No. 889), ethyl vanillin (No. 893), methyl salicylate (No. 899) and piperonal (No. 896), for which ADIs were previously established by the Committee, account for approximately 98% of the total annual volume in Europe and 99% in the USA. In Europe, the estimated daily per capita intakes of these compounds are 55 mg of vanillin, 6.2 mg of ethyl vanillin, 0.5 mg of methyl salicylate and 1.7 mg of piperonal. In the USA, the estimated daily per capita intakes are 150 mg of vanillin, 43 mg of ethyl vanillin, 44 mg of methyl salicylate and 3.2 mg of piperonal. The estimated daily per capita intakes of the other flavouring agents in this group are lower. Ethyl salicylate (No. 900) and *p*-methoxybenzaldehyde (No. 878) have the next highest daily per capita intakes; that of ethyl salicylate is 1.7 mg in the USA and that of *p*-methoxybenzaldehyde is 0.5 mg in both Europe and the USA. The remaining 40 flavouring agents have estimated daily per capita intakes of <100 µg, 10 of which are under 1 µg. The daily per capita intake of each substance in Europe and the USA is shown in Table 5.

4.1.4.2 Absorption, distribution, metabolism and elimination

The aromatic esters in this group can be expected to be hydrolysed extensively through the catalytic activity of the intestinal carboxylesterases, especially β-esterases, to benzyl alcohol or benzoic acid derivatives before absorption. Likewise, acetals of substituted benzaldehyde derivatives will be hydrolysed in gastric and intestinal fluids to yield benzaldehyde and aliphatic alcohols. The resulting hydroxy- and alkoxy-substituted benzyl derivatives are rapidly absorbed in the gut, metabolized in the liver and excreted in the urine.

Once absorbed, benzyl derivatives are oxidized to the corresponding benzoic acid derivative, which is subsequently excreted unchanged or as sulfate or glucuronide conjugates. Minor metabolic detoxication pathways include *O*-demethylation, reduction and decarboxylation. These pathways are used during enterohepatic cycling of conjugated benzyl metabolites and subsequent intestinal bacterial action. Piperonal is oxidized to piperonylic acid and excreted mainly as the glycine conjugate.

4.1.4.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1), the Committee assigned 36 of the 46 flavouring agents in this group to structural class I. These flavouring

agents are all either simple substituted aromatic compounds, cyclic acetals of benzaldehydes which are expected to be hydrolysed to aromatic aldehydes and simple aliphatic alcohols (Nos 870–875, 877–886, 889–891, 897–903, 908, 955–959), or compounds containing two aromatic rings which are expected to be hydrolysed to mononuclear residues with simple functional groups (Nos 876, 904, 905 and 907). The remaining 10 flavouring agents (Nos 887, 888, 892–896, 953, 954 and 960) are ethyl vanillin or piperonal derivatives that contain either an ethoxy or a methylene dioxy substituent. They are common components of food, or closely structurally related to common components of food, and were assigned to structural class II.

Step 2. At current levels of intake, the metabolic pathways of these flavouring agents can be predicted not to be saturated, and all can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded down the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 34 of the flavouring agents in structural class I and eight flavouring agents in structural class II were below the thresholds of concern for these classes (1800 μg and 540 μg, respectively). The Committee concluded that these 42 flavouring agents would not be expected to be of safety concern at currently estimated levels of use.

The estimated daily per capita intakes of vanillin (No. 889) and methyl salicylate (No. 899), which are in structural class I, exceed the threshold of concern for this class (1800 μg). The estimated daily per capita intakes of vanillin are 55 000 μg in Europe and 150 000 μg in the USA, and that of methyl salicylate is 44 000 μg in the USA. The estimated daily per capita intakes of ethyl vanillin (No. 893) and piperonal (No. 896), which are in structural class II, exceed the threshold of concern for this class (540 μg). The estimated daily per capita intakes of ethyl vanillin are 6200 μg in Europe and 43 000 μg in the USA, and those of piperonal are 1700 μg in Europe and 3200 μg in the USA.

The estimated daily per capita intakes of these four substances are below their respective ADI values. The daily intakes of vanillin in Europe and the USA, approximately 0.9 and 2.5 mg/kg of body weight respectively, do not exceed the ADI of 0–10 mg/kg of body weight for vanillin. The highest estimated daily intakes of ethyl vanillin (0.7 mg/kg of body weight in the USA) and piperonal (0.05 mg/kg of body weight in the USA) do not exceed the ADIs of 0–3 mg/kg of body weight for ethyl vanillin and 0–2.5 mg/kg of body weight for piperonal. The highest estimated daily intake of the remaining substance, methyl salicylate, is 0.7 mg/kg of body weight, which is approximately equal to its ADI of 0–0.5 mg/kg of body weight.

The estimates of intake derived from total annual volume of production are based on the assumption that only 10% of the population consumes the substance under consideration. The Committee reviewed an analysis of the intake of methyl salicylate which was based on individual dietary records of consumption of baked goods, chewing-gums, hard and soft sweets and beverages in which this agent is used in the USA. The analysis showed that more than 50% of the population would be expected to consume methyl salicylate. Use of this *measured* proportion of eaters in place of the default assumption of 10% yields an estimated intake of methyl salicylate of 0.1 mg/kg of body weight, which is still below the current ADI of 0–0.5 mg/kg of body weight.

Step A4. Vanillin (No. 889), methyl salicylate (No. 899), ethyl vanillin (No. 893) and piperonal (No. 896) are not endogenous in humans. The evaluation of these substances therefore proceeded to step A5.

Step A5. The ADI of 0–10 mg/kg of body weight for vanillin is based on a NOEL of 1000 mg/kg of body weight per day in a 2-year feeding study in rats. This NOEL provides a margin of safety, as it is more than 100 times the per capita intake of vanillin from its currently estimated use as a flavouring agent in Europe (0.9 mg/kg of body weight per day) or in the USA (2.5 mg/kg of body weight per day).

The ADI of 0–0.5 mg/kg of body weight for methyl salicylate is based on a NOEL of 50 mg/kg of body weight per day reported in a 2-year study in dogs. This NOEL is more than 1000 times greater than the intake of methyl salicylate from its currently estimated use as a flavouring agent in Europe (0.008 mg/kg of body weight per day) and is more than 100 times greater than the intake of methyl salicylate in the USA when intake is calculated on the basis of the measured portion of eaters of 50% (0.1 mg/kg of body weight per day).

A NOEL of 500 mg/kg of body weight per day for ethyl vanillin was reported in a 14-week feeding study in rats. This NOEL is more than 100 times greater than the intake of ethyl vanillin from its use as a flavouring agent in Europe (0.1 mg/kg of body weight per day) or in the USA (0.7 mg/kg of body weight per day).

A NOEL of 250 mg/kg of body weight per day for piperonal was reported in a 2-year study in rats. This NOEL is more than 1000 times the intake of piperonal from its use as a flavouring agent in Europe (0.03 mg/kg of body weight per day) and in the USA (0.05 mg/kg of body weight per day).

Table 5 summarizes the evaluations of the 46 hydroxy- and alkoxy-substituted benzyl derivatives used as flavouring agents.

4.1.4.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 36 flavouring agents in structural class I were to be consumed on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (1800 µg/day). In the unlikely event that all 10 flavouring agents in structural class II were to be consumed on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (540 µg/day). However, all 46 flavouring agents in this group are expected to be efficiently detoxicated, and the available detoxication pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

4.1.4.5 Conclusions

The Committee retained the previously established ADIs of 0–10 mg/kg of body weight for vanillin (No. 889), 0–3 mg/kg of body weight for ethyl vanillin (No. 893), 0–2.5 mg/kg of body weight for piperonal (No. 896) and 0–0.5 mg/kg of body weight for methyl salicylate (No. 899). The Committee noted that the estimated daily intake of 0.7 mg/kg of body weight of methyl salicylate, based on the production volume used, is approximately equal to its ADI of 0–0.5 mg/kg of body weight, within the precision of the intake estimates. The Committee reviewed an analysis of intake based on individual dietary records of consumption of mint-flavoured baked goods, chewing-gums, hard and soft sweets and beverages in which methyl salicylate is potentially used. This analysis showed that more than 50% of the population would be expected to consume methyl salicylate. The use of this *measured* proportion of eaters in place of the default assumption of 10% yields an estimated intake of methyl salicylate of 0.1 mg/kg of body weight, which is below the ADI of 0–0.5 mg/kg of body weight.

On the basis of the available data on metabolism and toxicity, the Committee concluded that the safety of the flavouring agents in this group would not raise concern at the currently estimated levels of use. Other data on toxicity, including studies on developmental toxicity and genotoxicity, were consistent with the results of the safety evaluations.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.5 ***Aliphatic acyclic diols, triols and related substances***

The Committee evaluated a group of 31 flavouring agents[1] that included aliphatic acyclic diols, triols and related substances

[1] During evaluation of these flavouring agents, the Committee questioned whether some substances in this group (Nos 909 and 914–926) were used as flavouring agents and therefore appropriately evaluated by this Procedure. Information to address this question will be sought from relevant manufacturers.

(Table 6) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All members of this group are aliphatic acyclic primary alcohols, aldehydes, acids or related esters with one or more additional oxygenated functional groups. The group comprised four subgroups: glycerol (No. 909) and 15 related glycerol esters and acetals (Nos 910–924); propylene glycol (No. 925) and four related esters, acetals and ketals (Nos 926–929); lactic acid (No. 930) and four lactate esters (Nos 931, 932, 934 and 935); and pyruvic acid (No. 936), its corresponding aldehyde (No. 937), two pyruvate esters (Nos 938 and 939) and one acetal of pyruvic acid (No. 933).

The Committee had previously evaluated three members of the group. Glycerol (No. 909) was considered at the twentieth meeting (Annex 1, reference *41*), when an ADI "not specified"[1] was established. Propylene glycol (No. 925) was considered at the seventh meeting (Annex 1, reference *7*), when an ADI of 0–20 mg/kg of body weight was established; it was further considered at the seventeenth meeting (Annex 1, reference *32*), when the ADI was increased to 0–25 mg/kg of body weight. Ethyl lactate (No. 931) was considered at the eleventh, twenty-third, twenty-fourth and twenty-sixth meetings (Annex 1, references *14*, *50*, *53* and *59*). At its twenty-sixth meeting, the Committee included ethyl lactate in the group ADI "not specified"[1] with lactic acid.

Nine of the 31 substances (Nos 909, 929–932, 934 and 936–938) have been detected as natural components of foods, in cocoa, milk, cider, cognac, asparagus, tomatoes and mushrooms.

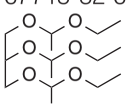
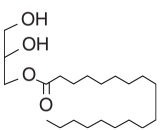
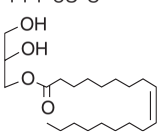
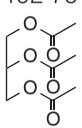
4.1.5.1 Estimated daily per capita intake

The total annual volume of production of the 31 flavouring agents in this group is 140 tonnes in Europe and 21 000 tonnes in the USA. These values are equivalent to a total estimated daily per capita intake of 20 000 µg in Europe and 2 800 000 µg in the USA. The large difference in the annual volume of production in Europe and the USA is due to the inclusion in the USA of figures on the use of glycerol, triacetin and propylene glycol as solvents in the preparation of flavour mixtures.

In Europe, 97% of the total daily per capita intake of this group of flavouring agents was accounted for by glycerol (17 000 µg), ethyl lactate (1900 µg) and butyl lactate (380 µg). In the USA, 96% of the total daily per capita intake was accounted for by glycerol (220 000 µg), triacetin (83 000 µg) and propylene glycol (2 400 000 µg).

[1] See footnote on page 32.

Table 6
Summary of results of the safety evaluations of aliphatic acyclic diols, triols and related substances[a]

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	Conclusion based on current intake
Structural class I					
Glycerol[c,d]	909	56-81-5 	Yes Europe: 17 000 USA: 220 000	Yes Glycerol is endogenous	Evaluation not finalized
1,2,3-Tris[(1′-ethoxy)-ethoxy]propane	913	67715-82-6 	No Europe: 0 USA: 140	NR	No safety concern
Glyceryl monostearate[c]	918	123-94-4 	No Europe: 0 USA: 230	NR	Evaluation not finalized
Glyceryl monooleate[c]	919	111-03-5 	No Europe: ND USA: 860	NR	
Triacetin[c]	920	102-76-1 	Yes Europe: ND USA: 83 000	Yes Expected to be hydrolysed to glycerol, which is endogenous	

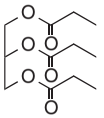
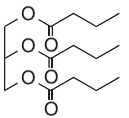
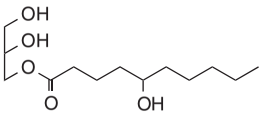
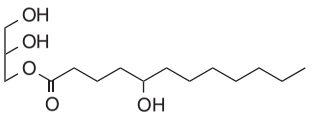
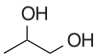
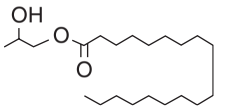
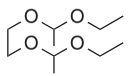
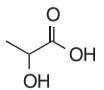
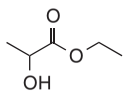
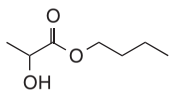
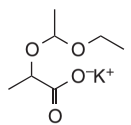
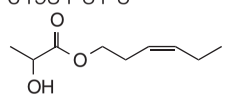
Glyceryl tripropanoate[c]	921	139-45-7 	No Europe: 0.1 USA: 280	NR	Evaluation not finalized
Tributyrin[c]	922	60-01-5 	No Europe: 31 USA: 2	NR	
Glycerol 5-hydroxy-decanoate[c]	923	26446-31-1 	No Europe: 4 USA: 0	NR	
Glycerol 5-hydroxy-dodecanoate[c]	924	26446-32-2 	No Europe: 4 USA: 0	NR	
Propylene glycol[c,e]	925	57-55-6 	Yes Europe: ND USA: 2400000	Yes Expected to be oxidized to lactic acid, which is endogenous	
Propylene glycol stearate[c]	926	142-75-6 	Yes Europe: ND USA: 66000	Yes Expected to be hydrolysed to propylene glycol and subsequently oxidized to lactic acid	

Table 6 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	Conclusion based on current intake
1,2-Di[(1-ethoxy)-ethoxy]propane	927	67715-79-1 	No Europe: 7 USA: 150	NR	
Lactic acid	930	598-82-3 	Yes Europe: ND USA: 47000	Yes Lactic acid is endogenous	
Ethyl lactate[f]	931	97-64-3 	Yes Europe: 1900 USA: 760	Yes Expected to be hydrolysed to lactic acid, which is endogenous	
Butyl lactate	932	138-22-7 	No Europe: 380 USA: 24	NR	No safety concern
Potassium 2-(1′-ethoxy)-ethoxypropanoate	933	100743-68-8 	No Europe: ND USA: 1400	NR	
cis-3-Hexenyl lactate	934	61931-81-5 	No Europe: 38 USA: 5	NR	

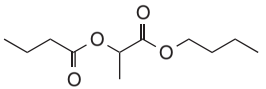
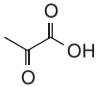
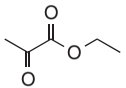
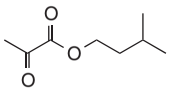
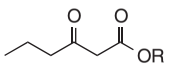
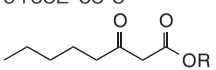
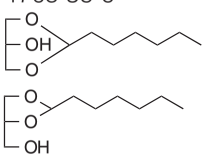
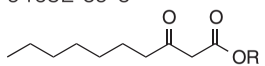
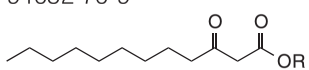
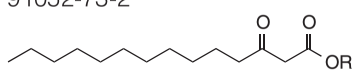
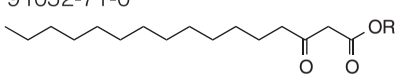
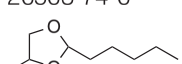
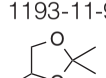
Butyl butyryllactate	935	7492-70-8 	No Europe: 280 USA: 1400	NR	No safety concern
Pyruvic acid	936	127-17-3 	No Europe: 35 USA: 69	NR	
Pyruvaldehyde	937	78-98-8 	No Europe: 120 USA: 3	NR	
Ethyl pyruvate	938	617-35-6 	No Europe: 1 USA: 20	NR	
Isoamyl pyruvate	939	7779-72-8 	No Europe: 0 USA: 0	NR	
Structural class III					
3-Oxohexanoic acid glyceride	910	91052-72-1 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous	No safety concern
3-Oxooctanoic acid glyceride	911	91052-68-5 	No Europe: 34 USA: 0	NR	
Heptanal glyceryl acetal (mixed 1,2 and 1,3 acetals)	912	1708-35-6 	No Europe: 4 USA: 0	NR	

Table 6 (*continued*)

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	Conclusion based on current intake
3-Oxodecanoic acid glyceride[c]	914	91052-69-6 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous	Evaluation not finalized
3-Oxododecanoic acid glyceride[c]	915	91052-70-9 	No Europe: 73 USA: 0	NR	
3-Oxotetradecanoic acid glyceride[c]	916	91052-73-2 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous	
3-Oxohexadecanoic acid glyceride[c]	917	91052-71-0 	No Europe: 43 USA: 0	NR	
4-Methyl-2-pentyl-1,3-dioxolane	928	26563-74-6 	No Europe: 0 USA: 0.2	NR	No safety concern
2,2,4-Trimethyl-1,3-oxyacyclopentane	929	1193-11-9 	No Europe: 0.3 USA: 0.2	NR	

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

[a] *Step 2*: All of the flavouring agents in this group are predicted to be metabolized to innocuous products.

[b] The threshold for human intake for structural classes I and III are 1800 μg/day and 90 μg/day, respectively. All intake values are expressed in μg/day.

[c] Further information is required to determine whether this substance is in current use as a flavouring agent.

[d] An ADI "not specified" was established for glycerol by the Committee at its twentieth meeting (Annex 1, reference *41*), which was maintained at the present meeting.

[e] An ADI of 0–25 mg/kg of body weight was established for propylene glycol by the Committee at its seventeenth meeting (Annex 1, reference *32*), which was maintained at the present meeting.

[f] Ethyl lactate was included in the group ADI "not specified" for lactic acid and its salts that was established by the Committee at its twenty-sixth meeting (Annex 1, reference *59*), which was maintained at the present meeting.

The daily per capita intake of each substance in Europe and the USA is shown in Table 6.

4.1.5.2 Absorption, distribution, metabolism and elimination

The aliphatic esters of propylene glycol (No. 925), lactic acid (No. 930) and pyruvic acid (No. 936) and their parent compounds would all be expected to be readily absorbed from the gut. Hydrolysis of the aliphatic esters is catalysed largely by hepatic esterases, to give the component alcohol and carboxylic acid or aldehyde. After hydrolysis of the glycerol esters in the intestine, glycerol is readily absorbed. Glycerol, pyruvic acid and lactic acid are endogenous in humans. Glycerol and pyruvic acid are metabolized completely and are not excreted. Lactic acid is also largely metabolized, although urinary excretion may occur if the blood concentration is high. Propylene glycol can be metabolized, but high doses are likely to be excreted largely unchanged in the urine.

Glycerol (No. 909) is converted in the liver to glycerol-3-phosphate, which is metabolized via the glycolytic pathway, by oxidation, to yield dihydroxyacetone phosphate, which is isomerized to glyceraldehyde-3-phosphate, eventually yielding pyruvic acid.

Pyruvic acid follows two primary routes of metabolism. Under aerobic conditions, it is converted to acetyl coenzyme A and enters the citric acid cycle. Under anaerobic conditions, primarily in muscles as a result of strenuous physical activity, pyruvic acid is reduced by lactic dehydrogenase to lactic acid.

Lactic acid diffuses through muscle tissue and is transported to the liver in the bloodstream. In the liver, it is converted to glucose by gluconeogenesis. Lactic acid can also be further catabolized in the lactic acid cycle (also known as the Cori cycle).

Propylene glycol can be oxidized to lactic acid via two biochemical pathways. If propylene glycol is phosphorylated, it can be converted to acetol phosphate, lactaldehyde phosphate, lactyl phosphate and then lactic acid. If it is not phosphorylated, propylene glycol is successively oxidized to lactaldehyde, methylglyoxal and lactic acid.

4.1.5.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Twenty-eight of the 31 flavouring agents in this group are linear, simple-branched aliphatic compounds. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1), the Committee assigned 22 of the agents to structural class I because they contain fewer than three types of functional group (Nos 909, 913,

918–927 and 930–939). The Committee assigned six of the substances to structural class III because they contain three or more types of functional group (Nos 910, 911 and 914–917). The three remaining substances were also assigned to structural class III because they are cyclic acetals and ketals (Nos 912, 928 and 929).

Step 2. The data on the metabolism of individual members of the group were sufficient to allow conclusions about their probable metabolic fate. The aliphatic esters of propylene glycol (No. 926), lactic acid (Nos 931, 932, 934 and 935) and pyruvic acid (Nos 938 and 939) can be expected to be hydrolysed to their component alcohols and carboxylic acids. The glycerol esters (Nos 910, 911 and 914–924) can be expected to be hydrolysed to glycerol and carboxylic acids. Esters of propylene glycol can be expected to be hydrolysed to propylene glycol and its component acid. Esters of lactic acid and pyruvic acid can be expected to be hydrolysed to lactic acid and pyruvic acid, respectively, and the corresponding alcohols. The acetals (Nos 912, 913, 927 and 933) can be expected to be hydrolysed to their component alcohols and aldehydes, while the ketals (Nos 928 and 929) can be expected to be hydrolysed to their component ketones and alcohols. Glycerol (No. 909), lactic acid (No. 930) and pyruvic acid (No. 936) are endogeneous and are metabolized through the glycolytic and citric acid pathways. Propylene glycol (No. 925) is oxidized to lactic acid. Because they are predicted to be metabolized to innocuous products, the evaluation of all the substances in this group proceeded via the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 17 of the substances in structural class I and five substances in structural class III are below the threshold of concern for these classes (1800 μg and 90 μg, respectively). The Committee concluded that these substances would not be expected to be of safety concern at their currently estimated levels of use. The daily per capita intakes of six substances in structural class I (Nos 909, 920, 925, 926, 930 and 931) and three substances in structural class III (Nos 910, 914 and 916) exceed the threshold of concern for these classes, and their evaluation therefore proceeded to step A4.

Step A4. Glycerol (No. 909), lactic acid (No. 930) and the hydrolysis products of ethyl lactate (No. 931) are endogenous in humans and their use as flavouring agents is therefore not expected to be of safety concern. Triacetin (No. 920), 3-oxohexanoic acid glyceride (No. 910), 3-oxodecanoic acid glyceride (No. 914) and 3-oxotetradecanoic acid glyceride (No. 916) are glycerol esters and are hydrolysed to glycerol.

Propylene glycol (No. 925) and propylene glycol stearate (No. 926) are not endogenous in humans; however, the ester is expected to be hydrolysed to propylene glycol and stearic acid. Propylene glycol is known to be oxidized to lactic acid in mammals. The safety of these substances would therefore not be expected to be of concern.

4.1.5.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 23 substances in structural class I were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (1800 μg). In the unlikely event that all eight substances in structural class III were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (90 μg). Given that the substances can be expected to be efficiently metabolized by known metabolic pathways, the Committee considered that the combined intake would not give rise to concern about safety.

4.1.5.5 Conclusions

On the basis of the predicted metabolism, the Committee concluded that the safety of the 31 aliphatic acyclic diols, triols and related substances in this group would not raise concern at the currently estimated levels of use as flavouring agents. The Committee noted that all of the available data on toxicity were consistent with the results of the safety evaluations.

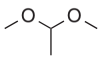
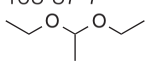
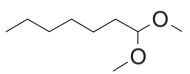
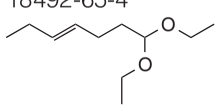
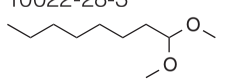
A monograph summarizing the safety data on this group of flavouring agents was prepared.

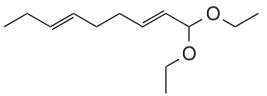
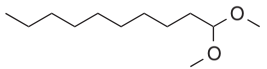
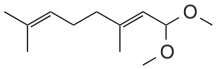
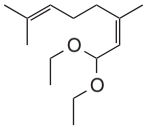
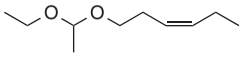
4.1.6 *Aliphatic acyclic acetals*

The Committee evaluated a group of 10 flavouring agents consisting of aliphatic acyclic acetals (Table 7) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). They all have acyclic structures that vary only in the length of their hydrocarbon chains and the number and placement of double bonds. None of these flavouring agents had been evaluated previously by the Committee.

Aliphatic acetals are geminal diethers in which two molar equivalents of alcohol are condensed with an aldehyde. Three of the 10 acetals in this group are formed from acetaldehyde and simple aliphatic alcohols (Nos 940, 941 and 943); the remaining seven acetals are formed from methanol or ethanol and aldehydes of carbon chain-length C7–C10 (Nos 942 and 944–949). Acetals are known to be hydrolysed in vivo to yield the corresponding alcohols and aldehydes. Of the component alcohols (methanol, ethanol and *cis*-3-hexen-1-ol)

Table 7
Summary of results of the safety evaluations of aliphatic acyclic acetals used as flavouring agents[a]

Flavouring agent	No.	CAS number and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
1,1-Dimethoxyethane	940	534-15-6 	No Europe: 71 USA: 11	Metabolized to acetaldehyde and methanol	No safety concern
Acetal	941	105-57-7 	No Europe: 240 USA: 640	Metabolized to acetaldehyde and ethanol	
Heptanal dimethyl acetal	947	10032-05-0 	No Europe: 0.04 USA: 0.26	Metabolized to heptanal and methanol	
4-Heptenal diethyl acetal	949	18492-65-4 	No Europe: 0.04 USA: 0	Metabolized to 4-heptenal and ethanol	
Octanal dimethyl acetal	942	10022-28-3 	No Europe: 1.1 USA: 0	Metabolized to octanal and methanol	

2,6-Nonadienal diethyl acetal	946	67674-36-6 	No Europe: 0.04 USA: 0.01	Metabolized to 2,6-nonadienal and ethanol	No safety concern
Decanal dimethyl acetal	945	7779-41-1 	No Europe: 0.03 USA: 0	Metabolized to decanal and methanol	
Citral dimethyl acetal	944	7549-37-3 	No Europe: 3 USA: 5	Metabolized to citral and methanol	
Citral diethyl acetal	948	7492-66-2 	No Europe: 4 USA: 0	Metabolized to citral and ethanol	
Acetaldehyde ethyl *cis*-3-hexenyl acetal	943	28069-74-1 	No Europe: ND USA: 0	Metabolized to acetaldehyde, ethanol and *cis*-3-hexen-1-ol	

CAS: Chemical Abstracts Service; ND: no data on intake reported.

[a] *Step 1*: All of the flavouring agents in this group are in structural class I.
Step 2: All of the flavouring agents in this group are expected to be metabolized to innocuous products.

[b] The threshold for human intake for structural class I is 1800 μg/day. All intake values are expressed in μg/day.

and aldehydes, acetaldehyde, heptanal, 4-heptenal, octanal and decanal had been considered previously by the Committee at its forty-ninth and fifty-first meetings (Annex 1, references *131* and *137*), when it concluded that their safety was of no concern at currently estimated levels of use as flavouring agents.

Three of the 10 flavouring agents in this group, 1,1-dimethoxyethane (No. 940), acetal (No. 941) and acetaldehyde ethyl *cis*-3-hexenyl acetal (No. 943), have been reported to occur as natural components of foods. They have been detected in orange juice, strawberries, cider, peas, coffee and cognac.

4.1.6.1 Estimated daily per capita intake

The total annual volume of production of the 10 aliphatic acyclic acetals is approximately 2.2 tonnes in Europe and 4.9 tonnes in the USA. About 97% of the total annual volume of production in Europe and 99% of that in the USA is accounted for by 1,1-dimethoxyethane (No. 940), formed from acetaldehyde and methanol, and acetal (No. 941), formed from acetaldehyde and ethanol.

4.1.6.2 Absorption, distribution, metabolism and elimination

In general, aliphatic acetals undergo acid-catalysed hydrolysis to their component aldehydes and alcohols. They are hydrolysed within 1–5 h in simulated gastric fluid in vitro and to a lesser extent in simulated intestinal fluid. Indirect evidence, from a study in which rabbits were given aliphatic acetals in aqueous suspension by stomach tube, indicated that rapid hydrolysis occurs in the stomach. The acetals formed from the reaction of alkyl-substituted pentanal with methanol, ethanol and isopropyl alcohol are metabolized to the corresponding alcohols and acids in rat liver homogenates by an oxidative mechanism involving cytochrome P450 enzymes. Aliphatic acetals can be expected to undergo similar metabolism in humans to the corresponding alcohols and acids. There are insufficient data to exclude the possibility that significant amounts of the parent acetals reach the general circulation; however, the parent compounds are all in structural class I. The low intake resulting from uses of these substances as flavours would not be expected to saturate metabolic enzyme pathways, and the acetals are metabolized to innocuous compounds by hydrolysis or oxidation.

On the basis of their recognized or presumed metabolic fate, the component alcohols and aldehydes can be grouped into three structural classes: linear, aliphatic, primary, saturated and unsaturated alcohols and aldehydes; α,β-unsaturated aldehydes; and branched-chain aliphatic aldehydes. The metabolic detoxication of linear, ali-

phatic, primary alcohols in vivo occurs primarily by oxidation to the corresponding aldehyde, with subsequent oxidation of the aldehyde to the corresponding carboxylic acid. The acid can serve as a substrate for fatty acid oxidation pathways and the citric acid cycle. In general, α,β-unsaturated aldehydes are metabolized by oxidation to the corresponding carboxylic acid, which may then participate in the fatty acid pathway. The aldehyde can also be conjugated with glutathione in a Michael-type addition. Branched-chain aliphatic aldehydes are oxidized primarily to polar metabolites, which are excreted mainly in the urine. The main urinary metabolites of branched aldehydes are diacids and hydroxyacids resulting from ω-oxidation, reduction and hydration of the alkene function and oxidation of the aldehyde function.

Although few studies on the absorption, distribution and elimination of aliphatic acyclic acetals have been reported, the metabolism of the component alcohols and aldehydes has been investigated. These studies are considered relevant to the safety evaluation of orally administered acetals which are expected to be hydrolysed in the acid environment of the stomach.

Citral is predicted to be a metabolite of citral dimethyl acetal (No. 944) and citral diethyl acetal (No. 948). The absorption, distribution and excretion of citral have been studied extensively in rats and mice. Citral underwent rapid absorption from the gut and was distributed uniformly throughout the body. Rapid elimination of citral and its metabolites occurred primarily in the urine and to a minor extent in exhaled air and faeces.

4.1.6.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1), the Committee assigned all 10 of the aliphatic acetals to structural class I.

Step 2. At current levels of intake, all of these flavouring agents can be predicted to be metabolized to their component aldehydes and alcohols, which are then metabolized to innocuous products,[1] and the pathways involved would not be expected to be saturated. Evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

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[1] Some aldehydes, including acetaldehyde, were genotoxic in vitro in a number of test systems, and acetaldehyde has been reported to produce tumours of the respiratory tract in rats and hamsters exposed to high doses by inhalation. The relevance of this observation to oral administration is questionable, as various metabolic processes in the intestinal wall and liver (i.e. oxidation and conjugation) are predicted to result in extensive first-pass metabolic inactivation, especially at the low concentrations expected from use of these substances as flavours.

Step A3. The estimated daily per capita intakes of all 10 substances in this group are below the threshold of concern for structural class I (1800 μg). The Committee concluded that their safety raises no concern at their currently estimated levels of use as flavouring agents.

Table 7 summarizes the results of the evaluations of the aliphatic acetals used as flavouring agents.

4.1.6.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 10 aliphatic acetals were consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for structural class I (1800 μg/day). All flavouring agents in this group are expected to be efficiently metabolized and the available metabolic pathways would not be saturated. Evaluation of all the data indicated there would be no safety concern associated with combined intake.

4.1.6.5 Conclusions

The Committee concluded that the safety of aliphatic acetals would not raise concern at the currently estimated levels of intake. Other data on the toxicity of aliphatic acetals were consistent with the results of the safety evaluation.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.2 Revision of certain specifications for purity

4.2.1 *Flavouring agents with specifications designated as "tentative" at previous meetings*

At its forty-sixth, forty-ninth, fifty-first, fifty-third and fifty-fifth meetings (Annex 1, references *122*, *131*, *137*, *143* and *149*), the Committee evaluated a total of 143 flavouring agents for which further information was required in order to complete the specifications. At its present meeting, the Committee reviewed new data on 95 of these flavouring agents and revised the specifications to take account of the new information. For 83 substances, the "tentative" designation was removed; for the remaining 12, the revised specifications were classified as "tentative" (see Annex 2).

For the remaining 47 substances, no further data were provided that would permit the preparation of satisfactory specifications, according to the criteria identified by the Committee at its fifty-third meeting (Annex 1, reference *143*). The flavour industry has indicated that many of these substances are highly noxious, sulfur-containing compounds which are not routinely handled in the pure form. It is there-

fore impracticable to provide information on, for instance, boiling-point or specific gravity. The Committee concluded that, in such cases, the additional criteria identified at its fifty-third meeting for the establishment of satisfactory specifications should not apply and that only the basic data on chemical identity, minimum assay and identity test were required.

The Committee reiterated that the evaluation of flavouring agents relies on adequate information about the identity and composition of products in commercial use. In future, specifications for flavouring agents will be withdrawn within 2 years of publication of tentative specifications, if the necessary information is not provided.

4.2.2 *Flavouring agents with minimum assay values less than 95%*

At its fifty-third meeting (Annex 1, reference *143*), the Committee announced its intention to re-examine specifications for flavouring agents for which the minimum assay values were <95% (see also section 2.6 of the present report), which were designated as "tentative". At its present meeting, the Committee considered information on all 62 remaining substances in this category with regard to the secondary components that might be present in commercial products.

The available information was sufficient for the Committee to revise the specifications for 27 flavouring agents (including two flavouring agents for which the specifications were revised for other reasons). The Committee therefore removed the "tentative" designations, on the basis of the general principle that no more than 5% of any commercial product should remain undefined after taking account of the flavouring agent and named secondary components.

The Committee will review this group of flavouring agents at a future meeting to confirm whether all the named secondary components are covered by existing safety evaluations. At the same time, the Committee will determine the need for further information to complete those specifications that remain tentative.

4.2.3 *Specifications for flavouring agents being reviewed for safety*

A total of 203 flavouring agents were reviewed for safety at the present meeting. For four of them, specifications had been drawn up at the forty-sixth meeting (Annex 1, reference *122*), and these were revised for use of these substances as flavouring agents. For six flavouring agents, information on specifications was not submitted. These will be considered at a future meeting when such data become available.

New specifications were drawn up for the remaining 193 flavouring agents. For 27 substances, the new specifications were designated as "tentative", either because they failed to meet the criteria drawn up at the fifty-third meeting (Annex 1, reference *143*) or because certain aspects required clarification.

5. Contaminants

5.1 Chloropropanols

Certain chlorinated propanols occur as contaminants in hydrolysed vegetable proteins. Processing of defatted vegetable proteins by traditional hydrochloric acid hydrolysis leads to the formation of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol. These two substances were evaluated by the Committee at its forty-first meeting (Annex 1, reference *107*), when it concluded that they are undesirable contaminants in food and expressed the opinion that their concentrations in hydrolysed vegetable proteins should be reduced as far as is technically achievable. The present evaluations were conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (*4*) for the Expert Committee to re-evaluate 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol on the basis of new data that had become available since the forty-first meeting.

5.1.1 *3-Chloro-1,2-propanediol*

5.1.1.1 Absorption, distribution, metabolism and excretion

3-Chloro-1,2-propanediol crosses the blood–testis barrier and the blood–brain barrier and is widely distributed in the body fluids. The parent compound is partly detoxified by conjugation with glutathione, resulting in excretion of the corresponding mercapturic acid, and is partly oxidized to β-chlorolactic acid and further to oxalic acid. Approximately 30% is broken down to carbon dioxide and exhaled. In the studies from which these data were derived, however, much of the administered dose was not accounted for. Intermediate formation of an epoxide has been postulated but not proven. There is some indication that microbial enzymes can dehalogenate halogenated alcohols to produce glycidol, a known genotoxin in vitro and in vivo.

5.1.1.2 Toxicological studies

The median lethal dose of 3-chloro-1,2-propanediol in rats after oral administration was 150 mg/kg of body weight.

In several studies in which 3-chloro-1,2-propanediol was administered orally to rats as repeated doses of >1 mg/kg of body weight per day, it

decreased sperm motility and impaired male fertility. At doses of ≥10–20 mg/kg of body weight per day, alterations in sperm morphology and epididymal lesions (spermatocoele) were found. The compound reduced fertility in males of several other mammalian species at slightly higher doses than in the rat.

In rats and mice, oral administration of 3-chloro-1,2-propanediol at doses of ≥25 mg/kg of body weight per day was associated with the development of dose-related lesions of the central nervous system, particularly in the brain stem.

In several short-term studies in rats and mice, the kidney was shown to be the target organ for toxicity. In a 4-week study in rats treated by gavage at 30 mg/kg of body weight per day and in a 13-week study in rats given an oral dose of 9 mg/kg of body weight per day, 3-chloro-1,2-propanediol increased the weight of the kidneys relative to body weight.

In the pivotal long-term study in Fischer 344 rats, the absolute weight of the kidney was reported to be significantly increased by administration of 3-chloro-1,2-propanediol in drinking-water, at all doses tested. The incidence of tubule hyperplasia in the kidneys of treated animals of both sexes was also higher than in controls. Although the incidence did not reach statistical significance at the lowest dose tested (1.1 mg/kg of body weight per day), the Committee concluded that it represented part of a compound-related dose–response relationship. Overt nephrotoxicity was seen at higher doses (5.2 and 28 mg/kg of body weight per day).

The results of most assays for mutagenicity in bacteria in vitro were reported to be positive, although negative results were obtained in the presence of an exogenous metabolic activation system from mammalian tissue. The results of assays in mammalian cells in vitro were also generally positive. It should be noted, however, that the concentrations used in all these assays were very high (0.1–9 mg/ml), so that their relevance might be questionable. The weight of the evidence indicates that 3-chloro-1,2-propanediol is not genotoxic in vitro at concentrations at which other toxic effects are not observed. The results of assays conducted in vivo, including a test for micronucleus formation in mouse bone marrow and an assay for unscheduled DNA synthesis in rats, were negative. The Committee concluded that 3-chloro-1,2-propanediol is not genotoxic in vivo.

Four long-term studies of toxicity and carcinogenicity were available. Three (two in mice and one in rats) did not meet modern standards of quality; nevertheless, none of these three studies indicated

carcinogenic activity. In the fourth study, conducted in Fischer 344 rats, oral administration of 3-chloro-1,2-propanediol was associated with increased incidences of benign tumours in some organs. These tumours occurred only at doses greater than those causing renal tubule hyperplasia, which had been selected as the most sensitive end-point.

5.1.1.3 Occurrence

3-Chloro-1,2-propanediol has been detected at concentrations >1 mg/kg in only two food ingredients: acid-hydrolysed vegetable protein and soya sauce. In both ingredients, a range of concentrations has been reported, from below the limit of quantification (0.01 mg/kg) with an analytical method that has been validated in various foods and food ingredients, up to 100 mg/kg in some samples of acid-hydrolysed vegetable protein and more than 300 mg/kg in some samples of soya sauce.

Formation of 3-chloro-1,2-propanediol in acid-hydrolysed vegetable protein has been found to be related to production processes, and the concentration can be reduced markedly by modifying the processes suitably. The source of 3-chloro-1,2-propanediol in soya sauce is being investigated; by analogy with hydrolysed vegetable protein, however, it may arise during acid hydrolysis in the manufacture of some products. Traditionally fermented soya sauces would not be contaminated with 3-chloro-1,2-propanediol.

3-Chloro-1,2-propanediol has also been quantified at concentrations generally <0.1 mg/kg in other foods and food ingredients, notably a number of cereal products that have been subjected to high temperatures during roasting or toasting. Concentrations ≤0.5 mg/kg have been found in food ingredients such as malt extracts, but the resulting concentrations in finished foods were <0.01 mg/kg.

5.1.1.4 Estimates of dietary intake

Information on the concentrations of 3-chloro-1,2-propanediol in foods, food ingredients and protein hydrolysates was submitted by the United Kingdom and the USA and by the International Hydrolyzed Protein Council. The USA supplied a national estimate of the intake of 3-chloro-1,2-propanediol, and information on the consumption of soya sauce in Australia, Japan and the USA was received.

The toxicological studies summarized above indicate that 3-chloro-1,2-propanediol would not be expected to have acute effects at any level of intake that might reasonably be expected. This analysis therefore addressed only long-term intake of 3-chloro-1,2-propanediol from foods.

Intake of 3-chloro-1,2-propanediol would be due predominantly to consumption of contaminated soya sauces. In a survey of 90 samples of commercial soya sauces, 50 samples contained <1 mg/kg, and the average concentration was 18 mg/kg. The results of this survey were taken as representative for all soya sauces for the purposes of the intake assessment.

The mean daily per capita consumption of soya sauce in Australia by persons consuming this product was about 11 g, and that of persons at the 95th percentile of consumption was about 35 g. Thus, the daily per capita intake of 3-chloro-1,2-propanediol would be 200 µg for mean consumption of soya sauce and 630 µg at the 90th percentile of consumption. The estimated mean daily per capita consumption of soya sauce in Japan (equivalent to consumption by consumers only, in view of its widespread use in that country) was about 30 g, resulting in a mean daily per capita intake of 3-chloro-1,2-propanediol of about 540 µg. Intake at the 95th percentile in Japan was estimated to be 1100 µg by assuming twice the mean consumption of soya sauce. The estimated mean daily per capita consumption of soya sauce in the USA by consumers of this product was 8 g, and that of consumers at the 90th percentile of consumption was 16 g. The resulting estimated daily per capita intake of 3-chloro-1,2-propanediol was 140 µg for mean consumption and 290 µg for consumption at the 90th percentile.

The data submitted by the United Kingdom showed that 3-chloro-1,2-propanediol occurs in some savoury foods, with about 30% of samples containing concentrations above the limit of detection of 0.01 mg/kg. The mean residual concentration in these savoury foods was 0.012 mg/kg.

In estimating the intake of 3-chloro-1,2-propanediol from foods other than soya sauce, the Committee assumed that about one-eighth of the diet, i.e. 180 g (on the basis of 1500 g/day of solid food), consists of savoury foods that might contain 3-chloro-1,2-propanediol and that the mean residual concentration of the compound in those foods is 0.012 mg/kg. On this basis, the daily per capita intake of 3-chloro-1,2-propanediol from foods other than soya sauce was estimated to be 2 µg.

5.1.1.5 Evaluation

The Committee chose tubule hyperplasia in the kidney as the most sensitive end-point for deriving a tolerable intake. This effect was seen in the long-term study of toxicity and carcinogenicity in rats in a dose-related manner, although the effect did not reach statistical significance at the lowest dose. The Committee concluded that the

lowest-observed-effect level (LOEL) was 1.1 mg/kg of body weight per day and considered this to be close to a NOEL.

The Committee established a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg of body weight for 3-chloro-1,2-propanediol on the basis of the LOEL of 1.1 mg/kg of body weight per day and a safety factor of 500, which included a factor of 5 for extrapolation from a LOEL to a NOEL. This factor was considered to be adequate to allow for the absence of a clear NOEL and to account for the effects on male fertility and for inadequacies in the studies of reproductive toxicity. Data available to the Committee indicated that the estimated mean intake of 3-chloro-1,2-propanediol by consumers of soya sauce would be at or above this PMTDI.

5.1.1.6 Impact of regulatory limits

As 3-chloro-1,2-propanediol is found infrequently in foods, a regulatory limit would be unlikely to have much effect on the overall intake by persons who do not consume soya sauce. However, because the distribution of residual 3-chloro-1,2-propanediol in soya sauce is highly skewed and because it is likely that brand loyalty could result in regular consumption of highly contaminated brands of soya sauce, a regulatory limit on the concentration of 3-chloro-1,2-propanediol in soya sauce could markedly reduce the intake by consumers of this commodity.

5.1.2 ***1,3-Dichloro-2-propanol***

5.1.2.1 Absorption, distribution, metabolism and excretion

Approximately 5% of an oral dose of 1,3-dichloro-2-propanol was excreted in the urine of rats as β-chlorolactate and about 1% of the dose as 2-propanol-1,3-dimercapturic acid. In another experiment, the urine of rats contained the parent compound (2.4% of the dose), 3-chloro-1,2-propanediol (0.35% of the dose) and 1,2-propanediol (0.43% of the dose). Epoxy-chloropropane (epichlorohydrin) was postulated to be an intermediate, and this compound may either undergo conjugation with glutathione to form mercapturic acid or be hydrolysed to 3-chloro-1,2-propanediol. The latter undergoes oxidation to β-chlorolactate, which is further oxidized to oxalic acid.

5.1.2.2 Toxicological studies

The median lethal dose of 1,3-dichloro-2-propanol in rats treated orally was 120–140 mg/kg of body weight.

In several short-term studies in rats, oral administration of 1,3-dichloro-2-propanol at doses of ≥10 mg/kg of body weight per day

caused significant hepatic toxicity. This was associated with oxidative metabolism, which yielded intermediates that reacted with and depleted glutathione.

In a 13-week study in rats, overt hepatotoxicity, including increased liver weights, histological changes and/or increased activity of serum alanine and aspartate transaminases, was seen after oral administration of 1,3-dichloro-2-propanol at doses of ≥10 mg/kg of body weight per day. These doses also caused histopathological changes in the kidney, increased kidney weights and alterations in urinary parameters. The NOEL was 1 mg/kg of body weight per day.

1,3-Dichloro-2-propanol has been reported to be hepatotoxic in humans exposed occupationally.

1,3-Dichloro-2-propanol was clearly mutagenic and genotoxic in various bacterial and mammalian test systems in vitro. The only available study in vivo showed no mutagenic effect in a wing spot test in *Drosophila melanogaster*.

The results of the one long-term study of toxicity and carcinogenicity in rats confirmed the hepatotoxicity and nephrotoxicity seen in the 13-week study. Furthermore, it demonstrated a clear carcinogenic effect of 1,3-dichloro-2-propanol at the highest dose tested, 19 mg/kg of body weight per day. The tumours (adenomas and carcinomas) occurred in liver, kidney, the oral epithelium and tongue and the thyroid gland. No increase in tumour incidence was seen at the lowest dose tested, 2.1 mg/kg of body weight per day. Treatment-related non-neoplastic lesions of the liver were observed, sinusoidal peliosis being found in all treated groups.

5.1.2.3 Occurrence

Information on the concentrations of 1,3-dichloro-2-propanol in soya sauce was submitted by the USA. Additional information was derived from a published report on the concomitant occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in soya sauces, which showed that 1,3-dichloro-2-propanol may be present at concentrations >1 mg/kg in samples of hydrolysed vegetable protein and soya sauce that contain 3-chloro-1,2-propanediol. In those products in which 1,3-dichloro-2-propanol was quantifiable, the ratio of the concentrations of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol was at least 20.

5.1.2.4 Estimates of dietary intake

A report from the USA was used by the Committee to estimate the intake of 1,3-dichloro-2-propanol present in soya sauces. Information

about the consumption of soya sauce was received from Australia, Japan and the USA.

The toxicological studies summarized above indicate that 1,3-dichloro-2-propanol would not be expected to have acute effects at any level of intake that might reasonably be expected. This analysis therefore addressed only long-term intake of the compound from foods.

The upper-bound 20:1 ratio of 3-chloro-1,2-propanediol:1,3-dichloro-2-propanol was used by the Committee to estimate the intake of 1,3-dichloro-2-propanol from consumption of soya sauce. As the average concentration of 3-chloro-1,2-propanediol in a survey of 90 commercially obtained soya sauce samples was 18 mg/kg, the residual concentration of 1,3-dichloro-2-propanol was assumed to be 0.9 mg/kg.

The mean daily per capita consumption of soya sauce in Australia was approximately 11 g, and that of persons at the 95th percentile of consumption was 35 g. The estimated daily per capita intake was therefore 10 µg for consumers at the mean and 30 µg at the 95th percentile. The daily per capita intake of soya sauce in Japan (equivalent to the consumption by consumers only, in view of its widespread use in that country) was 30 g, resulting in an estimated daily per capita intake of 1,3-dichloro-2-propanol of 27 µg. An intake of 55 µg/person per day was estimated for consumers in the upper percentile of consumption by assuming twice the mean consumption of soya sauce. The estimated mean daily per capita consumption of soya sauce in the USA by consumers of this product was 8 g, and that of consumers at the 90th percentile of consumption was 16 g. The resulting estimated daily per capita intake of 1,3-dichloro-2-propanol was 7 µg at the mean level of consumption and 14 µg at the 90th percentile.

The intake of 1,3-dichloro-2-propanol from foods other than soya sauce can be estimated roughly from data on residual concentrations of 3-chloro-1,2-propanediol in savoury foods and the upper-bound 20:1 ratio of 3-chloro-1,2-propanediol:1,3-dichloro-2-propanol. If it is assumed that about one-eighth of the diet, i.e. 180 g (on the basis of 1500 g/day of solid food), consists of savoury foods that might contain 1,3-dichloro-2-propanol and that the mean residual concentration of the compound in those foods is 0.6 µg/kg, the background daily per capita intake is approximately 0.1 µg.

5.1.2.5 Evaluation

Although only a few studies of kinetics, metabolism, short- and long-term toxicity and reproductive toxicity were available for evaluation,

the results clearly indicated that 1,3-dichloro-2-propanol was genotoxic in vitro, was hepatotoxic and induced a variety of tumours in various organs in rats. The Committee concluded that it would be inappropriate to estimate a tolerable intake because of the nature of the toxicity observed:

- The results of the long-term study of toxicity and carcinogenicity showed significant increases in the incidences of both benign and malignant neoplasms in at least three different tissues.
- It has been shown unequivocally that this contaminant can interact with chromosomes and/or DNA; however, the tests were confined to bacterial and mammalian test systems in vitro, and there were no data on intact mammalian organisms or humans.

The Committee noted that the dose that caused tumours in rats (19 mg/kg of body weight per day) was about 20000 times the highest estimated intake of 1,3-dichloro-2-propanol by consumers of soya sauce (1 µg/kg of body weight per day).

The available evidence suggests that 1,3-dichloro-2-propanol is associated with high concentrations of 3-chloro-1,2-propandiol in food. Regulatory control of the latter would therefore obviate the need for specific controls on 1,3-dichloro-2-propanol.

5.2 Polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls

5.2.1 *Introduction*

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are by-products of combustion and of various industrial processes, and they are found widely in the environment. Polychlorinated biphenyls (PCBs) were manufactured in the past for a variety of industrial uses, notably as electrical insulators or dielectric fluids and specialized hydraulic fluids. Most countries banned manufacture and use of PCBs in the 1970s; however, past improper handling of PCBs constitutes a continuing source of these compounds in the environment, and disposal of equipment containing these compounds poses some risk of further contamination.

Neither PCDDs nor PCDFs have been evaluated previously by the Committee. PCBs were evaluated by the Committee at its thirty-fifth meeting (Annex 1, reference *88*), when it concluded that it was impossible to establish a precise numerical value for a tolerable intake in humans because of limitations in the available data and the ill-defined nature of the materials that were used in feeding studies.

PCDDs, PCDFs and coplanar PCBs were evaluated at the present meeting in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (*4*) for the Expert Committee to evaluate the risks associated with their presence in food.

The Committee evaluated the PCDDs, PCDFs and coplanar PCBs for which toxic equivalency factors (TEFs) for mammals have been derived by WHO. Table 8 lists the compounds that were considered

Table 8
Compounds considered and their toxic equivalency factor assigned by WHO

Compound	Abbreviation	Toxic equivalency factor
Polychlorinated dibenzodioxins		
2,3,7,8-Tetrachlorodibenzodioxin	TCDD	1
1,2,3,7,8-Pentachlorodibenzodioxin	1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-Hexachlorodibenzodioxin	1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-Hexachlorodibenzodioxin	1,2,3,6,7,8-HxCDD	0.1
1,2,3,6,7,9-Hexachlorodibenzodioxin	1,2,3,6,7,9-HxCDD	0.1
1,2,3,4,6,7,8-Heptachlorodibenzodioxin	1,2,3,4,6,7,8-HpCDD	0.01
Octachlorodibenzodioxin	OCDD	0.0001
Polychlorinated dibenzofurans		
2,3,7,8-Tetrachlorodibenzofuran	2,3,7,8-TCDF	0.1
1,2,3,7,8-Pentachlorodibenzofuran	1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-Pentachlorodibenzofuran	2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-Hexachlorodibenzofuran	1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-Hexachlorodibenzofuran	1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-Hexachlorodibenzofuran	1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-Hexachlorodibenzofuran	2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-Heptachlorodibenzofuran	1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-Heptachlorodibenzofuran	1,2,3,4,7,8,9-HpCDF	0.01
Octachlorodibenzofuran	OCDF	0.0001
"Non-ortho" polychlorinated biphenyls		
3,3′,4,4′-Tetrachlorobiphenyl	3,3′,4,4′-TCB	0.0001
3,4,4′,5,-Tetrachlorobiphenyl	3,4,4′,5-TCB	0.0001
3,3′,4,4′,5-Pentachlorobiphenyl	3,3′,4,4′,5-PeCB	0.1
3,3′,4,4′,5,5′-Hexachlorobiphenyl	3,3′,4,4′,5,5′-HxCB	0.01
"Mono-ortho" polychlorinated biphenyls		
2,3,3′,4,4′-Pentachlorobiphenyl	2,3,3′,4,4′-PeCB	0.0001
2,3,4,4′,5-Pentachlorobiphenyl	2,3,4,4′,5-PeCB	0.0005
2,3′,4,4′,5-Pentachlorobiphenyl	2,3′,4,4′,5-PeCB	0.0001
2,3′,4,4′,5′-Pentachlorobiphenyl	2,3′,4,4′,5′-PeCB	0.0001
2,3,3′,4,4′,5-Hexachlorobiphenyl	2,3,3′,4,4′,5-HxCB	0.0005
2,3,3′,4,4′,5′-Hexachlorobiphenyl	2,3,3′,4,4′,5′-HxCB	0.0005
2,3′,4,4′,5,5′-Hexachlorobiphenyl	2,3′,4,4′,5,5′-HxCB	0.00001
2,3,3′,4,4′,5,5′-Heptachlorobiphenyl	2,3,3′,4,4′,5,5′-HpCB	0.00001

and their assigned TEF values. In the TEF approach, the toxicity of all chemicals in the series is related to that of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), one of the most potent of the dioxins and that for which most toxicological and epidemiological information is available. Use of the TEF approach is based on the assumption that PCDDs, PCDFs and coplanar PCBs have a common mechanism of action, which involves binding to the aryl hydrocarbon (Ah) receptor, an intracellular receptor protein. This binding is considered to be the necessary, but not sufficient, first step in the expression of the toxicity of these compounds. Many uncertainties exist in applying the TEF approach to the assessment of human risk, but it is the most feasible approach currently available.

PCDDs, PCDFs and coplanar PCBs were considered by a WHO consultation held in 1998 (*5*), which established a tolerable daily intake (TDI) of 1–4 pg/kg of body weight, applicable to the toxic equivalents of these compounds. The TDI was based on a number of studies of developmental toxicity and immunological toxicity. At its present meeting, the Committee used the assessment of the consultation as the starting point for its evaluation, taking into account the following additional data:

— the results of a study on the toxicokinetics of TCDD after single and repeated dosing;
— two new studies of developmental toxicity;
— new information on a study in rhesus monkeys that had been evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference *149*).

5.2.2 *Toxicokinetics*

5.2.2.1 Absorption and biotransformation

Coplanar compounds in dietary fat pass easily from the gut into the blood. Indeed, experiments in humans and laboratory animals given an oral dose of TCDD showed 50–90% absorption. This figure is comparable with the near-complete absorption of PCDDs, PCDFs and PCBs by nursing infants from their mothers' milk.

After absorption from the gut, TCDD enters the lymph in the form of chylomicrons and is cleared from the blood within 1 h, to appear mainly (74–81% of an administered dose) in the liver and adipose tissue. After clearance from the blood, coplanar compounds remain mainly in serum lipoproteins (very low density, low density and high density), and some are bound to serum proteins.

The Committee used the results of a study in which [^{3}H]TCDD was given to pregnant Long-Evans rats by gavage as a single dose of 50,

200, 800 or 1000 ng/kg of body weight on day 15 of gestation, and the concentration of the radiolabel measured in tissues 1 day after treatment. The average maternal body burdens (with the percentage of the dose in the four treatment groups) were 31 (60%), 97 (48%), 520 (65%) and 580 (59%) ng/kg of body weight, respectively. On the basis of this study, the Committee used a value of 60% for the percentage of TCDD retained in pregnant rats 1 day after administration of a single dose by gavage on day 15 of gestation.

The distribution of PCDDs and PCDFs between the blood and organs is governed by lipid partitioning and binding to plasma proteins. The concentrations of PCDDs and PCDFs in blood and adipose tissue are closely correlated. TCDD is distributed between blood and adipose tissue by lipid partitioning, whereas the distribution of hexachlorodibenzodioxins (HxCDDs), hexachlorodibenzofurans (HxCDFs), octachlorodibenzodioxins (OCDDs) and octachlorodibenzofurans (OCDFs) is also governed by binding to plasma proteins.

Binding to plasma proteins plays an important role in the uptake of coplanar compounds from the blood in the liver, even for lower chlorinated congeners. When rodents are exposed to increasing doses of TCDD, it is preferentially sequestered in the liver. After entering liver cells, TCDD either dissolves in the lipid fraction or binds to the Ah receptor or to cytochrome P450 (CYP) proteins, probably microsomal CYP 1A2. As the amounts of CYP 1A and CYP 1B proteins in cells are regulated by formation of the TCDD–Ah receptor complex, exposure to increasing amounts of TCDD results in increased formation of this complex, which leads to increased production of CYP 1A and CYP 1B mRNA and proteins (enzyme induction), and accumulation of TCDD by increased binding to the induced CYP proteins. Similar sequestration has been observed with higher chlorinated PCDDs and PCDFs and with coplanar PCBs.

The hepatic sequestration of coplanar compounds markedly affects their distribution in the body. For example, whereas the liver usually contributes 10% and the adipose tissue 60% of the body burden of TCDD in mice, these fractions may increase to 67% in liver and decrease to 23% in adipose tissue in mice in which hepatic CYP proteins have been fully induced. Similar results were found in rats, clearly indicating the non-linear character of the kinetics of TCDD at concentrations that induce hepatic CYP proteins.

As in rodents, preferential sequestration of PCDDs and PCDFs in the liver rather than in adipose tissue has been observed in humans exposed to background concentrations of these compounds. Although Ah receptor-dependent CYP induction has been observed in

human liver cells in vitro after exposure to TCDD, it occurred at concentrations that were several orders of magnitude higher than those observed in human blood. It is therefore likely that the sequestration is due to binding to constitutive CYP proteins.

5.2.2.2 Metabolism and excretion

In laboratory animals, PCDDs and PCDFs are excreted almost exclusively in the bile, excretion in the urine being a minor route. Whereas the parent compound is found primarily in the organs of rodents, only metabolites of PCDDs and PCDFs occur in bile, indicating hepatic metabolism, including hydroxylation and conjugation, of these compounds. Similar reactions were found in vitro when recombinant human CYP 1A1 was incubated with TCDD. Faecal excretion of unmetabolized PCDDs and PCDFs is also an important route of elimination in humans.

In rodents, the half-life of TCDD ranges from 8–24 days in mice to 16–28 days in rats. Humans eliminate PCDDs and PCDFs more slowly, the estimated mean half-life of TCDD ranging from 5.5 to 11 years. The half-lives of other PCDD congeners and of PCDFs and coplanar PCBs vary widely. These differences in the half-lives of different congeners are reflected in their TEFs (see Table 8).

5.2.2.3 Relationship between human intake and doses used in studies in laboratory animals

The biochemical and toxicological effects of PCDDs, PCDFs and coplanar PCBs are directly related to their concentrations in tissues, and not to the daily dose. The most appropriate measure of dose would therefore be the concentration at the target tissue; however, this is seldom known. The body burden, which is strongly correlated with the concentrations in tissue and serum, integrates the differences in half-lives between species. Thus, rodents require appreciably higher daily doses (100–200-fold) to achieve a body burden at steady state that is equivalent to that recorded in humans exposed to background concentrations. Toxicokinetically, estimates of body burden are therefore more appropriate measures of dose for interspecies comparisons than is the daily dose.

The long half-lives of PCDDs, PCDFs and coplanar PCBs in humans have several implications for the period of intake that is relevant to the assessment. First, the concentration of toxic equivalents in the body (or the internal toxic equivalents to which a target organ is exposed) will increase over time as more of the compounds are ingested. Second, after cessation of exposure, the body's concentration of stored toxic equivalents (and the exposure of internal organs) will

decline slowly, only half of the accumulated toxic equivalents disappearing over about 7 years, resulting in a pseudo-steady state only after decades. Third, because of this long-term storage in the body and the consequent daily exposure to the body's stored toxic equivalents, intake on a particular day will have a small or even negligible effect on the overall body burden. For example, in the unlikely event of food contamination that leads to an intake 100 times the amount present in a typical meal, the body burden of the adult eating that meal would increase by <3%. The rest of the body burden would be made up of the PCDDs, PCDFs and coplanar PCBs consumed in many thousands of meals over the previous decade or more.

Therefore, the Committee concluded that the appropriate period for evaluating the mean intake of these compounds is 1 month.

In order to transform an animal body burden into an equivalent human monthly intake (EHMI) that on a long-term basis would result in a similar body burden (at steady state), simple, classical toxicokinetic calculations can be used. The elimination of low doses of PCDDs was considered to follow first-order kinetics and to be independent of the body burden or dose. The Committee calculated the total body burden at steady-state using the following equation:

$$\text{Body burden at steady state (ng/kg of body weight)} = \frac{f \times \text{intake (ng/kg of body weight per day)} \times \text{half-life (days)}}{\ln(2)}$$

where f is the fraction of dose absorbed from food (assumed to be 50% in humans) and the estimated half-life of TCDD is 2774 days (7.6 years). For compounds that follow first-order kinetics, 4–5 half-lives will be required to approach steady state. For TCDD, this would be equivalent to more than 30 years.

This model is based on the assumption that PCDDs are distributed in only one compartment: the whole body. Although most of the body burden of PCDD is distributed in the lipid stores, at higher doses the liver also sequesters these compounds in both humans and animals. Predictions of body burden after intake of high doses that are based on lipid concentrations may therefore be underestimates of the total body burden (and the intake leading to that body burden), because of hepatic sequestration. Use of physiologically based pharmacokinetic models may be more appropriate under these circumstances. In order to transform the body burdens resulting from intake of the low concentrations to which the general population is exposed and from the low doses used in the pivotal toxicological studies into estimated

human daily intake, the Committee considered use of a less complicated, classical pharmacokinetic model to be appropriate.

5.2.2.4 Determinants of dose received by fetuses in studies of developmental toxicity

The time of dosing in several of the studies considered by the Committee, day 15 of gestation, marks the onset of the sensitive phase of sexual differentiation in rats and represents a critical time of fetal exposure. The determinant of the reproductive effects is the fetal concentration on days 15–16 of gestation, which in turn is determined by the maternal serum concentration. The latter concentration differs with a bolus dose (as in these studies) and with repeated doses providing the same total intake. As the serum concentration of TCDD after a bolus dose rises before distribution to the tissue compartments, the serum concentration is likely to be higher than that after long-term intake of a lower concentration.

The difference in the fetal body burden after a single bolus dose and after repeated administration of low doses resulting in a similar maternal body burden was addressed in a study in Long-Evans rats treated on day 16 of gestation (*6*, *7*). The rats were given [^{3}H]TCDD at 1, 10 or 30 ng/kg of body weight per day by gavage in corn oil, on 5 days per week for 13 weeks. They were then mated, and dosing was continued daily throughout gestation. The regimen produced a steady-state concentration of TCDD in the dams. The average maternal and fetal body burdens on day 16 of gestation after this treatment and after administration of a single dose of TCDD by gavage on day 15 of gestation are shown in Table 9.

As expected, a single dose on day 15 of gestation by gavage resulted in considerably higher fetal concentrations on day 16 than short-term administration of low daily doses leading to maternal steady-state body burdens of similar magnitude.

Using the data in Table 9, the Committee conducted least-squares linear fits of dose versus maternal and fetal body burdens. Since radiolabelled TCDD was used in both studies, a zero intercept was assumed for the fitted line. None of these fits showed what appeared to be any significant deviation from linearity. These data indicate that the ratio of fetal:maternal body burden resulting from a bolus dose would be 1.7 times that from multiple doses providing the same total dose. Kinetic data indicate that a linear dose–response relationship would be expected at the doses used in these studies. The fetal and maternal body burdens in both data sets were also fitted to power equations, which provided a better fit of the data obtained at the low end of the range of single doses. The factor used to convert maternal

Table 9
Average maternal and fetal body burdens after a single dose and after administration of repeated doses of TCDD to pregnant Long-Evans rats

Dose (ng/kg of body weight per day)	Body burden on day 16 of gestation (ng/kg of body weight per day)	
	Maternal	Fetal
Single dose		
50	30	5.3
200	97	13
800	520	39
1000	520	56
Repeated doses[a]		
0.71	20	1.4
7.1	120	7.5
21	300	15

Source: references *6* and *7*; used by permission.
[a] Daily dose, adjusted for continuous administration from 5 to 7 days per week.

body burden after single doses to a corresponding steady-state body burden with the power equations was 2.6.

5.2.3 *Toxicological studies*

5.2.3.1 Acute toxicity studies

In laboratory animals, the acute toxicity of TCDD and related PCDDs and PCDFs substituted in at least the C-2, C-3, C-7 and C-8 positions varies widely between and among species. For example, the median lethal dose in guinea-pigs treated orally was 0.6 μg/kg of body weight, while that in hamsters was >5000 μg/kg of body weight. Explanations for this variation include differences in Ah receptor functionality (size, transformation and binding of the PCDD response element), toxicokinetics (metabolic capacity and tissue distribution) and body fat content. While data on acute toxicity were available for various commercial PCB mixtures (median lethal doses usually >100 mg/kg of body weight), the data on individual coplanar PCB congeners in mammals were limited.

One of the more common symptoms associated with lethality induced by PCDDs is a generalized delayed wasting syndrome characterized by inhibition of gluconeogenesis, reduced feed intake and loss of body weight. Other toxic effects observed after a single exposure to PCDDs include haemorrhages in a number of organs, thymic atrophy, reduced bone-marrow cellularity and loss of body fat and lean muscle mass, although some differences in the frequency of these effects was seen among species.

5.2.3.2 Carcinogenicity studies

TCDD and other PCDDs induced tumours at multiple sites in laboratory animal species of each sex. In a series of assays in vivo and in vitro, TCDD promoted the growth of transformed cells (e.g. rat tracheal epithelium cells treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine), consistent with observations of cancer promotion in whole animals in vivo. In a long-term study of carcinogenicity with TCDD in rats, the LOEL for hepatic adenomas in females was 10 ng/kg of body weight per day, and the NOEL was 1 ng/kg of body weight per day. Several studies have shown that TCDD promotes tumours in laboratory animals, in particular liver tumours. Several other PCDDs, PCDFs and non-*ortho*- and mono-*ortho*-PCBs also promoted liver tumours. In a long-term study in rats in which the incidence of liver tumours was increased over that in controls, the LOEL of 10 ng/kg of body weight per day corresponded to a steady-state body burden of 290 ng/kg of body weight. In order for humans to attain a similar steady-state body burden, they would have to have a daily intake of 150 pg/kg of body weight (see the equation on page 126).

5.2.3.3 Genotoxicity studies

The results of several short-term assays for genotoxicity with TCDD, covering various end-points, were negative. Furthermore, TCDD did not bind covalently to DNA from the liver of mice. The Committee concluded that TCDD does not initiate carcinogenesis.

5.2.3.4 Developmental toxicity studies

A number of biochemical changes, including enzyme induction, altered expression of growth factors and enhanced oxidative stress, have been noted in laboratory animals with body burdens of TCDD within a lower range of 3–10 ng/kg of body weight. The Committee considered these biochemical effects to be early markers of exposure to PCDDs, PCDFs and coplanar PCBs, or events induced by these compounds in animals and in humans that may or may not result in adverse effects at higher body burdens.

The Committee reviewed the relevant studies (*8–14*) considered by the WHO consultation held in 1998 (*4*), as well as three recent studies (*15–17*). The Committee noted that the most sensitive adverse effects reported were on development in the male offspring of rats and immunological deficits in rats after prenatal exposure to TCDD (see Table 10).

The WHO consultation identified a study in which endometriosis was found after long-term administration of TCDD to rhesus monkeys. The Committee stressed that the findings in this study should be

Table 10
Summaries of studies in which the lowest NOELs and LOELs were identified for the most sensitive adverse effects of TCDD on developmental end-points in rats[a]

Dosing regimen	Strain	End-point	NOEL body burden (ng/kg of body weight)	LOEL body burden (ng/kg of body weight)	Reference no.
Single bolus by gavage on day 14 of gestation	Fischer 344	Immune suppression in offspring		50	*8*, *15*
Single bolus by gavage on day 15 of gestation	Holtzman	Reduced ventral prostate weight; decreased anogenital distance in male offspring	13	51	*17*
Single bolus by gavage on day 15 of gestation	Holtzman	Decreased sperm count in offspring		28	*13*
Single bolus by gavage on day 15 of gestation	Long-Evans	Accelerated eye opening and decreased sperm count in offspring		28	*9*
Loading and maintenance doses by subcutaneous injection	Wistar	Decreased sperm production and altered sexual behaviour in male offspring		25	*16*

[a] Body burdens estimated from a linear fit to the data in Table 9.

interpreted with caution, as the daily intake was not adequately reported. In addition, analyses conducted 13 years after the end of exposure showed high concentrations of coplanar PCBs in the blood of the monkeys with endometriosis, possibly from an unknown source (*14*). The Committee also noted that the LOELs in some of the pivotal studies in rats (Table 10) would result in EHMIs that were similar to or lower than that derived from the LOEL for endometriosis in monkeys.

In a recent study (*17*), pregnant Holtzman rats were given a single oral dose of TCDD at 0–800 ng/kg of body weight on day 15 of gestation, and the male offspring were examined on days 49 and 120 after birth. No changes were seen in testicular or epididymal weight nor in daily sperm production or sperm reserve at any dose. However, the weight of the urogenital complex, including the ventral prostate, was significantly reduced at doses of 200 and 800 ng/kg of body weight in rats killed on day 120. Moreover, the anogenital distance of male rats receiving doses ≥ 50 ng/kg of body weight and killed on day 20 was significantly decreased. The Committee noted that administration of TCDD at any dose resulted in a dose-dependent increase in 5α-reductase type 2 mRNA and a decrease in androgen receptor mRNA in the ventral prostate of rats killed at day 49 but not in those killed at day 120, with no adverse sequelae at the lowest dose of 12.5 ng/kg of body weight. On the basis of 60% absorption and an assumption of a linear relationship for the data in Table 9, the equivalent maternal body burden after multiple doses at this NOEL would be 13 ng/kg of body weight. Fitting the data in Table 9 into the power equation, the Committee estimated the body burden NOEL to be 19 ng/kg of body weight. The LOEL of 50 ng/kg of body weight per day corresponds to an equivalent body burden of 51 ng/kg of body weight with the linear model and 76 ng/kg of body weight with the power model.

The lowest LOEL reported for the reproductive system of male offspring was found in an experiment with Wistar rats (*16*). In this study, the dams were treated subcutaneously before mating and throughout mating, pregnancy and lactation. They received an initial loading dose of [^{14}C]TCDD at 25, 60 or 300 ng/kg of body weight 2 weeks before mating, and then a weekly maintenance dose of TCDD at 5, 12 or 60 ng/kg of body weight. The size of the maintenance doses was determined on the basis of a reported elimination half-life for TCDD of 3 weeks in adult rats. The effects on male reproductive end-points were studied on days 70 and 170 after birth. The number of sperm per cauda epididymis at puberty and in adulthood was lower in the offspring of all treated dams than in those of controls. Daily sperm production was permanently lower in offspring of treated dams than

in those of controls, as was the sperm transit rate, thus increasing the time required by the sperm to pass through the cauda epididymis. Moreover, the offspring of the treated groups showed increased numbers of abnormal sperm when investigated in adulthood. The latency periods to mounting and intromission were significantly greater in offspring of dams at the lowest and highest doses, but not of those at the intermediate dose, than in offspring of controls. The Committee noted the lack of clear dose–response relationships for most of these effects in the treated groups. In the male offspring of dams at the highest dose, the concentration of serum testosterone was decreased in adulthood, and permanent changes found in the testicular tubuli included pyknotic nuclei and the presence of cell debris in the lumen. The fertility of the male offspring was not affected in any of the treated groups.

In computing the long-term dose required to produce the fetal concentration found in the group given the initial loading dose of 25 ng/kg of body weight, the Committee noted that the dose would have been reduced to 20 ng/kg of body weight before the maintenance dose of 5 ng/kg of body weight given on day 14. On the basis of the linear fit to the data in Table 9, the fetal body burden resulting from the maternal body burden of 20 ng/kg of body weight would be 1.04 ng/kg of body weight. The maintenance dose of 5 ng/kg of body weight administered on day 14 of gestation would make an additional contribution to the fetal body burden of 0.27 ng/kg of body weight, resulting in a total fetal body burden of 1.31 ng/kg of body weight. On the basis of a linear fit to the data in Table 9, a maternal body burden of TCDD of 25 ng/kg of body weight at steady state would be required to produce this fetal body burden.

The studies summarized in Table 10 provide evidence that adverse effects on the reproductive system are induced in male offspring of pregnant rats given TCDD. The studies show reductions in daily sperm production, in the number of sperm in the cauda epididymides and in epididymal weight as well as accelerated eye opening, a reduction in anogenital distance and feminized sexual behaviour in male offspring associated with maternal steady-state body burdens of TCDD of ≥25 ng/kg of body weight. Reductions in the weights of the testes and the size of the sex accessory glands, such as the ventral prostate, in male offspring, development of external malformations of the genitalia in female offspring and reduced fertility in males and females required higher maternal body burdens.

The Committee noted that the most sensitive end-points differed between studies, perhaps reflecting strain differences in sensitivity and even minor differences in the experimental conditions, e.g. the

diet. The Committee also noted that, in one study, administration of a single dose of TCDD at 12.5 ng/kg of body weight to dams by gavage decreased the amount of androgen receptor mRNA in the ventral prostate of offspring at puberty on day 49 after birth, indicating reduced androgenic responsiveness. However, none of the other above-mentioned adverse effects were seen in male offspring at this dose, which corresponds to an estimated maternal steady-state body burden of TCDD of approximately 19 ng/kg of body weight (Table 10). The Committee considered the effect on androgenic responsiveness to be an early marker of exposure to TCDD, like enzyme induction, altered expression of growth factors and enhanced oxidative stress, or an event that may or may not result in adverse effects in animals at higher body burdens.

5.2.4 *Observations in humans*

5.2.4.1 Effects other than cancer

In two episodes of food poisoning in China (Province of Taiwan) and Japan, in which infants were exposed in utero to heat-degraded PCBs, a variety of adverse physical developmental abnormalities was observed, including decreased penis length and alterations of spermatozoa; neurodevelopmental abnormalities were also seen. The affected children in Taiwan, China, were born to mothers with estimated body burdens of toxic equivalents of PCBs of 2–3 µg/kg of body weight.

Environmental or background exposure of infants in Germany, the Netherlands and the USA was evaluated in several studies; for example, the mean concentration of toxic equivalents in human milk was 60 pg/g of lipid (range 25–155 pg/g) in a study in Groningen and Rotterdam, the Netherlands. Low birth weight and detriments in neurological development and alterations in thyroid hormones, the distribution of lymphocyte subpopulations and the frequency of infections and respiratory symptoms were observed. The observed neurodevelopmental deficits were subtle and the prevalence within the normal range; their potential consequences for future intellectual function are unknown. The associations observed were considered to be due to prenatal exposure rather than to postnatal intake (from milk). In one study of breastfed and bottle-fed infants, the intake of PCDDs and PCBs was inversely related to performance in neurobehavioural tests, breastfed infants having better scores than bottle-fed infants. These studies of low exposure related primarily to PCBs, and fewer data were available on the effects of PCDDs and PCDFs.

In adults, most of the effects other than cancer observed after exposure to PCDDs, PCDFs and coplanar PCBs, such as chloracne,

appeared only at doses several orders of magnitude greater than those generally received from background contamination of foods. In Seveso, Italy, more female children than expected were born to fathers who had serum TCDD concentrations >80 pg/g of lipid (16–20 ng/kg of body weight) at the time of conception.

5.2.4.2 Carcinogenicity

A working group convened by the International Agency for Research on Cancer (IARC) classified TCDD as a human carcinogen (Group 1). Other PCDDs and PCDFs were considered not to be classifiable as to their carcinogenicity to humans (Group 3).

The most informative studies for evaluating the carcinogenicity of TCDD are four cohort studies of herbicide producers (two in Germany and one each in the Netherlands and the USA) and one cohort study of residents of a contaminated area in Seveso, Italy. A multicountry cohort study from IARC included three of these four cohorts, other industrial cohorts, many of which had not been reported in separate publications, and a cohort of professional herbicide applicators.

In most of the epidemiological studies considered, exposure had been primarily to TCDD, with some exposure to mixtures of other PCDDs, as contaminants of phenoxy herbicides and chlorophenols. The studies involved persons with the highest recorded exposure to TCDD, the estimated geometric mean blood lipid concentrations after the last exposure ranging from 1100 to 2300 pg/g of lipid in the industrial cohorts; lower average concentrations were found in the population exposed in Seveso.

Low excess risks of the order of 40% were found for all neoplasms combined in all the studies of industrial cohorts in which the exposure assessment was adequate. The risks for cancers at specific sites were increased in some of the studies, but the results were not consistent between studies, and no single cancer site seemed to predominate. The results of tests for trends for increasing excess risks for all neoplasms with increasing intensity of exposure were statistically significant. Increasing risks for all neoplasms with time since first exposure were observed in those studies in which latency was evaluated. The follow-up of the Seveso cohort has so far been shorter than that of the industrial cohorts; however, the rate of death from all cancers has not been found to differ significantly from that expected in the general population. Excess risks were seen for cancers at some specific sites among persons in the most heavily contaminated zones at the time of the accident, but there were few cases.

In these well-conducted cohort studies, the intensity of exposure could be ascertained with precision because of the long biological half-life of TCDD in human tissues, and the relative risks increased significantly with increasing exposure. Although the excess cancer risk at the highest exposure was statistically significant, these results must be evaluated with caution, as the overall risks are not high and the strongest evidence is for industrial populations whose exposure was two to three orders of magnitude greater than that of the general population, and who also had heavy exposure to other chemicals; furthermore, lifestyle factors such as smoking were not evaluated. There are few precedents of carcinogens that increase the risk for cancer at all sites combined, with no excess risk for any specific tumour predominating.

A "benchmark dose" was calculated from the effective dose estimated to result in a 1% increase in cancer mortality (ED_{01}), on the basis of a meta-analysis of data for three industrial cohorts with well-documented exposure and comparison with the doses required for effects other than cancer. A statistically significant linear trend in risk with intensity of exposure was observed, which persisted even after exclusion of the groups with the greatest exposure. Within the range of reasonable assumptions, the ED_{01} differed quite widely and depended strongly on the assumptions made. Furthermore, a number of uncertainties would influence the predicted ED_{01}, including the exact exposure of the occupational cohorts and, to a lesser extent, the potential confounding effects of factors not considered in the studies.

5.2.5 *Sampling and analytical methods*

As no specific guidelines have been drawn up for sampling foods to be analysed for their PCDD, PCDF and coplanar PCB content, the basic guidelines for sampling for organic contaminants or pesticides should be used. The objective is to obtain a representative, homogeneous laboratory sample without introducing secondary contamination. Although PCDDs, PCDFs and coplanar PCBs are chemically stable, the samples should be stored and transported in such a way that they do not deteriorate. PCDDs, PCDFs and coplanar PCBs are usually found as complex mixtures of varying composition in different matrices. Their identification and quantification require a highly sophisticated method of analysis in order to separate the toxic congeners listed in Table 8 from the more prevalent, less toxic congeners. Usually, PCDDs, PCDFs and coplanar PCBs are determined by capillary gas chromatography with mass spectrometry.

No official method exists for the determination of these compounds in food. Reliable results have been obtained in the absence of official

methods when the method used has been shown to be suitable and to fulfil analytical quality criteria developed in other fields of residue analyses. The methods used to determine PCDDs and PCDFs in food must provide sufficient information to allow calculation of the results as toxic equivalents, at concentrations of 0.1–1 pg/g of fat in milk, meat and eggs, 10 pg/g of fat in fish or ≥100 pg/g of fat in cases of heavier contamination, and 0.1–0.5 pg/g of dry matter in food of vegetable origin. The patterns of congeners can vary between regions and foods.

When the method used is of insufficient sensitivity, the concentrations of PCDDs, PCDFs and coplanar PCBs in many foods may be near or below the limit of quantification. The method used to derive the concentrations of undetected congeners (the imputation method) can therefore have a variable effect on the summary toxic equivalent value for a food sample. In the most commonly used imputation methods, the contribution of each undetected congener to the toxic equivalent is considered to be either 0 ("lower-bound concentrations"), the limit of detection or limit of quantification ("upper-bound concentrations") or half the limit of detection or limit of determination. In methods with insufficient sensitivity, the lower- and upper-bound concentrations can differ by a factor of 10–100 or even more. If the sensitivity is appropriate, the differences between lower- and upper-bound concentrations are negligible. Therefore, low estimates of PCDDs, PCDFs and coplanar PCBs in a sample may represent truly low concentrations or be the result of use of zero as the value for undetected congeners in a food sample. Conversely, high estimates may be the result either of contamination or of use of the upper-bound concept with insufficient sensitivity.

Application of upper-bound or lower-bound concentrations leads to over- and underestimates of intake, respectively. Therefore, the Committee recommended that laboratories report their results as lower-bound, upper-bound and half-detection limits, in addition to values for individual congeners, thus providing all the necessary information for interpreting the results for specific requirements. Experts who are summarizing results based on toxic equivalents should indicate the way in which the toxic equivalents were calculated.

For analysis of food samples with normal background contamination by PCDDs, PCDFs or PCBs, gas chromatography with high-resolution mass spectrometry has been validated in collaborative studies and has been shown to provide the required sensitivity and specificity. Bioanalytical assays have been developed for rapid screening of sediments, soil, fly ash and various foods, but only the chemical-

activated luciferase gene expression (CALUX) assay has been used for food; validation of this assay has begun. While gas chromatography with mass spectrometry is the most powerful method for identifying and quantifying congeners and for recognizing congener-specific patterns, it does not allow direct measurement in a matrix of all congeners present that act through the Ah receptor pathway. The CALUX assay provides an indication of the toxic equivalents present in a certain matrix, including interactive (synergistic or antagonistic) effects; however, it cannot provide information on the pattern of congeners.

The Committee recognized that the available analytical data on PCDDs, PCDFs and coplanar PCBs are limited by the lack of generally accepted criteria for intra- and inter-laboratory validation. Mutual acceptance of analytical methods would be facilitated by international collaborative studies and proficiency testing programmes. For reliable analysis of concentrations in the range of normal background contamination, control laboratories must use sufficiently sensitive methods. General statistical parameters that have been established in other fields of residue analysis could be used. The requirements for acceptable analytical methods clearly need to be harmonized, so that data are comparable and can be used for risk management purposes.

5.2.6 *Levels and patterns of contamination of food commodities*

Data were submitted by Belgium, Canada, Japan, New Zealand, Poland and the USA and by the European Commission in a report containing data on Belgium, Denmark, Finland, France, Germany, Italy, the Netherlands, Norway, Sweden and the United Kingdom. In all countries in which a substantial number of samples had been analysed, the concentrations of PCDDs, PCDFs and coplanar PCBs in food were found to have decreased up to the late 1990s, but the decrease had slowed or was even partly reversed in some food categories in several countries owing to contamination of animal feed. For the present assessment of intake at the international level, only data collected after 1995 were considered.

As the Committee did not have access to the original analytical results, it was not possible to ascertain whether the results had been obtained by the lower- or upper-bound approach, and the concentrations used in the assessment were expressed as sums of congeners.

Insufficient individual data were available from most countries to allow construction of a full curve of the distribution of concentrations. Most data were submitted in an aggregated format. As recommended by a FAO/WHO workshop on assessing exposure to contaminants

Table 11
Weighted mean and derived median concentrations of PCDDs, PCDFs and coplanar PCBs in six food groups, expressed as toxic equivalents (pg/g whole food)

Region or country	Food category	PCDDs/PCDFs		Coplanar PCBs	
		Weighted mean	Derived median	Weighted mean	Derived median
North America	Dairy	0.10	0.07	0.02[a]	0.01[a]
	Eggs	0.17	0.14	0.04[a]	0.02[a]
	Fish	0.56	0.28	0.13[a]	0.08[a]
	Meat	0.13	0.10	0.14[a]	0.05[a]
Western Europe	Dairy	0.07	0.04	0.08	0.07
	Eggs	0.16	0.15	0.07	0.06
	Fish	0.47	0.31	2.55	0.90
	Meat	0.08	0.06	0.41	0.08
	Vegetable products	0.04	0.03	0.04	LOD
Japan	Dairy	0.06	0.04	0.04	0.02
	Eggs	0.07	0.03	0.06	0.04
	Fish	0.37	0.11	0.69	0.19
	Meat	0.09	0.01	0.04	0.009
	Vegetable products	0.003	0.002	0.02	0.003
New Zealand	Dairy	0.02	0.02	0.01	0.008
	Fish	0.06	0.05	0.09	0.07
	Meat	0.01	0.01	0.02	0.01
	Vegetable products	0.008	0.008	<LOD	<LOD
All	Fats and oils	0.21	0.10	0.07[a]	0.02

LOD: limit of detection.

[a] Data on PCBs frequently did not include mono-*ortho* PCBs.

(*18*), aggregated data were weighted as a function of the number of initial samples and then used to obtain a weighted mean concentration of PCDDs, PCDFs and PCBs in six major food groups: meat and meat products, eggs, fish and fish products, milk and milk products, vegetables and vegetable products, and fats and oils. National data were aggregated by region or country (North America, Western Europe, Japan and New Zealand) and are summarized in Table 11. Insufficient data were available for the rest of the world to permit a realistic estimate of the distribution of contaminants. The Committee recognized that there are significant differences within the food categories in Table 11, and that the data used in this analysis may not reflect the true mean for a food category. For example, the mean concentrations of PCDDs, PCDFs and coplanar PCBs and the rate of consumption vary considerably in different fish species, and it was not possible to determine if the mean represents the fish species most

commonly consumed. However, the data received were not sufficient to allow an analysis that might account for such variation.

In a second step, a log-normal distribution of contaminants in foods was assumed, and a model of distribution was constructed from the weighted mean and a geometric standard deviation of 3 derived from the concentrations in six broad food groups. On the basis of these derived distributions, the percentiles of consumption were determined. The derived median values (50th percentiles) are presented in Table 11.

5.2.7 *Estimated dietary intake*

Because of the long half-lives of PCDDs, PCDFs and coplanar PCBs, their hazard to health can be estimated only after consideration of intake over a period of months. Short-term variations in PCDD, PCDF and coplanar PCB concentrations in foods have much less effect on overall intake than might be the case for other food contaminants.

The distribution of long-term mean intake in various populations was calculated by the following procedure:

- The distributions of concentrations were constructed for various regions and food groups from the available data. The distributions were assumed to be log-normal.
- Data on food consumption from the GEMS/Food regional diets and national surveys were used to estimate mean consumption of six major food groups in each diet. A log-normal distribution was constructed from these data with a geometric standard deviation of 1.3 extrapolated from the results of the food consumption survey in the Netherlands in order to account for inter-individual variation in consumption. The average contributions of the six basic food groups to total food consumption were derived for each diet.
- The dietary intake of a particular population was assessed by combining the concentrations in foods and food consumption distributions for that population using a Monte Carlo approach. In each Monte Carlo trial, the dietary intake was estimated by multiplying random values for food consumption and concentrations in various food groups. The concentrations were weighted according to the contribution of the food group to total food consumption. The estimates of intake were combined to form a distribution of long-term mean dietary intake for each population studied. The distributions are characterized by a median and a 90th percentile intake. Calculations were performed for the sum of the toxic equivalents of PCDDs and PCDFs and for the sum of coplanar PCBs separately, because the data on occurrence of PCBs were obtained independently.

Table 12
Median and 90th percentile values of estimated long-term intake of PCDDs, PCDFs and coplanar PCBs,[a] based on the GEMS/Food regional diets

Source of data on concentrations[b]	Source of data on food consumption	Intake of PCDDs and PCDFs		Intake of coplanar PCBs	
		Median	90th percentile	Median	90th percentile
North America	Europe	68	160	14	35
Western Europe	Europe	54	130	57	150
Japan	Far East	7	15	7	19
New Zealand	Europe	18	36	10	22

[a] Expressed as toxic equivalents, pg/kg of body weight per month, assuming 60 kg of body weight.

[b] For North America, the data on concentrations in vegetables in Western Europe were used; for New Zealand, the data on concentrations in eggs in Japan were used.

Table 13
Median and 90th percentile values of estimated long-term intake of PCDDs, PCDFs and coplanar PCBs,[a] based on national food consumption data

Source of data on concentrations[b]	Source of data on food consumption	Intake of PCDDs and PCDFs		Intake of coplanar PCBs	
		Median	90th percentile	Median	90th percentile
North America	USA	42	100	9	25
Western Europe	France	40	94	47	130
	Netherlands	33	81	30	82
	United Kingdom	39	91	41	110

[a] Expressed as toxic equivalents, pg/kg of body weight per month, assuming 60 kg of body weight.

[b] For North America, the data on concentrations in vegetables in Western Europe were used.

The simulated intakes of PCDDs, PCDFs and coplanar PCBs in the GEMS/Food regional diets are presented in Table 12. These intakes are, however, likely to be overestimates, as the data on concentrations were derived from surveys (without random sampling) and from the GEMS/Food regional diets, which are based on data on food supply (apparent consumption) and which are known to overestimate food consumption by at least 15%.

More reliable estimates of intake (Table 13) were obtained by using national food consumption data rather than data on the food supply (apparent consumption) from the GEMS/Food regional diets. The simulated intakes presented in Table 13 are not strictly national estimates and are somewhat higher than the national estimates submitted by the European Commission.

The calculated contributions of various food categories to the intake of PCDDs, PCDFs and coplanar PCBs showed that the largest fraction (>70%) is from food of animal origin in both the GEMS/Food regional diets and the national diets.

Information was lacking on both the quality of data and geographical representativeness for some regions. More data are required on the occurrence of coplanar compounds in food products, particularly from geographical regions other than Europe, so that more representative estimates of intake can be made for all regions.

Breastfed infants have higher intakes of these compounds than bottle-fed infants or adults on a body-weight basis, although for only a small portion of their lives. Breast milk has beneficial effects, despite the risk of contamination. WHO has therefore repeatedly evaluated the health significance of contamination of breast milk with coplanar compounds. WHO recommends and supports breastfeeding but has concluded that continued and enhanced efforts should be directed towards identifying and controlling environmental sources of these substances.

5.2.8 *Evaluation*

In view of the long half-lives of PCDDs, PCDFs and coplanar PCBs, the Committee concluded that it would not be appropriate to establish an acute reference dose for these compounds.

The Committee concluded that a tolerable intake could be established for TCDD on the basis of the assumption that there is a threshold for all effects, including cancer. Carcinogenicity due to TCDD was not linked to mutagenicity or DNA binding, and it occurred at higher body burdens in animals than other toxic effects. The Committee concluded that the establishment of a tolerable intake based on effects other than cancer would also address any carcinogenic risk.

The studies listed in Table 10 were those considered by the Committee in choosing the lowest LOELs and NOELs for assessment of tolerable intake. The lowest LOEL and NOEL were provided by the studies of Faqi et al. (*16*) and Ohsako et al. (*17*), respectively. With the toxicokinetic conversions described in Table 9, these two studies indicate maternal body-burden LOELs and NOELs for effects on male rat offspring of 25 ng/kg of body weight and 13 ng/kg of body weight, respectively.

5.2.8.1 Background body burdens in laboratory animals

In the studies used to estimate body burden on the basis of the distribution of TCDD after multiple dosing, radiolabelled material

was used. Therefore, the known background concentrations of TCDD and other PCDDs and PCDFs in the tissues of laboratory rodents resulting from traces of these compounds in rat feed were ignored. The Committee identified two studies that could be used to predict the body burdens of rats resulting from the presence of coplanar compounds in laboratory feed. These studies were mutually consistent and predicted that "unexposed" laboratory rats had toxic equivalent body burdens of 3–12 ng/kg of body weight, depending on age. Thus, the maternal body burdens of TCDD seen in studies with radiolabelled material should be adjusted upwards by a minimum of 3 ng/kg of body weight to account for the background concentrations of unlabelled PCDDs and PCDFs. The maternal toxic equivalent body burden may still be underestimated, as 3 ng/kg of body weight was the minimum in the two studies, and in one of the studies coplanar PCBs were not included.

Addition of 3 ng/kg of body weight to the body burdens calculated from the linear model and the data in Table 9 resulted in estimated total toxic equivalent body burdens of 16 ng/kg of body weight for the NOEL and 28 ng/kg of body weight for the LOEL. These body burdens correspond to EHMIs of 240 and 420 pg/kg of body weight, respectively. Fitting the data in Table 9 into the power-model equation gave EHMIs of 330 pg/kg and 630 pg/kg of body weight, respectively.

5.2.8.2 Identification of safety factors

The safety factors considered in establishing acceptable levels of intake on the basis of the results of studies in laboratory animals usually include the following: a factor to convert a LOEL to a NOEL (if needed); a factor to extrapolate from animals to humans; and factors to account for inter-individual variations in susceptibility. Typically, factors of 10 have been used for extrapolation between species and for accounting for the human variation in susceptibility, and a factor of 3–10 for extrapolating from a LOEL to a NOEL.

As a NOEL was identified for effects in the male offspring of rats, no factor for conversion from a LOEL to a NOEL was needed for the EHMI derived from the study described above (*17*).

As concluded by the WHO consultation (*5*), use of body burdens to scale doses from studies in laboratory animals to equivalent human doses removes the need for safety factors to account for differences in toxicokinetics between animals and humans.

To account for inter-individual differences in toxicokinetics among humans, a safety factor should be applied. The Committee noted that limited data were available on the toxicokinetics of TCDD in humans and considered that the default factor of 3.2 was appropriate.

The Committee observed that humans may be less sensitive than rats to some effects. However, the conclusion is less certain for other effects and the possibility that the most sensitive humans might be as sensitive to the adverse effects of TCDD as rats were in the pivotal studies cannot be excluded. Therefore, the Committee concluded that no safety factor in either direction need be applied for differences in toxicodynamics among humans.

Use of a LOEL instead of a NOEL indicates the need for an additional safety factor. As the LOEL for the sensitive end-point was considered to be close to a NOEL and represented marginal effects, the Committee applied a factor of 3 to account for use of a LOEL instead of a NOEL. This resulted in an overall safety factor of 9.6 (3×3.2).

The Committee concluded that a total safety factor of 3.2 should be applied to the EHMI associated with the NOEL, and a total safety factor of 9.6 should be applied to the EHMI associated with the LOEL.

5.2.8.3 Tolerable intake

As stated in the discussion of toxicokinetics, the long half-lives of PCDDs, PCDFs and coplanar PCBs mean that each daily ingestion has a small or even a negligible effect on overall intake. In order to assess long- or short-term risks to health due to these substances, total or average intake should be assessed over months, and tolerable intake should be assessed over a period of at least 1 month. To encourage this view, the Committee decided to express the tolerable intake as a monthly value in the form of a provisional tolerable monthly intake[1] (PTMI).

As shown in Table 14, use of the linear model to extrapolate the maternal body burden at the NOEL, obtained with a single dose, to that expected at multiple doses gives a EHMI of 237 pg/kg of body weight, which would be expected to result in a body burden that is lower than that which had effects in animals. The PTMI derived by application of the safety factor of 3.2 to this EHMI is 74 pg/kg of body weight.

Similarly, as shown in Table 14, the PTMI derived by application of the safety factor of 9.6 to the EHMI derived from the study that

[1] By analogy with the provisional tolerable weekly intake (PTWI), the end-point used for safety evaluations by the Committee for food contaminants with cumulative properties. Its value represents the permissible human monthly exposure to those contaminants unavoidably associated with otherwise wholesome and nutritious foods.

Table 14
Summary of four calculations of PTMI

	Linear model		Power model	
	NOEL	LOEL	NOEL	LOEL
Administered dose (ng/kg of body weight)	12.5[a]		12.5[a]	
Maternal body burden (ng/kg of body weight)	7.6	25[b]	7.6	25[b]
Equivalent maternal body burden with long-term dosing (ng/kg of body weight)	13[c]	25[c]	19[d]	39[d]
Body burden from feed (ng/kg of body weight)	3	3	3	3
Total body burden (ng/kg of body weight)	16[e]	28[e]	22[e]	42[e]
EHMI (pg/kg of body weight per month)	237	423	330	630
Safety factor	3.2	9.6	3.2	9.6
PTMI (pg/kg of body weight per month)	74	44	103	66

[a] Bolus dose (NOEL).
[b] Target maternal body burden from repeated dosing (LOEL).
[c] Assuming a linear relationship between fetal and maternal body burden (based on data in Table 9).
[d] Assumes a non-linear relationship between fetal and maternal body burden (based on data in Table 9).
[e] Assuming, for humans, a half-life of 7.6 years and 50% uptake from food (see equation on page 126).

provided the LOEL is 44 pg/kg of body weight. As also shown in Table 14, use of the power model to extrapolate the maternal body burden with single doses to multiple doses would result in PTMIs of 103 pg/kg of body weight for the NOEL and 66 pg/kg of body weight for the LOEL. The range of PTMIs derived from the two studies, with either the linear or the power model to extrapolate the maternal body burden with single to multiple doses, is thus 40–100 pg/kg of body weight per month. The Committee chose the mid-point of this range, 70 pg/kg of body weight per month, as the PTMI. Furthermore, in accordance with the conclusions of the WHO consultation (*5*), the Committee concluded that this tolerable intake should be applied to intake of PCDDs, PCDFs and coplanar PCBs expressed as TEFs.

5.2.8.4 Comparison of PTMI with estimated intake from food

In the GEMS/Food regional diets, the range of estimated intake of toxic equivalents of PCDDs and PCDFs is 7–68 pg/kg of body weight per month at the median and 15–160 pg/kg of body weight per month at the 90th percentile of mean lifetime exposure, and that for coplanar PCBs is 7–57 pg/kg of body weight per month at the median and 19–150 pg/kg of body weight per month at the 90th percentile of consumption. The intakes estimated from national food consumption data were lower: 33–42 pg/kg of body weight per month at the median and 81–100 pg/kg of body weight per month at the 90th percentile for PCDDs and PCDFs, and 9–47 pg/kg of body weight per month at the

median and 25–130 pg/kg of body weight per month at the 90th percentile for coplanar PCBs. Estimates could not be made for the sum of PCDDs, PCDFs and coplanar PCBs, because data on concentrations were submitted separately by countries.

The median and 90th percentile of the derived distribution of intakes were considered to describe long-term intake. A Monte Carlo calculation was used to predict these intakes for coplanar PCBs on the basis of two sets of distribution curves generated from information on mean concentrations in six major food groups and corresponding data on mean food consumption from several sources, by applying geometric standard deviations of 3 and 1.3 to the respective means. The geometric standard deviation for the food consumption curves accounted for long-term consumption patterns. As the mean intakes of the whole population tend not to change with the duration of a survey, use of mean consumer intakes to generate the curves for major food groups, rather than individual commodities, approximates the mean intakes of the whole population, as nearly all respondents were consumers.

5.2.8.5 Uncertainties

Several sources of uncertainty were identified in the data used to assess intake, which suggest that they are likely to be overestimates at both the median and the 90th percentile levels of consumption. Despite the uncertainties, the results suggest that a considerable fraction of the population will have a long-term mean intake above the PTMI.

Furthermore, despite the large amount of information on toxicity, substantial uncertainties remain which should be considered in applying the risk assessment and in interpreting the estimates of intake of PCDDs, PCDFs and coplanar PCBs. The Committee used the overall data to identify a level of intake of coplanar compounds in food that represents no appreciable risk to humans. The safety assessment includes adjustment for a number of uncertainties, including estimates of TEFs within orders of magnitude in order to relate the potency of 28 relatively poorly studied compounds to that of one well-studied compound, TCDD. Moreover, the relative proportion of TCDD and the other 28 compounds varies; TCDD typically constitutes a small percentage of the total toxic equivalents in foods.

The PTMI is not a limit of toxicity and does not represent a boundary between safe intake and intake associated with a significant increase in body burden or risk. Long-term intakes slightly above the PTMI would not necessarily result in adverse health effects but would erode the safety factor built into the calculations of the PTMI. It is not

possible, given current knowledge, to define the magnitude and duration of excess intake that would be associated with adverse health effects.

5.2.8.6 Effect of maximum limits on intake, risk and food availability

The concentrations of PCDDs, PCDFs and coplanar PCBs vary among foods. In establishing regulatory limits, the possible undesired consequences of their enforcement should be taken into account, such as reductions in the food supply. The Committee explored the theoretical effects of various maximum regulatory limits on compliance and on long-term average reduction of intake. On the basis of this analysis, the Committee concluded that, in order to achieve, for example, a 20% reduction in intake of coplanar compounds from food, the intake of a wide range of foods would have to be reduced by a similar percentage. This relationship exists because these contaminants are present at relatively high levels in major food types. Furthermore, in view of the half-times of these compounds in humans, setting regulatory limits on the basis of the PTMI would have no discernible effect on body burdens for several years.

In contrast, long-term reductions could be gained by identifying and eliminating the routes by which these compounds pass from the environment into food supplies. The Committee was informed that studies of environmental concentrations over time in several countries suggest that measures to control emissions to the environment generally have had a substantial impact on both the amounts of PCDDs and PCDFs present in the environment and the body burdens of the general public.

6. Future work

1. The Committee has been asked on several occasions to advise the Codex Committee on Food Additives and Contaminants on the relative risks associated with alternative proposed maximum limits for contaminants in foods. However, a maximum limit will not, in many cases, have a substantial effect on the long-term intake of the contaminant by the general population, nor will it have a measurable impact on public health unless a substantial proportion of the food supply is removed from the market. Nevertheless, maximum limits could have a positive influence on agricultural and industrial practices and contribute to reducing the intake of some contaminants for which the distribution is highly skewed. The Committee recommended more detailed consideration of this issue at a future meeting.

2. The Committee strongly reiterated its recommendation made at its fifty-fifth meeting for revision of the *Guide to specifications* (Annex 1, reference *100*). This revision is urgently required, so that significant developments in methods of analysis can be included.
3. The Committee recommended continuation of its activity to update limits for heavy metals in food additives and concluded that acidity regulators and colours should be reviewed at its next meeting on food additives and contaminants.
4. The Committee recommended that the monograph that covers specifications for 16 modified starches should be divided into smaller monographs, as changes to one specification mean that the entire monograph must be changed.

7. Recommendations

1. In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, the important role that the recommendations of the Committee play in the development of international food standards and of regulations in many countries, and the need for maintaining consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.
2. The Codex Alimentarius Commission has adopted International Numbering System (INS) numbers 472e and 472f for diacetyltartaric and fatty acid esters of glycerol and for tartaric, acetic and fatty acid esters of glycerol, mixed, respectively. At its fifty-first meeting (Annex 1, reference *137*), the Committee established one specification under the name "diacetyltartaric and fatty acid esters of glycerol" to cover these two substances, and at the present meeting a temporary ADI was established. The specifications have been combined because, even if diacetyltartaric and fatty acid esters of glycerol and tartaric, acetic and fatty acid esters of glycerol, mixed, are manufactured from different raw materials, they meet all the criteria of the specifications and cannot be distinguished from each other by currently available analytical methods. The Committee recommended that the Codex Committee on Food Additives and Contaminants consider whether it would be more appropriate to have only one INS number for labelling purposes.
3. The Committee recognized that the revised "General specifications and recommendations for enzyme preparations used in food processing" in the *Compendium of food additive specifications*, *addendum 9* (FAO Food and Nutrition Paper, No. 52, Add. 9, 2001)

contain many criteria for safety evaluations that are more appropriate for inclusion elsewhere. The Committee recommended that the project to update and consolidate principles and methods for the assessment of chemicals in food (see section 2.3) include the safety assessment of enzymes intended for use in food and a subsequent removal of these guidelines from the general specifications.

4. In view of the complexity of the analytical methods for determining PCDDs, PCDFs and coplanar PCBs, the Committee recommended that a specific validation protocol be developed. Laboratories involved in such analytical work should be encouraged by FAO/WHO to participate in collaborative studies and proficiency testing.
5. A clear definition of "flavouring agent" has not been elaborated by the Committee, which recommended to FAO and WHO that such a definition be developed when updating principles for the assessment of chemicals in food.

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Annex 1
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

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[1] The full text is available electronically on the Internet at http://www.who.int/pcs.

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Annex 2

Acceptable Daily Intakes, other toxicological information and information on specifications

Food additives evaluated toxicologically

Food additive	Specifications[a]	Acceptable daily intake (ADI in mg/kg of body weight) and other toxicological recommendations
Emulsifiers		
Diacetyltartaric and fatty acid esters of glycerol	R	0–50 (temporary)[b]
Tartaric, acetic and fatty acid esters of glycerol, mixed	W[c]	ADI "not limited" withdrawn[c]
Quillaia extracts	R, T[b]	0–5 (temporary)[b,d]
Enzyme preparation		
Invertase from *Saccharomyces cerevisiae*	N	Acceptable[e]
Food colours		
β-Carotene from *Blakeslea trispora*	N, T[b]	0–5 (group ADI)[f]
Curcumin	R	0–1 (temporary)[b]
Food salts		
Calcium dihydrogen diphosphate	N	Included in the maximum tolerable daily intake of 70 mg/kg of body weight for phosphates, diphosphates and polyphosphates
Monomagnesium phosphate	N, T[b]	
Sodium calcium polyphosphate	N	
Trisodium diphosphate	N, T[b]	
Glazing agent		
Hydrogenated poly-1-decene	R	0–6
Preservative		
Natamycin (pimaricin)	R, T[b]	0–0.3
Sweetening agent		
D-Tagatose	S	0–80
Thickening agents		
Carrageenan	R	ADI "not specified"[g] (group ADI)[h]
Processed *Eucheuma* seaweed	R	
Curdlan	R	ADI "not specified"[g]
Miscellaneous substances		
Acetylated oxidized starch	N, R[i]	ADI "not specified"[g]
α-Cyclodextrin	N	
Sodium sulfate	S	

[a] N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing, new or revised specifications are tentative and new information is needed; W, existing specifications withdrawn.

[b] See Annex 3.

[c] The ADI was withdrawn because the specifications for tartaric, acetic and fatty acid esters of glycerol, mixed, were combined with those for diacetyltartaric and fatty acid esters of glycerol under the latter name at the fifty-first meeting (Annex 1, reference *137*).
[d] Applicable only to the unpurified extract.
[e] Invertase from *Saccharomyces cerevisiae* that meets the specifications developed at the present meeting was considered to be acceptable because *S. cerevisiae* is commonly used in the preparation of food. Its use should be limited by good manufacturing practice.
[f] Group ADI for β-carotene from *Blakeslea trispora* and synthetic β-carotene.
[g] ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.
[h] Group ADI for carrageenan and processed *Eucheuma* seaweed.
[i] The new specifications for acetylated oxidized starch were incorporated into the revised specifications for modified starches.

Food additives considered for specifications only

Food additive	Specification[a]
Acesulfame K (potassium salt)	R
Blackcurrant extract	R
DL-Malic acid	R
Oxystearin	W
Pectins	R
Smoke flavourings	R
Tagetes extract	R

[a] R, existing specifications revised; W, existing specifications withdrawn.

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Pyrazine derivatives			
Structural class II			
2-Methylpyrazine	761	N	No safety concern
2-Ethylpyrazine	762	N	
2-Propylpyrazine	763	N	
2-Isopropylpyrazine	764	N	
2,3-Dimethylpyrazine	765	N	
2,5-Dimethylpyrazine	766	N	
2,6-Dimethylpyrazine	767	N	

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
2-Ethyl-3-methylpyrazine	768	N	
2-Ethyl-6-methylpyrazine	769	N	
2-Ethyl-5-methylpyrazine	770	N	
2,3-Diethylpyrazine	771	N	
2-Methyl-5-isopropylpyrazine	772	N	
2-Isobutyl-3-methylpyrazine	773	N	
2,3,5-Trimethylpyrazine	774	N	
2-Ethyl-3,(5 or 6)-dimethylpyrazine	775	N	
3-Ethyl-2,6-dimethylpyrazine	776	N	
2,3-Diethyl-5-methylpyrazine	777	N	
2,5-Diethyl-3-methylpyrazine	778	N	
3,5-Diethyl-2-methylpyrazine	779	N	
2,3,5,6-Tetramethylpyrazine	780	N	No safety concern
5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine	781	N	
6,7-Dihydro-2,3-dimethyl-5*H*-cyclopentapyrazine	782	N	
Acetylpyrazine	784	N	
2-Acetyl-3-ethylpyrazine	785	N	
2-Acetyl-3,(5 or 6)-dimethylpyrazine	786	N	
Methoxypyrazine	787	N	
(2 or 5 or 6)-Methoxy-3-methylpyrazine	788	N	
2-Ethyl-(3 or 5 or 6)-methoxypyrazine	789	N	
2-Methoxy-(3 or 5 or 6)-isopropylpyrazine	790	N	
2-Methoxy-3-(1-methylpropyl)pyrazine	791	N	
2-Isobutyl-3-methoxypyrazine	792	N	
2-Acetyl-3-methylpyrazine	950	N	
Structural class III			
(Cyclohexylmethyl)pyrazine	783	N	
2-Methyl-(3 or 5 or 6)-ethoxypyrazine	793	N	
2-(Mercaptomethyl)pyrazine	794	N	
2-Pyrazinylethane thiol	795	N	
Pyrazinylmethyl methyl sulfide	796	N	No safety concern
(3 or 5 or 6)-(Methylthio)-2-methylpyrazine	797	N	
5-Methylquinoxaline	798	N	
Pyrazine	951	N	
5,6,7,8-Tetrahydroquinoxaline	952	N	
Aromatic substituted secondary alcohols, ketones and related esters			
Structural class I			
α-Methylbenzyl alcohol[b]	799	N	
α-Methylbenzyl formate	800	N	
α-Methylbenzyl acetate	801	N	
α-Methylbenzyl propionate	802	N	No safety concern
α-Methylbenzyl butyrate	803	N	
α-Methylbenzyl isobutyrate	804	N	
p, α-Dimethylbenzyl alcohol	805	N	

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Acetophenone	806	N	No safety concern
4-Methylacetophenone	807	N	
p-Isopropylacetophenone	808	N	
2,4-Dimethylacetophenone	809	N	
Acetanisole	810	N	
1-(*p*-Methoxyphenyl)-2-propanone	813	N	
α-Methylphenethyl butyrate	814	N, T	
4-Phenyl-2-butanol	815	N	
4-Phenyl-2-butyl acetate	816	N	
4-(*p*-Tolyl)-2-butanone	817	N, T	
4-(*p*-Methoxyphenyl)-2-butanone	818	N	
4-Phenyl-3-buten-2-ol	819	N	
4-Phenyl-3-buten-2-one	820	N	
3-Methyl-4-phenyl-3-buten-2-one	821	N	
1-Phenyl-1-propanol	822	N	
α-Ethylbenzyl butyrate	823	N	
Propiophenone	824	N	
α-Propylphenethyl alcohol	825	N	
1-(*p*-Methoxyphenyl)-1-penten-3-one	826	N	
Ethyl benzoylacetate	834	N	
Ethyl 2-acetyl-3-phenylpropionate	835	N	
Structural class II			
4-Acetal-6-*tert*-butyl-1,1-dimethylindan	812	N	Additional data required
α-Isobutylphenethyl alcohol	827	N	No safety concern
4-Methyl-1-phenyl-2-pentanone	828	N	
1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one	829	N	
3-Benzyl-4-heptanone	830	N	
1-Phenyl-1,2-propanedione	833	N	
Structural class III			
Methyl β-naphthyl ketone	811	N	No safety concern
Benzophenone	831	N	
1,3-Diphenyl-2-propanone	832	N	
Benzoin	836	N	
Benzyl derivatives			
Structural class I			
Benzyl alcohol[c]	25	R	No safety concern
Benzyl formate	841	N	
Benzyl acetate[c]	23	R	
Benzyl propionate	842	N	
Benzyl butyrate	843	N	
Benzyl isobutyrate	844	N	
Benzyl isovalerate	845	N	
Benzyl *trans*-2-methyl-2-butenoate	846	N	
Benzyl 2,3-dimethylcrotonate	847	N, T	

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Benzyl acetoacetate	848	N	No safety concern
Benzyl benzoate[c]	24	R	
Benzyl phenylacetate	849	N	
Benzaldehyde[c]	22	R	
Benzaldehyde dimethyl acetal	837	N	
Benzaldehyde glyceryl acetal	838	N	
Benzaldehyde propylene glycol acetal	839	N	
Benzoic acid[c]	850	N	Evaluation not finalized[d]
Methyl benzoate	851	N	No safety concern
Ethyl benzoate	852	N	
Propyl benzoate	853	N	
Hexyl benzoate	854	N	
Isopropyl benzoate	855	N	
Isobutyl benzoate	856	N	
Isoamyl benzoate	857	N	
cis-3-Hexenyl benzoate	858	N	
Linalyl benzoate	859	N	
Geranyl benzoate	860	N	
Glyceryl tribenzoate	861	N, T	Evaluation not finalized[d]
Propylene glycol dibenzoate	862	N, T	
Methylbenzyl acetate (mixed *ortho*-, *meta*- and *para*-isomers)	863	N	No safety concern
p-Isopropylbenzyl alcohol	864	N	
4-Ethylbenzaldehyde	865	N	
Tolualdehydes (mixed *ortho*-, *meta*- and *para*-isomers)	866	N, T	
Tolualdehyde glyceryl acetal	867	N	
Cuminaldehyde	868	N	
2,4-Dimethylbenzaldehyde	869	N	
Benzyl 2-methoxyethyl acetal	840	N	
Hydroxy- and alkoxy-substituted benzyl derivatives			
Structural class I			
4-Hydroxybenzyl alcohol	955	—[e]	No safety concern
4-Hydroxybenzaldehyde	956	—[e]	
4-Hydroxybenzoic acid	957	—[e]	
2-Hydroxybenzoic acid	958	—[e]	
Butyl *p*-hydroxybenzoate	870	N, T	Evaluation not finalized[d]
Anisyl alcohol	871	N	No safety concern
Anisyl formate	872	N, T	
Anisyl acetate	873	N	
Anisyl propionate	874	N	
Anisyl butyrate	875	N	
Anisyl phenylacetate	876	N	

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Veratraldehyde	877	N	
p-Methoxybenzaldehyde	878	N	
p-Ethoxybenzaldehyde	879	N	
Methyl *o*-methoxybenzoate	880	N	
2-Methoxybenzoic acid	881	N	
3-Methoxybenzoic acid	882	N	
4-Methoxybenzoic acid	883	N	
Methyl anisate	884	N	
Ethyl *p*-anisate	885	N	
Vanillyl alcohol	886	N	
Vanillin[f]	889	N	
4-Hydroxy-3-methoxybenzoic acid	959	—[e]	
Vanillin acetate	890	N	No safety concern
Vanillin isobutyrate	891	N	
Salicylaldehyde	897	N	
2-Hydroxy-4-methylbenzaldehyde	898	N	
Methyl salicylate[g]	899	N	
Ethyl salicylate	900	N	
Butyl salicylate	901	N	
Isobutyl salicylate	902	N	
Isoamyl salicylate	903	N	
Benzyl salicylate	904	N	
Phenethyl salicylate	905	N	
o-Tolyl salicylate	907	N	
2,4-Dihydroxybenzoic acid	908	N	
Structural class II			
Vanillyl ethyl ether	887	N	
Vanillyl butyl ether	888	N	
Ethyl vanillin[h]	893	N	
Vanillin *erythro*- and *threo*-butan-2,3-diol acetal	960	—[e]	
Ethyl vanillin isobutyrate	953	N	No safety concern
Ethyl vanillin propylene glycol acetal	954	N, T	
Piperonyl acetate	894	N	
Piperonyl isobutyrate	895	N	
Piperonal[i]	896	N	
Ethyl vanillin β-D-glucopyranoside	892	N	
Aliphatic acyclic diols, triols and related substances			
Structural class I			
Glycerol[j]	909	N, T	Evaluation not finalized[d]
1,2,3-Tris[(1′-ethoxy)ethoxy]propane	913	N	No safety concern
Glyceryl monostearate	918	N, T	
Glyceryl monooleate	919	N, T	Evaluation not finalized[d]
Triacetin	920	N, T	
Glyceryl tripropanoate	921	N, T	

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Tributyrin	922	N, T	Evaluation not finalized[d]
Glycerol 5-hydroxydecanoate	923	N, T	
Glycerol 5-hydroxydodecanoate	924	N, T	
Propylene glycol[k]	925	N, T	
Propylene glycol stearate	926	N, T	
1,2-Di[(1-ethoxy)ethoxy]propane	927	N	No safety concern
Lactic acid	930	N	
Ethyl lactate[l]	931	N	
Butyl lactate	932	N	
Potassium 2-(1′-ethoxy)ethoxypropanoate	933	N	
cis-3-Hexenyl lactate	934	N	
Butyl butyryllactate	935	N	
Pyruvic acid	936	N	
Pyruvaldehyde	937	N, T	
Ethyl pyruvate	938	N	
Isoamyl pyruvate	939	N	
Structural class III			
3-Oxohexanoic acid glyceride	910	N, T	No safety concern
3-Oxooctanoic acid glyceride	911	N, T	
Heptanal glyceryl acetal (mixed 1,2 and 1,3 acetals)	912	N	
3-Oxodecanoic acid glyceride	914	N, T	Evaluation not finalized[d]
3-Oxododecanoic acid glyceride	915	N, T	
3-Oxotetradecanoic acid glyceride	916	N, T	
3-Oxohexadecanoic acid glyceride	917	N, T	
4-Methyl-2-pentyl-1,3-dioxolane	928	N	No safety concern
2,2,4-Trimethyl-1,3-oxacyclopentane	929	N	
Aliphatic acyclic acetals			
Structural class I			
1,1-Dimethoxyethane	940	N	No safety concern
Acetal	941	N	
Heptanal dimethyl acetal	947	N	
4-Heptenal diethyl acetal	949	N	
Octanal dimethyl acetal	942	N	
2,6-Nonadienal diethyl acetal	946	N	
Decanal dimethyl acetal	945	N	
Citral dimethyl acetal	944	N	
Citral diethyl acetal	948	N	
Acetaldehyde ethyl *cis*-3-hexenyl acetal	943	N, T	

[a] N, new specifications prepared; R, existing specifications revised; T, the existing, new or revised specifications are tentative and new information is needed.

[b] An ADI of 0–0.1 mg/kg of body weight was established for α-methylbenzyl alcohol by the Committee at its forty-first meeting (WHO Technical Report Series, No. 837, 1993), which was maintained at the present meeting.

[c] A group ADI of 0–5 mg/kg of body weight for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate and benzyl alcohol, expressed as

benzoic acid equivalents, was confirmed by the Committee at its forty-sixth meeting (WHO Technical Report Series, No. 868, 1997) and extended to include benzyl benzoate at the present meeting.

[d] Further information is required to determine whether this substance is in current use as a flavouring agent.

[e] Specifications will be considered at the fifty-ninth meeting of the Committee.

[f] An ADI of 0–10 mg/kg of body weight was established for vanillin by the Committee at its eleventh meeting (WHO Technical Report Series, No. 383, 1968), which was maintained at the present meeting.

[g] An ADI of 0–0.5 mg/kg of body weight was established for methyl salicylate by the Committee at its eleventh meeting (WHO Technical Report Series, No. 383, 1968), which was maintained at the present meeting.

[h] An ADI of 0–3 mg/kg of body weight was established for ethyl vanillin by the Committee at its forty-fourth meeting (WHO Technical Report Series, No. 859, 1995), which was maintained at the present meeting.

[i] An ADI of 0–2.5 mg/kg of body weight was established for piperonal by the Committee at its eleventh meeting (WHO Technical Report Series, No. 383, 1968), which was maintained at the present meeting.

[j] An ADI "not specified" was established for glycerol by the Committee at its twentieth meeting (WHO Technical Report Series, No. 599, 1976), which was maintained at the present meeting.

[k] An ADI of 0–25 mg/kg of body weight was established for propylene glycol by the Committee at its seventeenth meeting (WHO Technical Report Series, No. 539, 1974), which was maintained at the present meeting.

[l] Ethyl lactate was included in the group ADI "not specified" for lactic acid and its salts that was established by the Committee at its twenty-sixth meeting (WHO Technical Report Series, No. 683, 1982), which was maintained at the present meeting.

Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications[a]
Allyl tiglate	10	R
Allyl cyclohexane acetate	12	R
Allyl cyclohexane butyrate	14	R
Allyl cyclohexane valerate	15	R
Allyl cyclohexane hexanoate	16	R
Isoamyl formate	42	R
Isoamyl 2-methylbutyrate	51	R
Geranyl acetate	58	R
Rhodinyl propionate	64	R
Geranyl hexanoate	70	R
Geranyl isobutyrate	72	R
Rhodinyl isobutyrate	74	R
Rhodinyl isovalerate	77	R
3,7-Dimethyl-2,6-octadien-1-yl 2-ethylbutanoate	78	R
Heptanal	95	R
Nonanal	101	R
Undecanal	107	R
Lauric acid	111	R, T
Myristic acid	113	R, T
Palmitic acid	115	R, T
Stearic acid	116	R, T
Propyl formate	117	R
n-Amyl formate	119	R
Isobutyl formate	124	R
n-Amyl heptanoate	170	R

Flavouring agent	No.	Specifications[a]
Isobutyl heptanoate	172	R
Nonyl octanoate	178	R
Methyl laurate	180	R
Isoamyl laurate	182	R, T
Butyl stearate	184	R
trans-3-Heptenyl 2-methyl propanoate	191	R
Methyl 2-methylbutyrate	205	R
2-Methylbutyl 2-methylbutyrate	212	R
ω-6-Hexadecenlactone	240	R
cis-4-Hydroxy-6-dodecenoic acid lactone	249	R
2-Methylpentanal	260	R
2-Methylhexanoic acid	265	R
5-Methylhexanoic acid	266	R
2-Methyloctanal	270	R
2,6-Dimethyloctanal	273	R
2-Methylundecanal	275	R
Isopropyl formate	304	R
Isopropyl propionate	306	R
Isopropyl hexanoate	308	R
cis-5-Octen-1-ol	322	R
cis-5-Octenal	323	R
cis-6-Nonenal	325	R
4-Decenal	326	R
9-Decenoic acid	328	R
10-Undecenal	330	R
Methyl 3-hexenoate	334	R
Butyl 10-undecenoate	344	R
2-Methyl-3-pentenoic acid	347	R
2,6-Dimethyl-6-hepten-1-ol	348	R
Ethyl 2-methyl-3-pentenoate	350	R
Hexyl 2-methyl-3(4)-pentenoate (mixture)	352	R
Terpinyl formate	367	R
Terpinyl butyrate	370	R
Terpinyl isovalerate	372	R
p-Menth-8-en-1-ol	374	R
α-Ionone	388	R
γ-Ionone	390	R, T
Allyl α-ionone	401	R
α-iso-Methylionone	404	R
5-Hydroxy-4-octanone	416	R
2-Hydroxy-2-cyclohexen-1-one	424	R
(+)-Neo-menthol	428	R
p-Menth-1-en-3-ol	434	R
2-Ethyl-1,3,3-trimethyl-2-norbornanol	440	R
Methyl 1-acetoxycyclohexyl ketone	442	R
1-Ethylhexyl tiglate	448	R
(1-Buten-1-yl) methyl sulfide	457	R
3-(Methylthio)propanol	461	R
3-(Methylthio)propyl acetate	478	R
Allyl thiopropionate	490	R, T

Flavouring agent	No.	Specifications[a]
2-Propanethiol	510	R
2-Naphthalenethiol	531	R
Trithioacetone	543	R
2,5-Dimethyl-2,5-dihydroxy-1,4-dithiane	562	R
2-Methyl-2-(methyldithio)propanal	580	R
Ethyl 2-(methyldithio)propionate	581	R
Methyl 2-oxo-3-methylpentanoate	591	R
Geranyl acetoacetate	599	R
3-(Hydroxymethyl)-2-heptanone	604	R, T
1,4-Nonanediol diacetate	609	R, T
Aconitic acid	627	R, T
3-Phenylpropyl hexanoate	642	R, T
3-Phenylpropionaldehyde	645	R
Cinnamaldehyde ethylene glycol acetal	648	R
Cinnamyl butyrate	652	R
Cinnamaldehyde	656	R
Propyl cinnamate	660	R
Butyl cinnamate	663	R
Heptyl cinnamate	666	R
Phenethyl cinnamate	671	R
3-Phenylpropyl cinnamate	672	R
Cinnamyl cinnamate	673	R
α-Amylcinnamyl formate	676	R
α-Amylcinnamyl acetate	677	R
α-Amylcinnamyl isovalerate	678	R, T
α-Amylcinnamaldehyde dimethyl acetal	681	R
o-Tolyl acetate	698	R
p-Vinylphenol	711	R
Guaiacyl phenylacetate	719	R
Hydroquinone monoethyl ether	720	R
4-Ethyl-2,6-dimethoxyphenol	723	R
4-Propyl-2,6-dimethoxyphenol	724	R
4-Allyl-2,6-dimethoxyphenol	726	R
Dihydroxyacetophenone	729	R, T
Vanillylidene acetone	732	R
Furfuryl propionate	740	R
Furfuryl pentanoate	741	R
Furfuryl octanoate	742	R
Furfuryl 3-methylbutanoate	743	R
Amyl 2-furoate	748	R
Hexyl 2-furoate	749	R
Octyl 2-furoate	750	R
2-Phenyl-3-carboethoxyfuran	752	R, T
Furfuryl butyrate	759	R
Cinnamyl benzoate	760	R

[a] R, existing specifications revised; T, the existing, new or revised specifications are tentative and new information is required.

Contaminants

Contaminant	Tolerable intake and other toxicological recommendations
3-Chloro-1,2-propanediol	Provisional maximum tolerable daily intake: 2 μg/kg of body weight
1,3-Dichloro-2-propanol	Establishment of a tolerable intake was considered to be inappropriate because of the nature of the toxicity observed (tumorigenic in various organs in rats and interacts with chromosomes and/or DNA); the Committee noted that the dose that caused tumours in rats (19 mg/kg of body weight per day) was about 20 000 times the highest estimated intake of 1,3-dichloro-2-propanol by consumers of soya sauce (1 μg/kg of body weight per day).
Polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls	Provisional tolerable monthly intake: 70 pg/kg of body weight

Annex 3

Further information required or desired

Toxicological information

Diacetyltartaric and fatty acid esters of glycerol

The following information relating to the 2-year study on toxicity in rats is required for evaluation in 2003:

1. In order to determine whether some of the adverse effects that were observed were treatment-related, the groups treated with diacetyltartaric and fatty acid esters of glycerol should be compared with both untreated and monoglyceride-treated controls, and the control groups should be compared with one another.
2. Additional information on the incidence of mycocardial fibrosis and adrenal medullary hyperplasia in animals at the lowest and intermediate doses should be provided.

Curcumin

The results of a study on reproductive toxicity with a substance complying with the specifications for curcumin, known to be in progress, is required for evaluation in 2003.

Information on specifications

β-Carotene from Blakeslea trispora

Information is required on the method of analysis for residual solvents (ethyl acetate and isobutyl acetate). This information is required for evaluation in 2003.

Monomagnesium phosphate and trisodium diphosphate

Information is required on the loss on drying, loss on ignition, test method for loss on ignition and assay method for the hydrates. This information is required for evaluation in 2003.

Natamycin

Information is required on the level and determination of water content, limit for lead, specific rotation, assay value and method of assay for the commercial product. Comments on other aspects of the monograph are invited. This information is required for evaluation in 2003.

Quillaia extracts

The existing specifications for quillaia extracts were revised in order to clarify the differences between unpurified and semi-purified extracts. As additional information on composition (minimum and

maximum percentages of saponins in unpurified and semi-purified extracts) is necessary, the specifications were designated as tentative. Once the requested information has been received, the Committee will consider whether separate specifications for unpurified and semi-purified extracts are required. This information is required for evaluation in 2003.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

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 (70 pages)

Safety evaluation of certain mycotoxins in food.
WHO Food Additives Series, No. 47, 2001 (707 pages)

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 (117 pages)

Safety evaluation of certain food additives and contaminants.
WHO Food Additives Series, No. 46, 2001 (392 pages)

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 (136 pages)

Safety evaluation of certain food additives and contaminants.
WHO Food Additives Series, No. 44, 2000 (539 pages)

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 (176 pages)

Safety evaluation of certain food additives.
WHO Food Additives Series, No. 42, 1999 (494 pages)

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 (104 pages)

Further information on these and other WHO publications can be obtained from Marketing and Dissemination, World Health Organization, 1211 Geneva 27, Switzerland.

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives and contaminants, with a view to recommending Acceptable Daily Intakes (ADIs) and tolerable intakes, respectively, and to prepare specifications for the identity and purity of food additives.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (diacetyltartaric and fatty acid esters of glycerol, quillaia extracts, invertase from *Saccharomyces cerevisiae*, β-carotene from *Blakeslea trispora*, curcumin, phosphates, diphosphates and polyphosphates, hydrogenated poly-1-decene, natamycin, D-tagatose, carrageenan, processed *Eucheuma* seaweed, curdlan, acetylated oxidized starch, α-cyclodextrin and sodium sulfate), flavouring agents and contaminants (3-chloro-1,2-propanediol, 1,3-dichloro-2-propanol, and a large number of polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives and tolerable intakes of the contaminants considered, changes in the status of specifications of these food additives and specific flavouring agents, and further information required or desired.

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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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Keywords: SCCS, scientific opinion, labelling, fragrance allergens, directive 76/768/ECC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), opinion on fragrance allergens in cosmetic products, 26-27 June 2012

Table of contents

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 prehaptens 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 prehaptens. While in the case of prohaptens the possibility of becoming activated is inherent to the molecule and cannot be avoided, the activation of prehaptens 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 suffcient 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 disabeling problem. Prevention is possible as it is an environmetal 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 exisiting 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 furoumarins 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 contactc 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 mimick 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 secion 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 achive 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 sensitiser 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 perfuming[1], 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: *Evernia prunastri*)	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 1 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 sampleadults, 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, *Evernia prunastri*, *Myroxylon pereirae* *	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	*Myroxylon pereirae*	1141	2.4
Denmark (88)	Population sample, age 18-69	1990 2006	*Myroxylon pereirae*	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 clincial 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	
Myroxylon pereirae	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)
Myroxylon pereirae	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)
Myroxylon pereirae	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)
Evernia prunastri	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)
Cananga odorata (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 - prehaptens and prohaptens

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 (autoxidation) 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 prehaptens and prohaptens. 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 autoxidation 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 (autoxidation and metabolic oxidation) (119, 120). When haptens are formed by both pathways, the impact on the sensitisation potency depends on the degree of autoxidation in relation to the amount of metabolic oxidation.

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

5.1. Prehaptens

Autoxidation is a free radical chain reaction in which hydrogen atom abstraction in combination with addition of oxygen forms peroxyl 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 autoxidation 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 autoxidation 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 fragrence 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 clincal 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)
Linalool, "stabilised" *		10	7/2401 (0.3%)	§ (74)
		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	

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 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 prohapten 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 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.
- Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation include limonene, linalool, and linalyl acetate.
- Fragrance substances of clinical importance known to be prohaptens 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 prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) 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 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.

  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 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 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 prehaptens and/or prohaptens 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 clincial 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 Toxicology[2]" 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 prehaptens by, e.g. autoxidation outside the body as well as metabolic activation in the skin of compounds able to act as prohaptens (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 *posible 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 sensiting 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 a 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:

+	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**
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.)
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.)

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	++

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

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 exeption, 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 fragrence 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 fragrence 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.

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)

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%.

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)
AMYLCYCLOPENTANONE	4819-67-4	0 / 178	(171)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(70)
2-TERT-BUTYLCYCLOHEXYL 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)
3α,4,5,6,7,7α-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

[#] Annex III, part 1

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, $perfuming name according to CosIng[4]) in bold; plant part / type of extract (partly indicative) in plain font	**CAS number**	**Comment: see text**
CANANGA ODORATA and Ylang-ylang oil	83863-30-3; *8006-81-3*	+++
CEDRUS ATLANTICA *BARK OIL*	*92201-55-3; 8000-27-9*	++
CINNAMOMUM CASSIA *LEAF OIL* ***CINNAMOMUM ZEYLANICUM*** *BARK OIL*	*8007-80-5* *84649-98-9*	++ (r.t.)
CITRUS AURANTIUM AMARA *FLOWER / PEEL OIL*	*8016-38-4; 72968-50-4*	++
CITRUS BERGAMIA *PEEL OIL EXPRESSED*$	*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 [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/

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

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.	*diverse*	++
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.)	*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	++

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)

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-lable 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 test[6] and the local lymph node assay (LLNA)[7]. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH[8]. 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 labelling[9], 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 (CLP[10]) "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 sensitiser (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 ≥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 sensitiser (EC3 value ≤ 0.2); strong sensitiser (EC3 > 0.2 - ≤ 2); and moderate sensitiser (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).

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[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)
Evernia prunastri extract oak moss	90028-68-5	3.9	n/a	(164)
Balsam oil, Peru *(Myroxylon pereirae 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)

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 *(Grandiflorum)*	8022-96-6	5.9	N/a	(164)
Dibenzyl ether	103-50-4	6.3	0.32	(164)
Cananga odorata 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)
Camellia sinensis 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)
Evernia furfuracea 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 (µg/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 (μg/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.

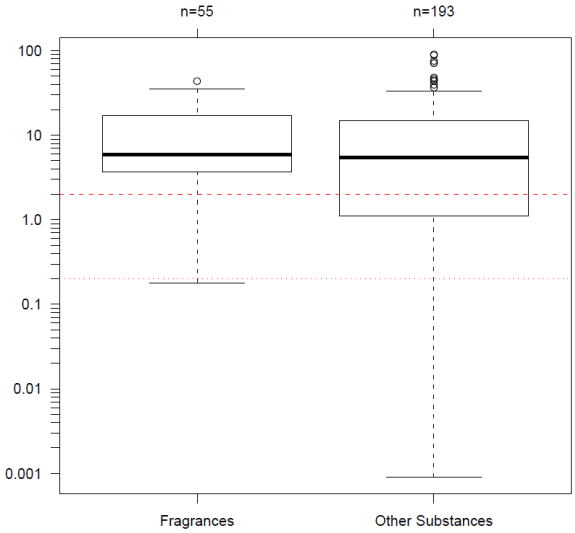

Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in 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 - ≤ 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 intitially 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 quantitiative 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§	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

Isoamyl salicylate	87-20-7	Acyltranfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehapten
Nerol	106-25-2	Prehapten and prohapten
Nerolidol (isomer not specified)	7212-44-4	Possible prehapten
Oxacyclohexadecenone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehapten

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

Substance (INCI) name	**CAS number**	**Structural alerts**
Ambrettolide	7779-50-2	Possible prehapten
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohapten via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehapten
Cuminaldehyde	122-03-2	Schiff base and possible prehapten
alpha-Cyclohexylidene benzeneacetonitrile	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 prehapten
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehapten
Dihydromyrcenol	18479-58-8	Possible prehapten
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehapten and possible prohapten
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehapten
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehapten
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehapten
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehapten
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohapten
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl	54830-99-8	Possible prehapten

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 prohapten
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	

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 carbinyl 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
d-Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
Evernia prunastri/oak moss	90028-68-5	0
Evernia furfuracea/tree moss	90028-67-4	0

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

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.
Evernia prunastri▪/oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
Evernia furfuracea▪/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.

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%)
Lyral™	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%)
Evernia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evernia 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; Lyral™, hydroxy-isohexyl-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
Citonellol	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

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
Evernia prunastri▪/oak moss	0.8
Evernia furfuracea▪/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.

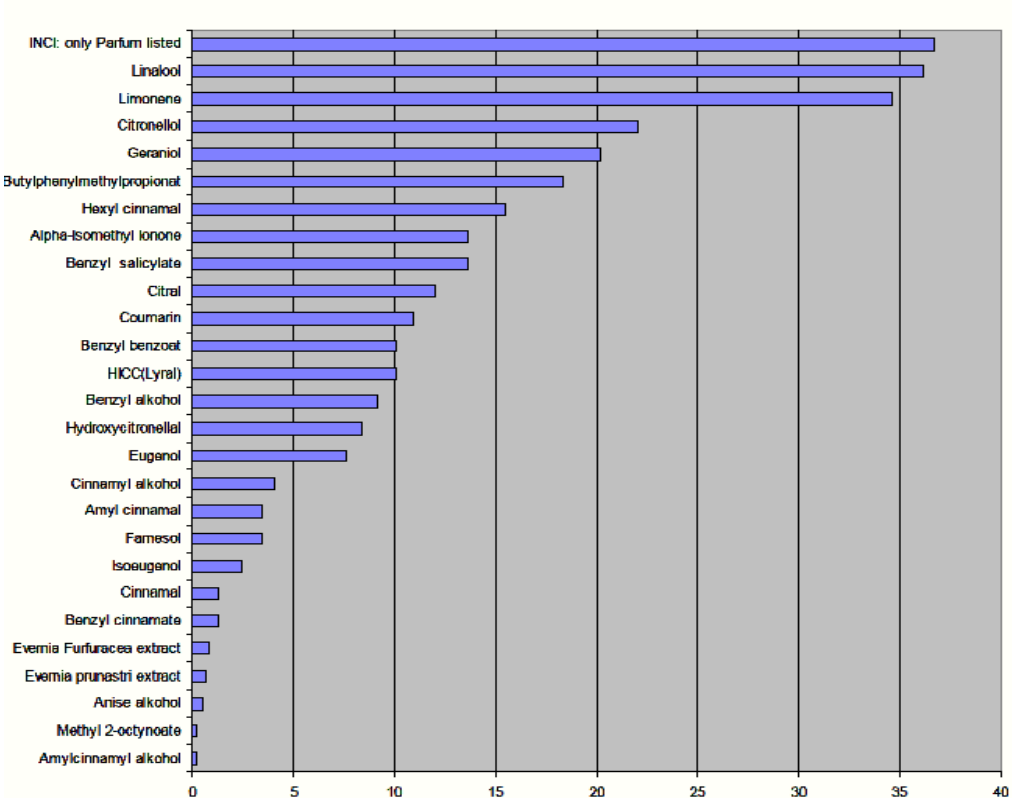

Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

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 themself, 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 dermatitits (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/cm^2/day is based on 8.5 mg/cm^2/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: EDxx(ROAT)=0.0296 *EDxx(patch test) based on testing nickel and methyldibromo 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 µg/cm²) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in µg/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

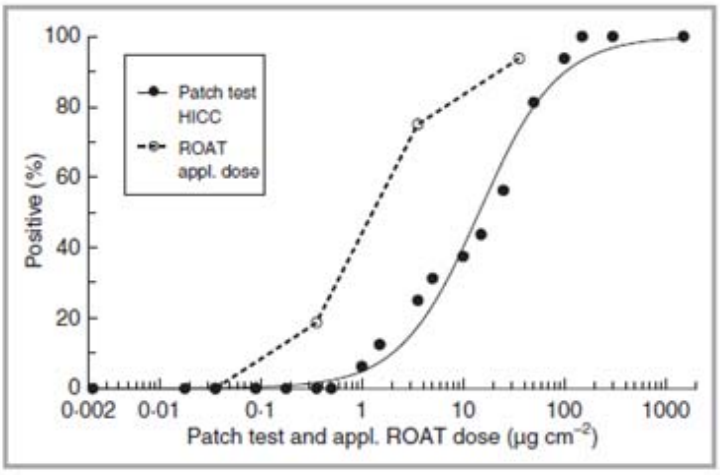

Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded 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 ($\mu g/cm^2$) 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):

$$\text{Log } (p_{40}) = - (T_B - 40)Tr/2.303RT$$

Where p is in atmospheres, T_B is in °C, R is the gas constant, Tr is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to 22 $cal.deg^{-1}$ 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 = 146-149°C, p_{40} = 17 mmHg) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal (T_B = 248°C, p_{40} = 0.5 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

(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			

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/cm^2 to 0.125 µg/cm^2 in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm^2.

Cinnamal

In ROAT a dose of 0.26 µg/cm^2 gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm^2.

The ED50 in patch testing was 96 µg/cm^2.

HICC

In ROAT a dose of 0.0357 µg/cm^2 gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm^2 to 1.2 µg/cm^2. The dose in a cream base was 4.9 µg/cm^2.

In ROAT a dose of 15.3 µg/cm^2 to 126.2 µg/cm^2 in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm^2.

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm^2 gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm^2.

The no-effect level in patch testing was below 0.036 µg/cm^2.

Isoeugenol

In ROAT a dose of 2.2 µg/cm^2 a response in 42% and 9.0 µg/cm^2 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/cm^2 caused a response in 23% and 77% reacted to 1.67 µg/cm^2.

The ED50 in patch testing was 32 µg/cm^2.

The no-effect in patch testing was below 0.15 µg/cm^2.

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/cm^2 if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 μg/cm^2 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/cm^2 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/cm^2 (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 µg/cm^2)/daily exposure to product (µg/cm^2/day)) x 100 (for %).

Table 11-5: Concentration limits in different product types based on 0.8 µg/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 /cm^2/day in grams**	**Exposure /cm^2/day in µg (1g= 1x10^6 µg)**	**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 mg/cm^2/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 ™ CG) (284), nickel (285), methyldibromo 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**	**ED$_{10}$ (µg/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.

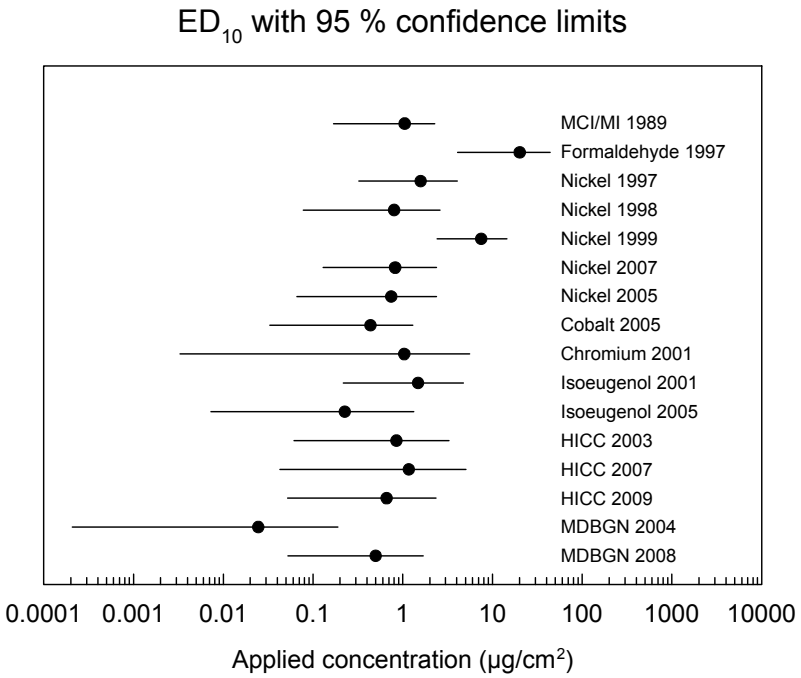

Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

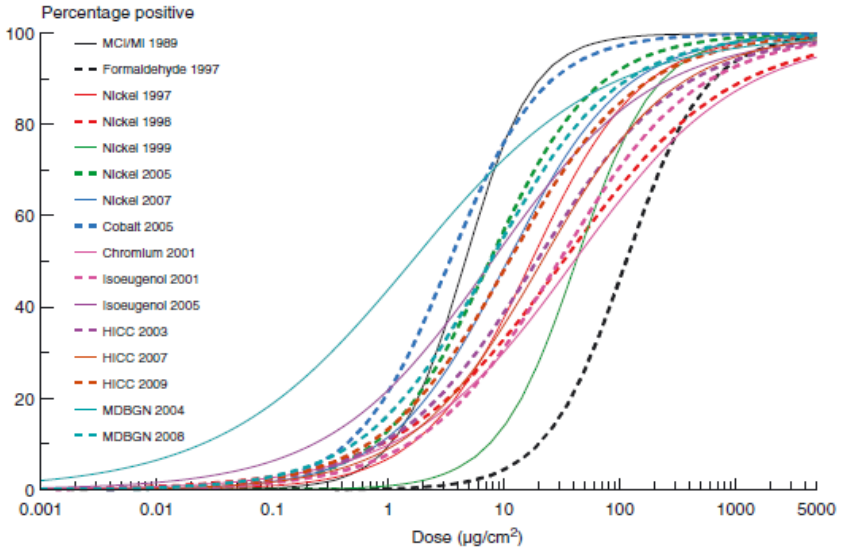

Figure 11-3: The fitted dose-response curves from the studies in Table 11-6**,** which are the basis for estimation of the ED10 value, after (280).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was 0.8 µg/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 µg/cm^2 and 0.89 µg/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 µg/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 µg/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 ug/cm² 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, dimetyl 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 extremly 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 (see11.1). 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	Hydroalcoholics for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcoholics 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

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 dermatitits 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.

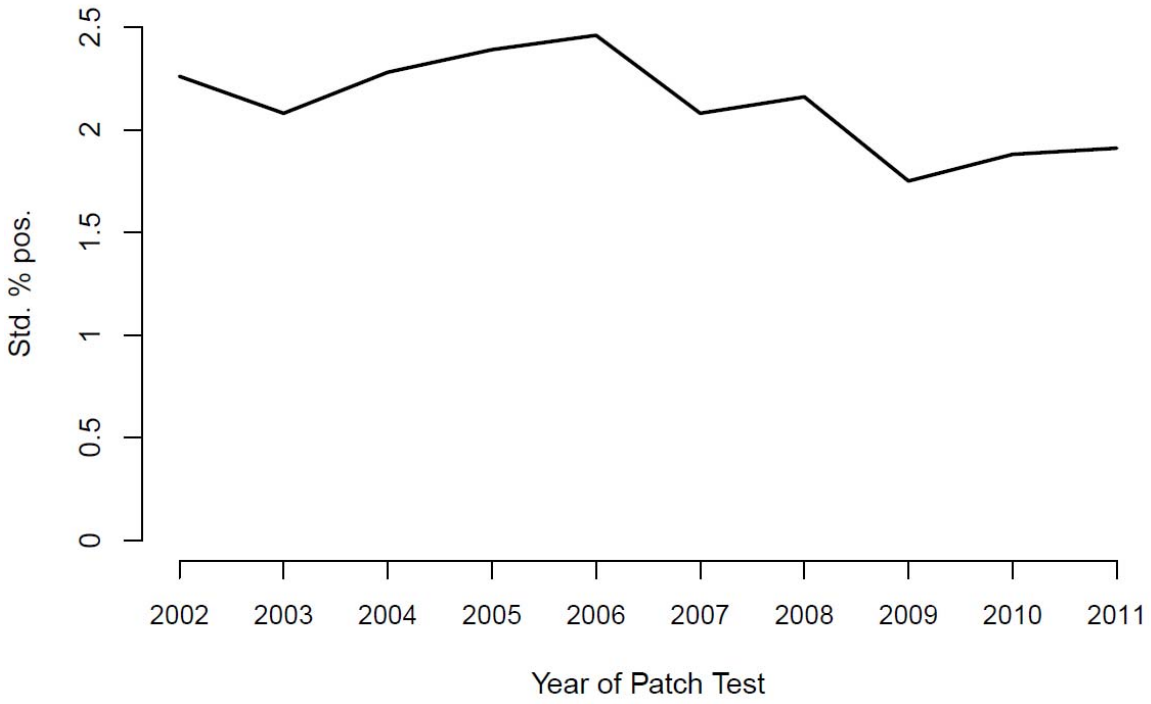

Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

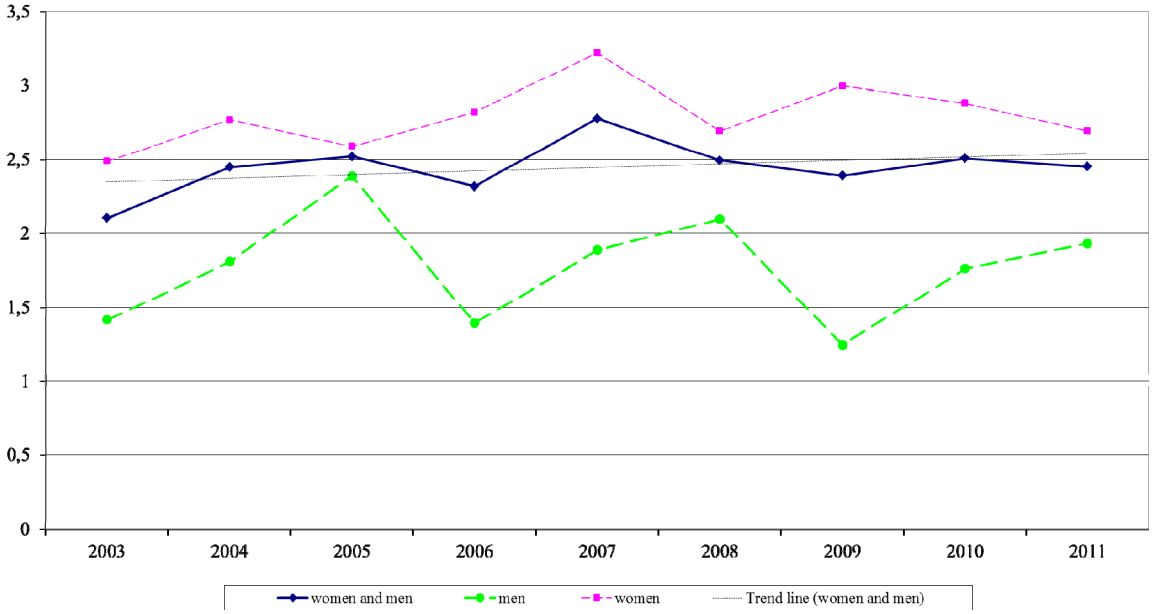

Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

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 µg/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 extremly 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.: +

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 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	+ (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.)

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;	++

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
	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	**8007-00-9;**	**++++**
NARCISSUS SPP.	diverse	++
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.)	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 hydrocinnamaldehdye	6658-48-6	none	9.5

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	+

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
AMYLCYCLOPENTANONE	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	+

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 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	
ILLICIUM VERUM FRUIT OIL	84650-59-9	Limited	none	
LAVANDULA SPICA	97722-12-8	Limited	none	

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/cm^2 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/cm^2 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 hithereto 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 $\mu g/cm^2$ (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 idendified 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 sensisitation potency is markedly increased by air exposure due to oxidation (autoxidation). 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 autoxidation 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 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. 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 prohapten, 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/cm^2 (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 prehapten and a prophapten. 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 prohapten, 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 prehaptens 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	*Myroxylon pereirae* 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/)

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 Committe on Consumer Products
UK	United Kingdom
US(A)	United States (of America)
UV	Ultraviolet

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Annex I - Catalgoue of fragrance allergens

Contents

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 Cometics 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.	*Evernia prun*.	Isoeug.	Hydroxy citron.	Cinnamal	Cinnamyl alcohol	Eugenol	Gera-niol	Alpha-amyl cinnamal
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

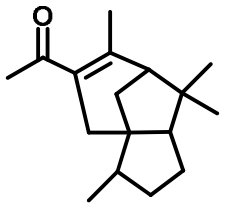
IVDK 2010b (11)	Germany, Switzerland and one centre in Austria	2005-2008 n=35633 tested with FM II, n=2217 with all SC	

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)

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

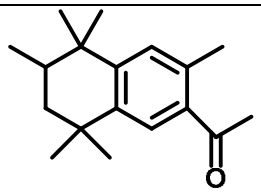
Catalogue of single chemicals evaluated

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-(3α,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 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".

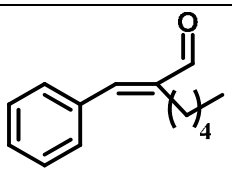
Additional information:
Acetyl cedrene (Vertofix®, IFF) is a complex mixture obtained from cedar wood oil by the acetylation of terpenes. The principal component of acetyl cedrene is methyl cedryl ketone (CAS 32388-55-9). It is a "top 100" substance (IFRA, pers. comm.2010)

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).

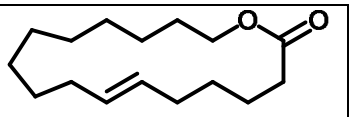
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; 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).

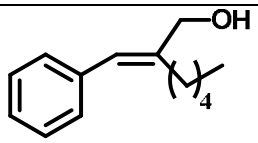
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

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).

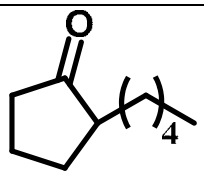
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol,	
2-Benzylidene- (6CI,8CI)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

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).

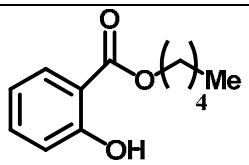
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

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: /

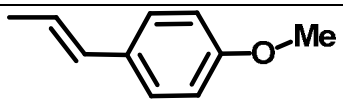
AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

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)

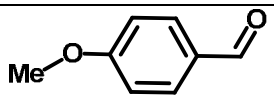
trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, 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

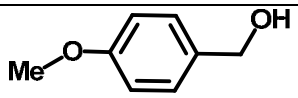
and fennel oils (38)

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).

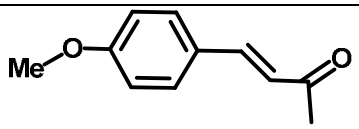
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy-α-hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methyloxy)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).

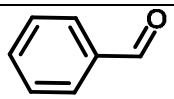
Additional information: /

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)

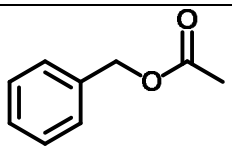
Additional information: /

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 Wöhrl 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: /

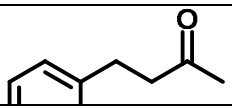
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl actetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl aceteate 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).

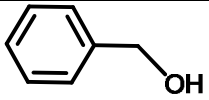
BENZYL ACETONE	
CAS # 2550-26-7	

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).

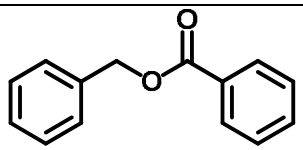
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

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 Directivr 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:

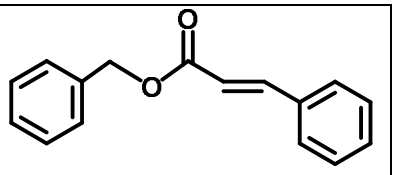
In the "background information" section of the 1999 opinion, benzyl benzoate is classified as "less frequently reported allergen"; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

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).

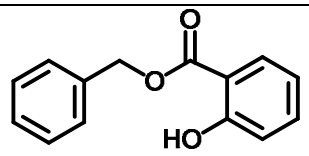
BENZYL CINNAMATE	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

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

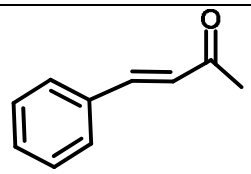
Clinical data:

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 Wöhrl 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).

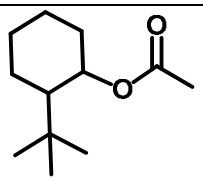
BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; 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: /

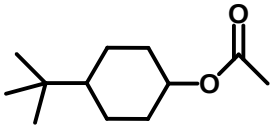
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

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).

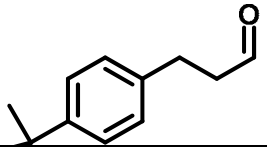
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinol A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; 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	

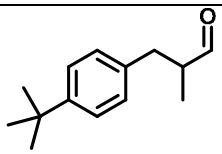
4-(1,1-Dimethylethyl)-benzenepropanal	
p-tert-Butyl-hydrocinnamaldehyde; 3-(4-tert-Butylphenyl)propanal; Bourgeonal; p-tert-Butyldihydrocinnamaldehyde	

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:

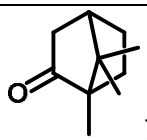
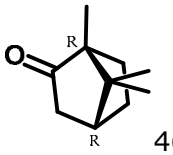
In the "background information" section of the 1999 opinion, lilial is classified as "less frequently reported allergen"; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were 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:

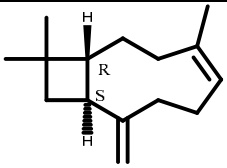
It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2  464-49-3
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.	

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	

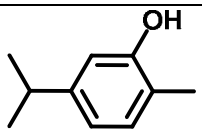
Current regulation: /

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 capciaty, 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	

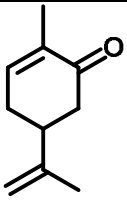
Current regulation: /

Clinical data:

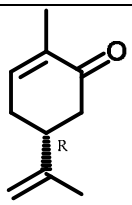
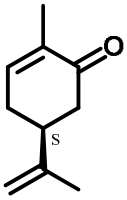
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an "excited back syndrome" and are thus of limited evidence (25). Meynadier et al. [11] patch tested 28 patients with contact 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	 99-49-0
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).

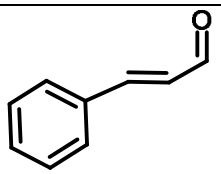
"Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis 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

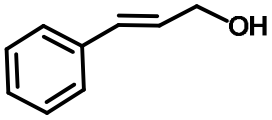
Clinical data:
In the "background information" section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

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	

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; Styrone; Styryl alcohol; Styryl carbinol; γ-Phenylallyl alcohol	

Current regulation: Annex III, part 1, n° 69

Clinical data:

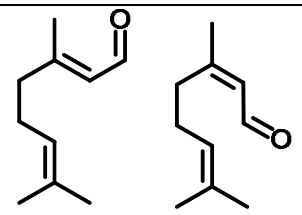
In the "background information" section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

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	
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	**Citral** = isomeric mixture of Geranial and Neral

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).

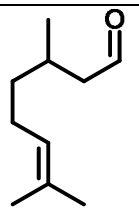
Additional information:

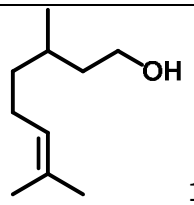
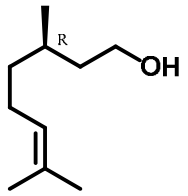
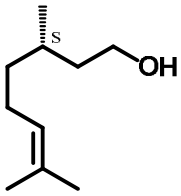
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

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	 106-22-9  1117-61-9  7540-51-4
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: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronellol 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 **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).

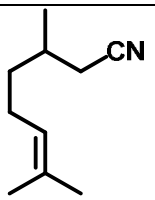
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronellol" (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%, 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).

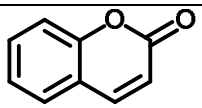
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLYL NITRILE	
CAS # 51566-62-2	
EC # 257-288-8	
3,7-Dimethyl-6-octenenitrile	
3,7-Dimethyl-6-octenonitrile (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).

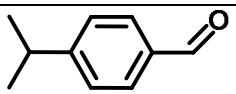
V. Mutterer et al. present the case of a 44 year old patient in whom coumarin was identified as culprit allergen by controlled ROAT testing with 1%, after having caused dermatitis by the use of a deodorant containing coumarin at 0.23% and an EdT (90).

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).

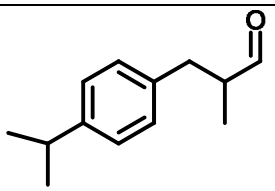
Coumarin is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

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; Cuminyl aldehyde; 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: …

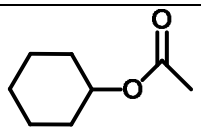
CYCLAMEN ALDEHYDE	
CAS # 103-95-7	
EC # 203-161-7	
α-Methyl-4-(1-methylethyl)-benzenepropanal	
p-Isopropyl-α-methyl-hydrocinnamaldehyde; 2-Methyl-3-(4-isopropylphenyl)propionaldehyde; 2-Methyl-3-(p-isopropylphenyl)propionaldehyde; 3-(4-Isopropylphenyl)-2-methylpropanal; 3-(p-Isopropylphenyl)-2-	

methylpropionaldehyde; 3-p-Cumenyl-2-methylpropionaldehyde(REACH, EINECS); 4-Isopropyl-α-methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl-α-methylhydrocinnamaldehyde; α-Methyl-4-(1-methylethyl)benzenepropanal; α-Methyl-p-isopropylhydrocinnamaldehyde	

Current regulation: …

Clinical data:
/

Additional information: It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

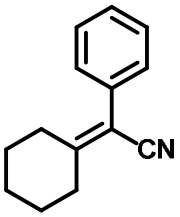
CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	

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).

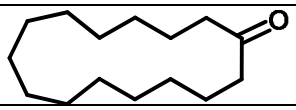
Additional information: A RIFM review is available (92).

alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	
CAS # 10461-98-0	
EC # 423-740-1	
α-Cyclohexylidenebenzeneacetonitrile	
alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ1α-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).

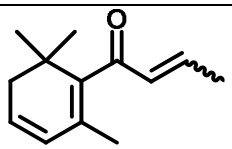
CYCLOPENTADECANONE	
CAS # 502-72-7	

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 pateints with known contact allergy to fragrance ingredients (19).

Additional information: …

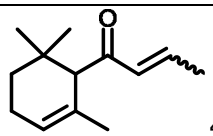
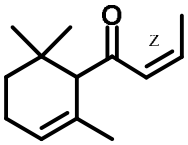
DAMASCENONE ***ROSE KETONE-4*** (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 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).

alpha-DAMASCONE (TMCHB)	 43052-87-5  23726-94-5
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**: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)-α-Damascone; cis-α-Damascone	

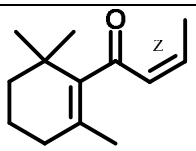
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

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.

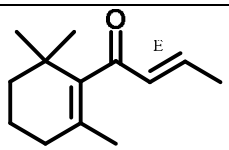
cis-beta-DAMASCONE	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)-β-Damascone	

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.

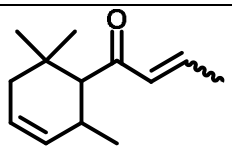
trans-beta-DAMASCONE	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)-β-Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans-β-Damascone; β-Damascone	

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%).

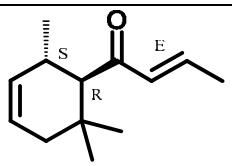
delta-DAMASCONE	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ-Damascone	

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.

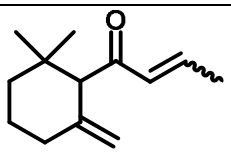
trans-trans-delta-DAMASCONE	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1α(E),2β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans-δ-Damascone; δ-Damascone; trans, trans-δ-Damascone	

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%).

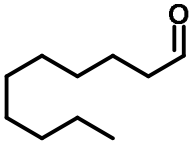
gamma-DAMASCONE	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

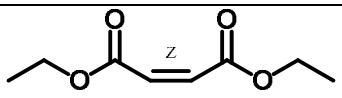
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

DECANAL	
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).

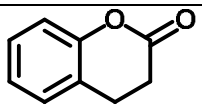
DIETHYL MALEATE	
CAS # 141-05-9	
EC # 205-451-9	
(**2Z)-Diethyl but-2-enedioate**	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

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: /

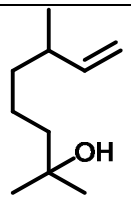
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ-lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

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)	

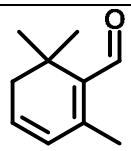
Current regulation: /

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).

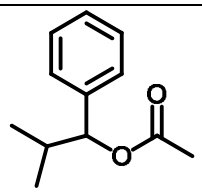
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

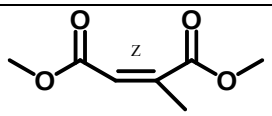
Additional information:

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; Benzyldimethylcarbinol acetate; Benzyldimethylcarbinyl 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).

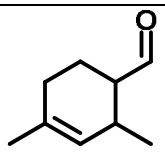
DIMETHYL CITRACONATE	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

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: …

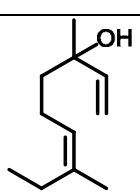
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

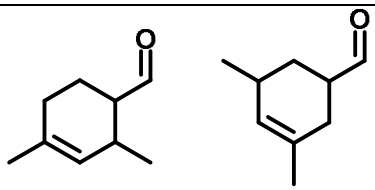
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyllinalool	

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).

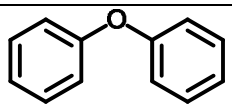
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde **3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde**	2,4- 3,5-
Hivertal; Vertocitral	

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).

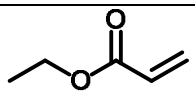
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryl 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	

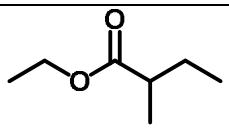
Current regulation: Annex II, n° 435

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).

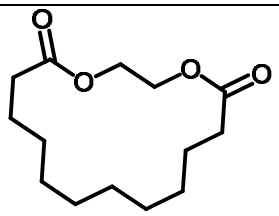
Additional information: /

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).

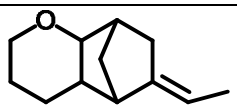
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

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).

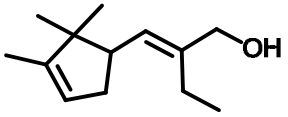
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

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).

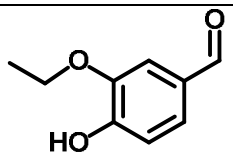
Additional information: /

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).

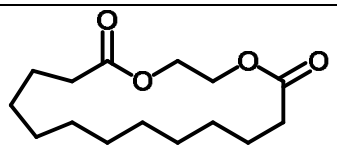
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; 3-Ethoxy-4-hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde 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).

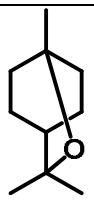
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

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	

6171; Terpan; p-Cineole	

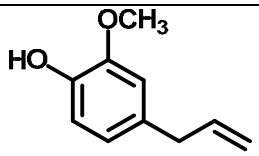
Current regulation: /

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; Eugenic 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).

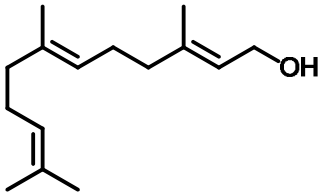
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (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 Wöhrl 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

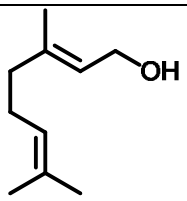
Clinical data:

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 Wöhrl 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).

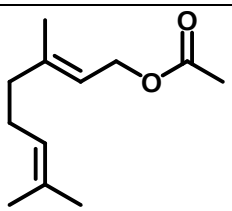
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman 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 autoxidization at air exposure and via metabolic oxidation. Geranial and neral together with hydroperoxide have been identified as oxidation products when geraniol autoxidizes (84). Geranial 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).

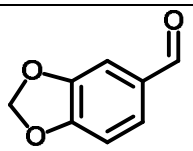
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic 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).

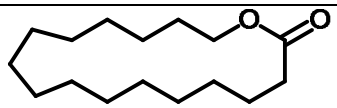
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Geliotropin; 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).

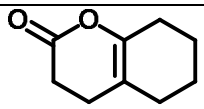
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid;1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; 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: /

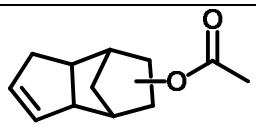
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ-Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ-1,6-2-Oxabicyclo(4.4.0)decen-3-one	

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.

3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxydihydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.02,6]dec-3-enyl acetate; Tricyclodecenyl acetate	

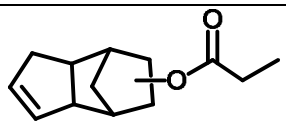
Current regulation: /

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).

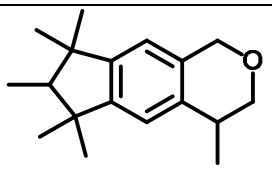
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenol propanoate	

3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.02,6]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: /

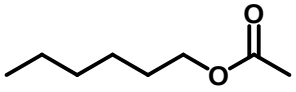
Clinical data:

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).

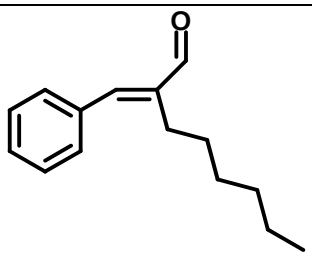
HEXYL ACETATE	
CAS # 142-92-7	
EC # 205-572-7	
Hexyl ethanoate	
Acetic acid, hexyl ester, Hexyl alcohol, acetate; 1-Hexyl acetate; Exceed 600; Hexyl acetate; Hexyl ester acetic	

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:

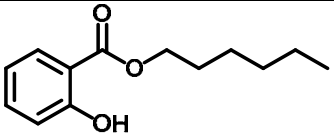
In the "background information" section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as "less frequently reported allergen"; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products 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	

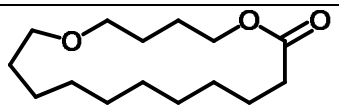
Current regulation: /

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).

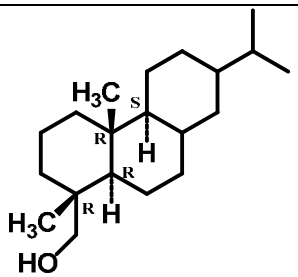
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

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)- 1-henanthrenemethanol; Tetrahydroabietyl alcohol	

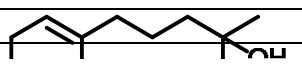
Current regulation: AnnexII, n° 440

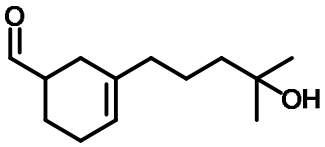
Clinical data:

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 tetrahydroabitetyl 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; Lyral	

Current regulation: Annex III, part 1, n° 79

Clinical data:

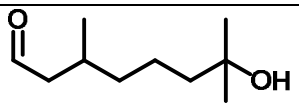
In the "background information" section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

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-; Cyclalia; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguettine principle; NSC	

406740; Phixia	

Current regulation: Annex III, part 1, n° 72

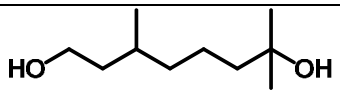
Clinical data:

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	

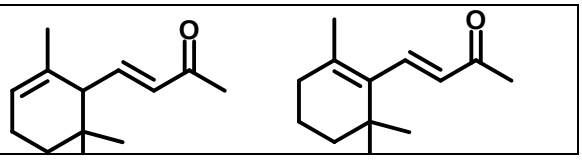
Current regulation: /

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	

Ionone	
Irisone, mixture of alpha- and beta ionone	

Current regulation: /

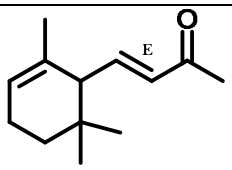
Clinical data: / (see single isomers)

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.

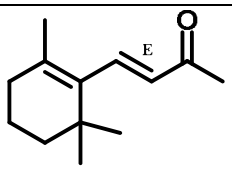
alpha-IONONE	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; 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).

beta-IONONE	
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: /

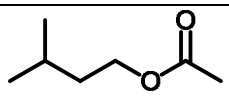
Clinical data:

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).

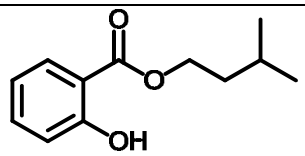
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic 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)

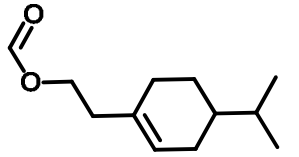
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; 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).

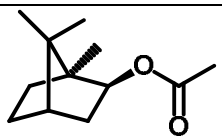
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletly defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

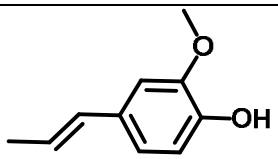
Current regulation: Annex III, part 1, n° 170

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	

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:

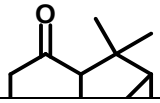
In the "background information" section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been 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: /

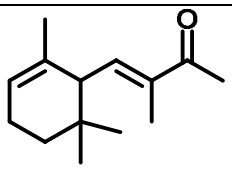
Clinical data:

The Larsen 2001 study idenfied 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

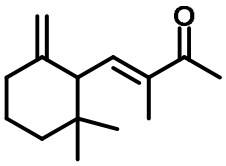
Additional information:

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".

alpha-ISOMETHYL IONONE	
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

gamma-Methylionone	
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:

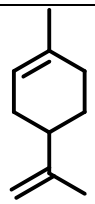
In the "background information" section of the 1999 opinion, "gamma-methylionone" is classified as "less frequently reported allergen"; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

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 sponateously 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

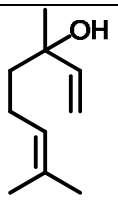
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- 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 rational 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; Linanool; 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:

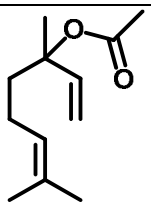
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

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).

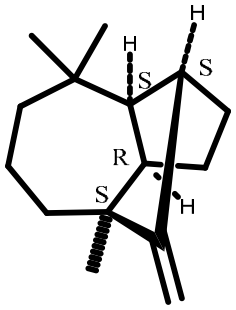
Additional information:

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 hydoperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be 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: /

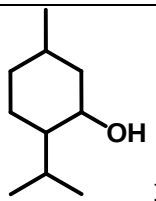
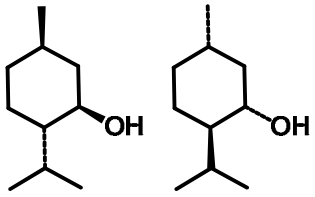
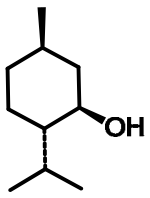
Additional information:

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  89-78-1  2216-51-5
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**: (1α,2β,5α)-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	

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1α,2β,5α)]-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).

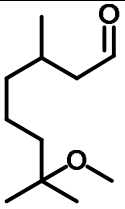
A case of contact allergy to "peppermint and menthol" in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155).Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application 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).

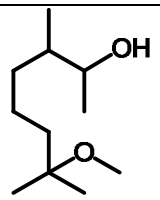
METHOXYCITRONELLAL	
CAS # 3613-30-7	
EC # 222-784-5	
7-Methoxy-3,7-dimethyl-octanal	
7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal	

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	

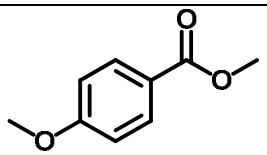
Current regulation: /

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).

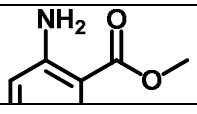
METHYL p-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

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	

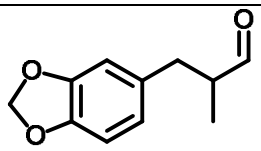
EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; Rejex-iT 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).

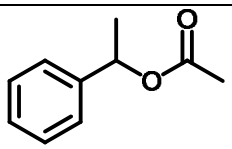
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

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).

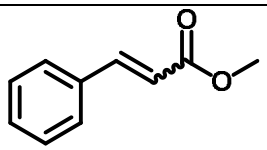
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl phenyl carbinyl 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	

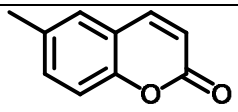
Current regulation: /

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	

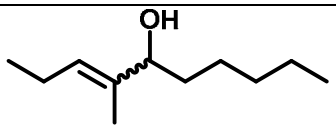
Current regulation: Annex III, part 1, n° 46

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 Portugese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

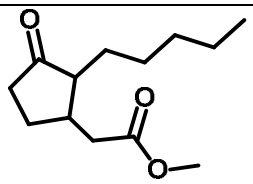
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

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	

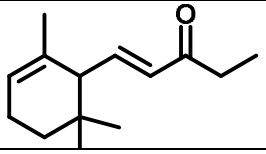
Current regulation: /

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	

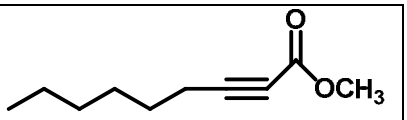
Current regulation: /

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).

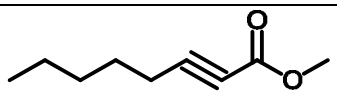
METHYL OCTINE CARBONATE	
CAS # 111-80-8	
EC #	
Methyl 2-octynoate	
Methyl 2-Nonynoate, MOC	

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 artificial	

Current regulation: Annex III, part 1, n° 89

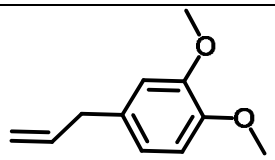
Clinical data:

In the "background information" section of the 1999 opinion, methyl 2-octynoate is classified as "less frequently reported allergen"; with only single cases of reported contact allergy, but the observation of this compound being a strong sensitizer according to IFRA (33), as also reported by Hostynek and Maibach (173)

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.49%) positive reactions in 2401 consecutively PTed patients (1% pet.) (4). The IVDK 2010 study, n=1 weak positive reaction was observed in 988 patients tested with 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 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-	

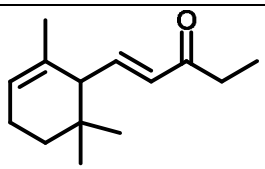
Current regulation: Annex II, 451

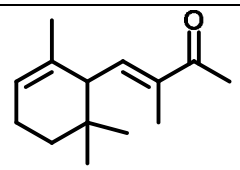
Clinical data:

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**	

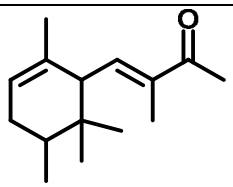
8-Methyl-α-ionone-10-methyl-α-ionone mixt.; Iralia Mixture	

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 alpha-IONONE ISOMERS); other names: Methyl-alpha-cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41456, last accessed 2010-07-14).

5-METHYL-alpha-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	

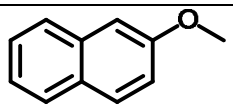
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to alpha-irone (10% pet.) (17).

Additional information:

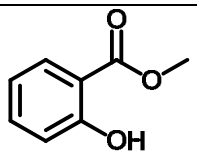
A RIFM review is available (176), citing a (negative) human maximisation test and the study results quoted.

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	

Current regulation: /

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: /

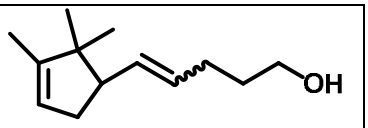
Clinical data:

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").

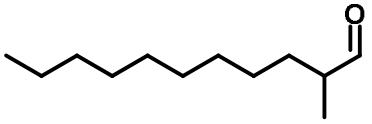
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

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).

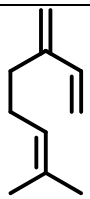
Additional information: /

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	

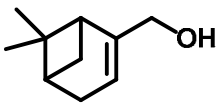
Current regulation: /

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	

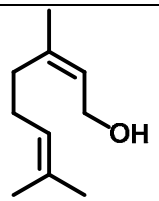
(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol

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: /

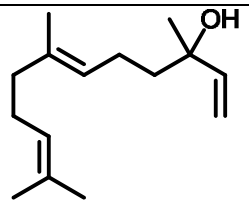
Clinical data:

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.

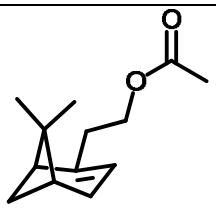
Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	

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.

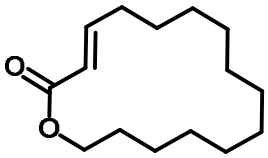
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol 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).

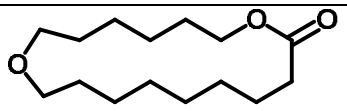
Additional information: /

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	

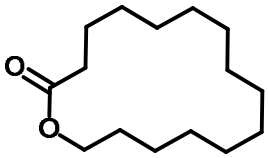
Current regulation: /

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).

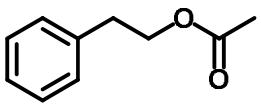
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ-lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; 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.

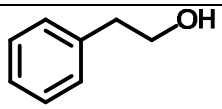
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbinyl acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl acetate; β-Phenylethanol acetate; β-Phenylethyl 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).

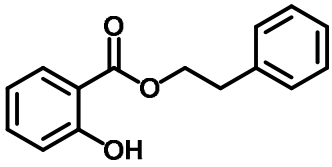
PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol; 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).

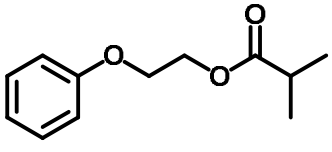
PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201-732-5	
2-Phenylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	

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	

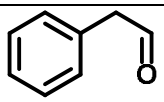
406209; Phenoxyethyl isobutyrate; β-Phenoxyethyl isobutyrate	

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	

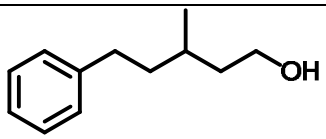
Current regulation: /

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:

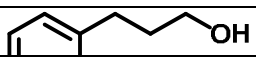
SCCS opinion: 1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assesment " (QRA: Citral, farnesol and phenylacetaldehyde)

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	

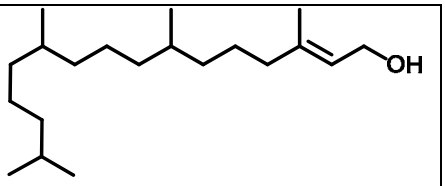
CAS # 122-97-4	
EC # 204-587-6	
3-Phenylpropan-1-ol	
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; 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: …

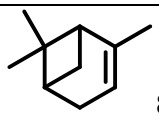
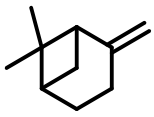
PHYTOL	
CAS # 150-86-7	
EC # 205-776-6	
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
Phytol; (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol	

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	 80-56-8  127-91-3
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	
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)	
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)	

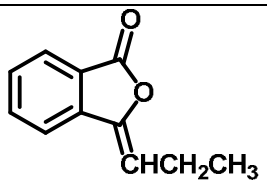
80-56-8: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene **127-91-3**: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 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 at 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: /

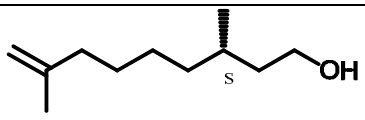
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; 3-Propylidenephthalide; Celeriax; Propylidenephthalide	

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)-	

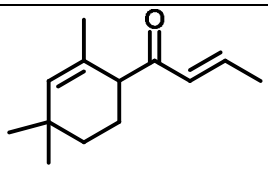
α-Citronellol	

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])"

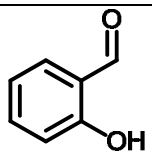
trans-ROSE KETONE-5	
CAS # 39872-57-6	
EC # 254-663-8	
(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one	
alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	

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	

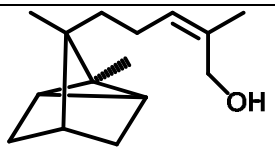
Current regulation: /

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 Wöhrl 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).

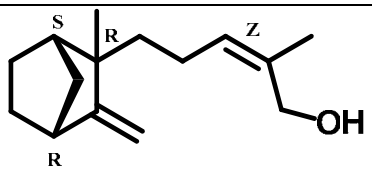
Additional information: Along with other derivates of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

alpha-SANTALOL	
CAS # 115-71-9	
EC # 204-102-8	
(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methylPent-2-en-1-ol	
2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.02,6]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.

beta-SANTALOL	
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 (MJCDRG, 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-dimethyl tricyclo[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).

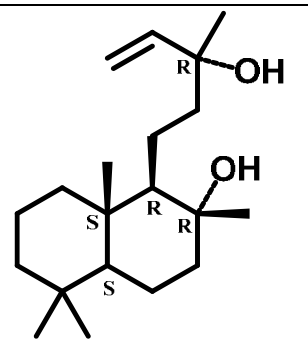
Additional information: "There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)" (A.M. Api, pers. comm., 2010).

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,4aβ,8aα]] - 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.,4a.beta.,8a.alpha.]]-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-.alpha.-vinyldecahydronaphthalene-1-propan-1-ol	

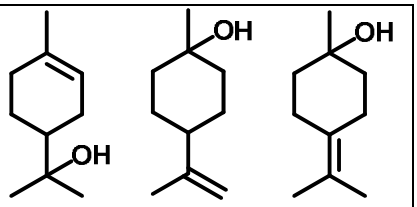
Current regulation: /

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 clincial data, but several maximisation studies, one of which was positive in a few volunteers, which was apparently due to an impurity.

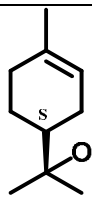
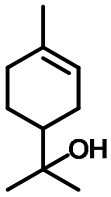
0986/06 - Opinion on Sclareol (sensitisation only) (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

TERPINEOL	
CAS # 8000-41-7	
EC # 232-268-1	
Mixtures of isomers	
Terpineol 318, mixture of terpineol isomers alfa, beta, gamma	alfa beta gamma

Current regulation: /

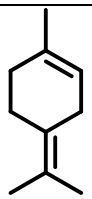
Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study "Takenaka 1986", finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding 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).

alpha-TERPINEOL	 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; Terpineol 350; dl-α-Terpineol; α,α,4-Trimethyl-3-cyclohexene-1-methanol; α-Terpineol	 98-55-5
Current regulation: /	

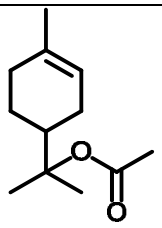
Clinical data:
A RIFM review is available (201) specifically on (-)-alpha-terpineol stating that "no data is available" regarding skin sensitisation. Another RIFM review is available on alpha-terpineol (202). In the Frosch 2002 b study, 1 of 1606 consecutive patients showed a positive reaction, but 11 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 terpineol (mixed isomers) (25). In 63 patients positive to the FM I, 2 had a positive PT reaction to alpha terpineol, 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 terpineol (mixture of isomers). Comments on turpentine under pinene.

Terpinolene	
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.,

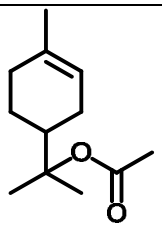
gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204). Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

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)

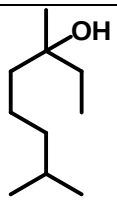
alpha-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate; 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)

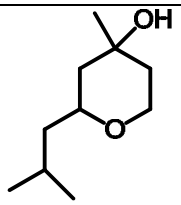
Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	

Current regulation: /

Clinical data:
/

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010). A RIFM

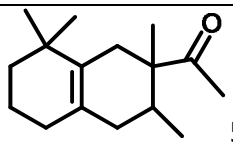
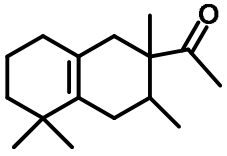
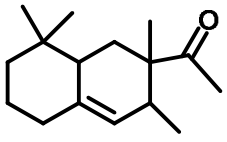
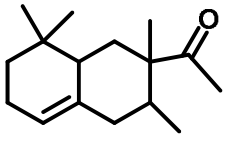
review is available (205) quoting 1 negative human maximisation test.

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).

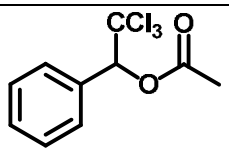
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	 54464-57-2
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9	
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2) **1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone** (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)	 54464-59-4  68155-66-8  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'-acetonaphthone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemone 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).

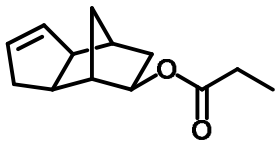
It is a "top 100" substance (IFRA, pers. comm. 2010)

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, 3a,4,5,6,7,7a-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-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).

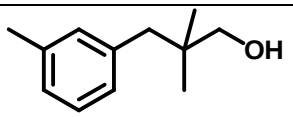
3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL	

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-isocamphyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of "synthetic sandalwood oil".

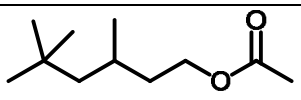
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

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).

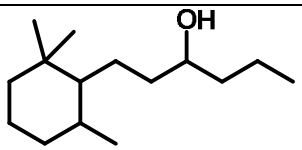
Additional information: /

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)

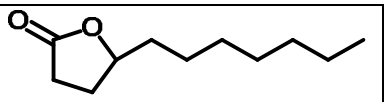
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethyl-cyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; Finotimber; 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: /

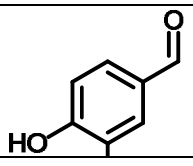
gamma-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ-lactone; (RS)-γ-Undecalactone; (±)-γ-Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 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: /

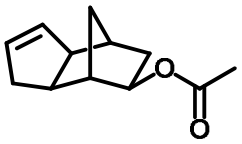
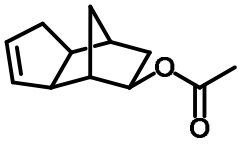
Clinical data:

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 Wöhrl 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	
3α,4,5,6,7,7α-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6) **3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-5-yl acetat** (5413-60-5)	2500-83-6  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[5.2.1.02,6]dec-3-ene; Greenyl acetate;	

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[5.2.1.02,6]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[5.2.1.02,6]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.A 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). Essenctial oil of fruit juice is „obtained by from a fruit juice during its concntration 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 varous 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 solvant"[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 sovant"[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 elimitated"[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 Cometics 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 potenial 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 Portugese 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 know 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 OIL* — *CAS 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 (Cinnamonum 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 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: *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 *Cinnamonum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamonum 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 Cinnamom, 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 Cinnamonum 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 *(Bergamot, ext.)*
Bergamot Oil, Bergamot Orange Oil	*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=search.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 Wöhrl 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); ß-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 an the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

	Type		Mediterranean		Equatorial
Parameter	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	8–128–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geranial	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*
Grapefruit oil, expressed

CAS 8016-20-4 ; EC /
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) PEEL OIL EXPRESSED*

CAS 97766-30-8, 8008-57-9, EC 307-891-8 *(Orange, sweet,*

	Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 *(Orange, sweet, ext.)*
(Sweet) Orange oil	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 Wöhrl 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 *…*	*CAS 223748-44-5; EC /*
Oil of tangerine	[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*

Coriander oil

CAS 84775-50-8; EC 283-880-0 *(Coriander, ext.)*

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. Watson[25]

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 Wöhrl 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 flexosus* (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.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 linallol 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 Wöhrl 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 *Szyigium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Speng.) 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 exctract 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 Wöhrl 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 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 *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, Jasminum grandiflorum, ext. = INCI)*
Jasmine abs.	
JASMINUM OFFICINALE *FLOWER OIL*	*CAS 90045-94-6; EC* 289-960-1 *(Jasmine, Jasminum officinale, 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, Jasminium 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); phytyl 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 Wöhrl 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); ß-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 Herb Oil is an essential oil distilled from the flowering herbs of the Lavendin, *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 "Lavendula 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 (≤ 1%) camphor (≤ 0.5%), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] (≥ 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*

Cornmint oil

CAS 68917-18-0 ; EC /

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 (Spearmint, ext.)

Spearmint oil

INCI: MENTHA VIRIDIS EXTRACT

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to “spearmint 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 spearmint (246).

Additional information:

ISO 4720:2009 nomenclature: Mentha spicata L. Mentha Spicata Oil is an essential oil obtained from the herbs of the Spearmint, 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 (spearmint) (53) and had been tested with positive results in “toothpaste cases”, even at a concentration as low as 0.067% (68).

For Oil of spearmint -- Part 1: Native type (Mentha spicata L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearmint -- Part 2: Chinese type (80 % and 60 %) (Mentha viridis L. var. crispa Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearmint -- Part 3: Indian type (Mentha spicata L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearmint -- 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 peruvianium) 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 is 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%) [§]

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 aera. 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.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=searc

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 norpatchoulenol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-ß-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); (-)-ß-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulenol (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	--
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 OIL*	*CAS 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 OIL*	*CAS 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 (Carribean)), 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, ß-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 OIL* *CAS 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 OIL* *CAS 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) [§]

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. hand dermatitis	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 Zizanoides Root Oil is an essential oil distilled from the dried roots of the grass *Vetiveria zizanoides* (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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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					EC3 value *					
INCI name *(other name)*	**CAS no.**	**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**	**%**	**µg/cm²**	**M**	**lowest for the substance (%)**	**Comment (deviation from OECD 429 etc)**	**Reference**
Allyl phenoxyacetate	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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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

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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
p-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

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)

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
Dibenzyl ether	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 *Treemoss absolute*	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 *Treemoss absolute*	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 *Oakmoss*	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
trans-2-Hexenal	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
trans-2-Hexenal	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
p-Isobutyl-α-methyl hydrocinnamaldehdye	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
Isocyclocitral	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
Isocyclogeraniol	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
Jasmine absolute (Grandiflorum)	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
*d-Limonene***	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
*d-Limonene***	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
*d-Limonene***	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
*d-Limonene***	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
*d-Limonene***	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

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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 *p-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 *p-Mentha-1,8-dien-7-al*	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (Myroxylon pereirae 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

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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 *Majantol*	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

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Annex III - Tabular summary of dose-elicitation studies in sensitised patients

Contents

Chloroatranol

Chloroatranol (allergen in oak moss absolute: *Evernia prunastri*) (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).

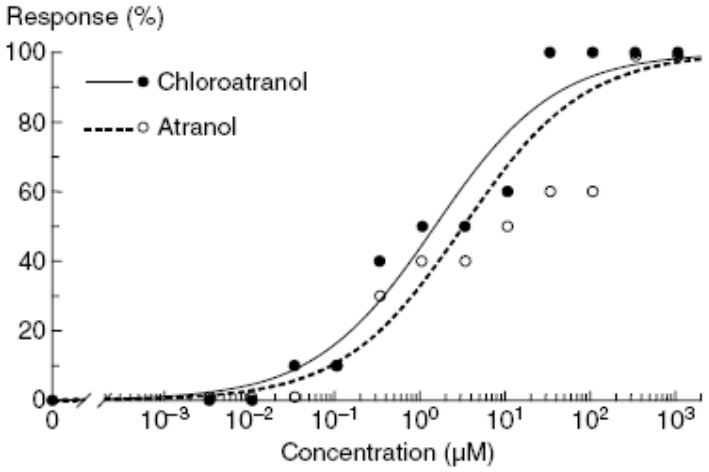


Fig. 1. Observed response rates and fitted parallel logistic dose–response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

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

	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)

Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application (μg HICC cm^{-2})	Number of applications after 1 week	Total accumulated dose after 1 week (μg HICC cm^{-2})	Number of applications after 2 weeks	Total accumulated dose after 2 weeks (μg HICC cm^{-2})	Number of applications after 3 weeks	Total accumulated dose after 3 weeks (μg 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.

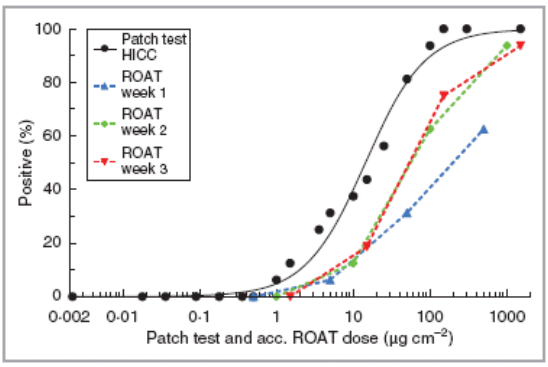


Fig 3. The fitted dose–response curve for the patch test (n = 16) and the 1-week, the 2-week 3-week accumulated ROAT doses.

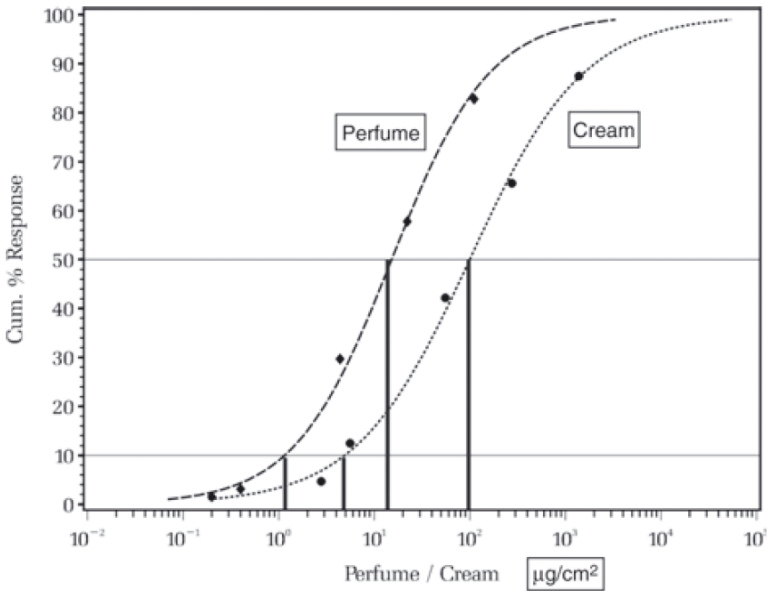
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

In a study be 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/cm² in cream 5.6 µg/cm² in cream 55.6 µg/cm² in cream 277.8 µg/cm² in cream 1388.9 µg/cm² in cream	0.2 µg/cm² in ethanol 0.4 µg/cm² in ethanol 4.4 µg/cm² in ethanol 22.2 µg/cm² in ethanol 111.1 µg/cm² 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/cm²: 4.7%	Ethanol preparation: 0.2 µg/cm²:1.6%

	5.6 µg/cm²: 12.5% 55.6 µg/cm²: 42.2% 277.8 µg/cm²: 65.6% 1388.9 µg/cm²: 87.5%	0.4 µg/cm²: 3.1% 4.4 µg/cm²: 29.7% 22.2 µg/cm²: 57.8% 111.1 µg/cm²: 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 µg/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 µg/cm^2
PT no effect level (observed)	< 0.01% = 0.4 µg/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 elicitating 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)

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Evaluation of certain food additives

Eighty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives

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Evaluation of certain food additives

Eighty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives

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Contents

[1] For use in formulas for special medical purposes intended for infants.

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Rome, 4–13 June 2019

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List of abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AL-HMF	acidified liquid human milk fortification
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATBC	Alpha-Tocopherol, Beta Carotene Cancer Prevention
$AUC_{0-\infty}$	area under the concentration–time curve from time 0 to infinity
BMD	benchmark dose
bw	body weight
CARET	Beta-Carotene and Retinol Efficacy Trial
CAS	Chemical Abstracts Service
CCFA	Codex Committee on Food Additives
CCFA51	Fifty-first Session of the Codex Committee on Food Additives
CI	confidence interval
CIFOCOss	FAO/WHO Chronic Individual Food Consumption database – summary statistics
CITREM	citric and fatty acid esters of glycerol
C_{max}	maximum concentration
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EHC 240	Environmental Health Criteria, No. 240
F_1	first filial generation
FAIM	Food Additive Intake Model
FAO	Food and Agriculture Organization of the United Nations
FSMP	formulas for special medical purposes for infants
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GIFT	Global Individual Food consumption data Tool
GMP	Good Manufacturing Practice
GSFA	(Codex) General Standard for Food Additives
HMF	human milk fortification
HPLC	high-performance liquid chromatography
hPXR	human pregnane X receptor
INS	International Numbering System for Food Additives
IU	International Units
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level

MOE	margin of exposure
mPXR	mouse pregnane X receptor
NAL-HMF	non-acidified liquid human milk fortification
NHANES	National Health and Nutrition Examination Survey (USA)
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PTWI	provisional tolerable weekly intake
RIVM	Dutch National Institute for Public Health and the Environment
rPXR	rat pregnane X receptor
RR	relative risk
T_3	triiodothyronine
T_4	thyroxine
T_{max}	time to reach the maximum concentration
TSH	thyroid stimulating hormone
USA	United States of America
WHO	World Health Organization

Monographs containing summaries of relevant data and toxicological and dietary exposure evaluations are available from WHO under the title:

Safety evaluation of certain food additives. WHO Food Additives Series, No. 78, 2019.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 23, 2019.

Dedication

Ms Inge Meyland

Institute of Food Safety and Nutrition, Denmark (retired)

It was with great sadness that the Committee noted the passing of Ms Inge Meyland. Inge was an active member of JECFA until 2016 and played a vital role in shaping the approaches to the development of specifications for food additives. She was instrumental in developing the collection of JECFA specifications published in Monograph 1, *Combined Compendium of Food Additive Specifications*, reproducing for the first time all the specifications monographs from the 1st to the 65th meeting (1956–2005) of JECFA. She continued to serve as an expert and chaired many meetings with an unwavering dedication to scientific excellence and collegiality. Inge was a cornerstone of JECFA over many years, and her deep knowledge of the subject matter and long-standing experience made her famous for her "institutional memory". Her warm personality, bright mind and great sense of humour will always be remembered.

Inge will be thoroughly missed by her peers and friends in the scientific community. In recognition of her service, the Committee dedicates this report to the memory of Ms Inge Meyland.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome from 4 to 13 June 2019. The meeting was opened on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) by Dr Markus Lipp, Head of Food Safety and Quality, Agriculture and Consumer Protection Department, FAO.

Dr Lipp preceded his opening remarks by welcoming Dr Yongxiang Fan, Chairperson of the Codex Committee on Food Additives (CCFA), and all other meeting participants. Dr Lipp highlighted the roles and responsibilities that JECFA has in the framework of the international food safety standard development work of the Codex Alimentarius Commission. He reminded the JECFA experts about their responsibility to elaborate the most unbiased and best scientific advice possible.

Dr Lipp emphasized that participants had been invited not as representatives of their employer or country, but to serve solely in their capacity as scientific experts to provide sound and independent scientific advice to generate food standards designed to be protective of health for all consumers and trade-inclusive for all regions and countries. He finished by urging the attendees to be as open and transparent as possible and emphasizing that scientific excellence will require the input from all and the courage to ask critical questions.

1.1 Declarations of interests

The Joint Secretariat informed the Committee that all experts participating in the eighty-seventh meeting had completed declaration of interest forms. No conflicts of interest were identified.

1.2 Modification of the agenda

No data were submitted on β-apo-8′-carotenoic acid methyl and ethyl esters, and these were removed from the evaluation of carotenoids (see agenda item 7.1 in Annex 4). The Committee also renamed the remaining carotenoids on the agenda (β-carotene, β-carotene from *Blakeslea trispora* and β-apo-8′-carotenal) as carotenoids (provitamin A). β-Carotene-rich extract from *Dunaliella salina* was included in the group of carotenoids (provitamin A).

Citric and fatty acid esters of glycerol (CITREM) was added to agenda item 7.3 for revision of specifications.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 *(1)*, there have been 86 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the eighty-sixth meeting (Annex 1, reference *241*).

The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives (section 2);
- review and prepare specifications for certain food additives (including flavouring agents) (sections 3 and 4 and Annex 2);
- undertake safety evaluations of certain food additives (section 3 and Annex 2).

2.1 Report from the Fifty-first Session of the Codex Committee on Food Additives (CCFA)

Dr Yongxiang Fan, Chairperson of CCFA, supported by the Codex Secretariat, provided the Committee with an update on the work of CCFA since the eighty-sixth meeting of JECFA (Annex 1, reference *241*).

The Fifty-first Session of CCFA (CCFA51) noted the conclusions of the eighty-sixth meeting of JECFA on the safety of nine substances and 69 flavourings *(2)*. CCFA51 agreed to include basic methacrylate copolymer (International Numbering System for Food Additives [INS] 1205), lutein from *Tagetes erecta* (INS 161b(i)) and zeaxanthin (synthetic) (INS 161h(i)) in Table 3 (Additives Permitted for Use in Food in General, Unless otherwise Specified, in Accordance with GMP [Good Manufacturing Practice]) of the Codex *General Standard for Food Additives* (GSFA) (CODEX STAN 192-1995) *(3)*. CCFA51 solicited members to provide more information or data to JECFA to allow the Committee to complete its evaluations of anionic methacrylate copolymer (INS 1207), neutral methacrylate copolymer (INS 1206) and spirulina extract (INS 134) and noted that no action was necessary for other substances.

CCFA51 forwarded specifications for the identity and purity of six food additives (one new specification and five revised specifications) and 27 flavouring agents (20 new specifications and seven revised specifications) prepared by the eighty-sixth meeting of JECFA and recommended them to the Forty-second Session of the Codex Alimentarius Commission for adoption. CCFA51 agreed on a revised priority list of substances for evaluation (or re-evaluation) by JECFA, which included 24 food additives (10 food additives were ranked as the highest

priority), 76 flavouring agents and 29 processing aids. CCFA51 agreed to amend the circular letter on the priority list for the purpose of clarification.

CCFA51 also made recommendations on 155 provisions already in the Codex step procedure and/or already adopted and discussed 102 proposed new and/or revised provisions of the GSFA. CCFA51 made major progress on replacing Note 161 by developing alternative wording for Note 161 relating to the use of sweeteners. CCFA51 agreed to establish both ingoing and residue levels for nitrates and nitrites in the GSFA.

CCFA51 agreed to delete red 2G (INS 128) and distarch glycerol (INS 1411) from the *Class Names and the International Numbering System for Food Additives* (CXG 36-1989) *(4)*. The name of INS 160a(iv) was changed from "Carotenes, beta-, algae" to "β-carotene-rich extract from *Dunaliella salina*". CCFA51 also completed the work on the alignment of the food additive provisions related to 23 commodity standards (13 standards for milk and milk products, two standards for sugars, two standards for natural mineral waters, three standards for cereals, pulses and legumes, three standards for vegetable proteins).

CCFA51 considered the issue of group food additives. It was understood that JECFA was going to re-evaluate two groups of food additives (carotenoids and 2-phenylphenols or *ortho*-phenylphenols) and had a general discussion on the use of the terms "group ADI [acceptable daily intake]" and "group PTWI [provisional tolerable weekly intake]" as well as how JECFA assigns group ADIs. The outputs of JECFA will guide the future considerations of CCFA in this regard.

The Fifty-second Session of CCFA will continue its routine work, including the development of the GSFA, alignments of food additive provisions in the Codex commodity standards with the corresponding provisions of the GSFA *(3)* and revisions to the *Class Names and the International Numbering System for Food Additives* (CXG 36-1989) *(4)*.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, the Committee took into consideration the principles established and contained in the publication *Principles and methods for the risk assessment of chemicals in food* (Environmental Health Criteria, No. 240 [EHC 240]), published in 2009 *(5)*.

2.2.1 Application of group ADIs

At the Fiftieth Session of CCFA, the Codex Secretariat noted that some food additives – such as provitamin A carotenoids (i.e. synthetic β-carotenes, β-carotene from *Blakeslea trispora,* β-apo-8′-carotenal and methyl and ethyl esters of β-apo-8′-carotenoic acid); chlorophylls and chlorophyllins, copper complexes;

and polyoxyethylene sorbitan esters (i.e. polyoxyethylene (20) sorbitan esters of lauric, stearic, palmitic and oleic acids and triesters of stearic acid) – were listed under the same food additive heading in the GSFA, despite not being included in a group ADI. The Codex Secretariat sought clarification from the present Committee on the application of group ADIs.

In making recommendations on the safety of food additives, the Committee takes into consideration the principles regarding group ADIs contained in EHC 240 *(5)*.

The Committee noted that most of the food additives about which CCFA had sought advice had been last considered as groups at several meetings up to and including the twenty-third meeting in 1980 and that the Committee did not explicitly use the term group ADI at those early meetings. Of these food additives, the Committee was able to confirm that group ADIs should have been established for the chlorophylls and chlorophyllins (copper complexes), polyoxyethylene sorbitan esters (polysorbates), ascorbyl esters, ethylenediaminetetraacetates, thiodipropionates, ferrocyanides, tartrates, stearoyl lactylates and iron oxide food additives.

For nitrates and nitrites, the respective ADIs are expressed as the ions and therefore encompass the different salts. The group ADI for steviol glycosides, expressed as steviol, includes the whole family of steviol glycosides. The Committee was also able to confirm that the PTWI of 2 mg/kg body weight (bw) for aluminium and its salts, when expressed as aluminium, refers to all aluminium salts used in food additives, as well as other sources of aluminium.

An "unconditional" ADI of 0–0.2 mg/kg bw for 2-phenylphenol was first established by JECFA at its eighth meeting in 1964. According to FAO documents, 2-phenylphenol and sodium *o*-phenylphenate were first evaluated by the 1962 JECFA for their use as a post-harvest treatment of fruits and vegetables to protect against microbial damage during storage and distribution. The current FAO specifications still refer to this use. In 1999, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) established an ADI of 0–0.4 mg/kg bw for 2-phenylphenol; an ADI was not established for the sodium salt because it rapidly dissociates to 2-phenylphenol *(6)*. 2-Phenylphenol has a minor use as a flavouring agent, and, during its evaluation at the fifty-fifth meeting of JECFA, the Committee cited the most recent ADI established by JMPR for its risk assessment (Annex 1, reference *149*). In view of its major use as a post-harvest treatment of fruits and vegetables, the Committee is seeking advice from Codex on its current usage as a food additive.

The Committee noted that provitamin A carotenoids were evaluated at the current meeting (see section 3.1.3).

2.2.2 Clarification of ADI "not specified"

Codex requested clarification of the use of the term "ADI 'not specified'" by JECFA, particularly with respect to addition of food additives to Table 3 of the GSFA (Additives Permitted for Use in Food in General, Unless otherwise Specified, in Accordance with GMP).

The Committee confirmed its definition of "ADI 'not specified'" *(5)*:

> A term applicable to a food substance of very low toxicity that, on the basis of the available chemical, biochemical and toxicological data as well as the total dietary intake of the substance (from its use at the levels necessary to achieve the desired effect and from its acceptable background in food), does not, in the opinion of the Joint FAO/WHO Expert Committee on Food Additives, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of Good Manufacturing Practice: that is, it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance.

Thus, the definition is based upon information on both toxicity and dietary exposure (intake). A conclusion that a substance is of very low toxicity could be based, for example, upon evidence that the substance did not show adverse effects at the highest doses tested in relevant toxicological studies, is poorly absorbed and does not bioaccumulate, and does not contain toxicologically relevant impurities. The estimate of total dietary exposure (intake) is based upon the uses proposed at the time of the evaluation.

The Committee noted that Guideline 2 (Food Additives with an ADI of "Not Specified") of the GSFA (CODEX STAN 192-1995) *(3)* specifies:

> When an additive has been allocated an ADI "not specified" it could in principle, be allowed for use in foods in general with no limitation other than in accordance with Good Manufacturing Practices (GMP). It should, however, be born [*sic*] in mind that ADI not specified does not mean that unlimited intake is acceptable. The term is used by JECFA in case [*sic*] where "on the basis of the available data (chemical, biochemical, toxicological, and other) the total daily intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of the Committee, represent a hazard to health". If, therefore, a substance is used in larger amounts and/or in a wider range of foods than originally envisaged by JECFA it may be necessary to consult JECFA to ensure that the new uses fall within the evaluation. For example a substance may have been evaluated as a humectant

> without including a later use as a bulk sweetener, which could give considerable [*sic*] higher intake.

The Committee endorses Guideline 2 of the GSFA and recommends that it be applied by addition of appropriate qualifications in Table 3 of the GSFA.

2.2.3 Update of guidance on evaluation of enzyme preparations (EHC 240)

The Committee was informed about activities of an expert working group established in 2018 to discuss available information on the safety of enzymes used in food and current practices of the food enzyme industry. This activity is being undertaken within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) *(5)*.

The starting point of the discussion was a background document prepared from a review of the current literature and conversations with representatives of the food enzyme industry and their technical experts.

It was noted that the current JECFA guidance on the evaluation of enzyme preparations was designed to address the potential toxicity of secondary metabolites generated by some enzyme sources (e.g. *Aspergillus* species) under certain growth conditions. The guidance includes a requirement to conduct genotoxicity tests as well as 90-day oral toxicity tests in animals.

After nearly 15 years of using this guidance to assess the safety of enzyme preparations, JECFA has not identified any that were toxic. The expert working group proposed that the safety of enzyme preparations could be assessed with methodologies using fewer animals (e.g. metabolic profiling of microbial fermentation products, genomic DNA sequencing identifying mycotoxin synthesis genes). The expert working group focused on enzymes from genetically modified microorganisms and the information requirements for their safety evaluation.

The expert working group will propose changes to the relevant sections of EHC 240 and produce a checklist of information required in enzyme submissions for future JECFA evaluations.

The Committee urges the expert working group to finalize its work and make the output available for public comment in time for the JECFA meeting in 2020.

2.2.4 Update of guidance on evaluation of genotoxicity of chemical substances in food (section 4.5 of EHC 240)

The Committee was informed about activities of an FAO/WHO expert working group established in 2018 to update and extend the guidance on evaluation of genotoxicity of chemical substances in food. This activity is being undertaken

within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) *(5)*. The aim of the expert working group is to provide guidance on interpretation of test results, in addition to general descriptions of genotoxicity tests, special considerations for data-poor substances, and considerations for chemically related substances and mixtures. The expert working group will also address recent developments and future directions.

This work is ongoing. A public consultation is intended before finalization.

2.2.5 Update of guidance on dose–response assessment and derivation of health-based guidance values (Chapter 5 of EHC 240)

At the eighty-third meeting of the Committee (in 2016), some general considerations regarding dose–response modelling were discussed. The Committee recommended that an expert working group be established to develop detailed guidance for the application of the methods most suitable to its work, in particular for the use of the benchmark dose (BMD) approach (Annex 1, reference *233*). The Committee asked that the expert working group address several aspects, including the use of constraints when fitting models, the use of model averaging, the use of non-parametric methods as alternatives for dose–response risk assessment, the use of biological information for selection of models and transparent presentation of modelling outcomes in JECFA publications.

The Committee was informed that the recommended expert working group was established in 2017 to update and extend the guidance on dose–response assessment and derivation of health-based guidance values. This activity is being undertaken within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) *(5)*.

The work was undertaken electronically and culminated in a meeting of experts in March 2019 in Geneva to revise and update Chapter 5 of EHC 240, including the preparation of more detailed advice on the BMD approach. The draft revised chapter will include guidance on the use of the freely available BMD software (both the United States Environmental Protection Agency Benchmark Dose Software suite of models and PROAST, which was developed by the Dutch National Institute for Public Health and the Environment [RIVM], now available through the European Food Safety Authority [EFSA] as a web tool). The draft guidance will encourage the use of the BMD approach wherever possible and appropriate, but will acknowledge that in some situations, use of the no-observed-adverse-effect level (NOAEL)/lowest-observed-adverse-effect level (LOAEL) approach may still be appropriate. The draft guidance will include a decision-tree to aid decision-making about which approach should be followed.

It is anticipated that a revised draft of Chapter 5 of EHC 240 will be ready in June 2019, to be reviewed by the expert working group. The draft will then go out for public consultation, will be revised if necessary and will be published online as a standalone chapter.

2.2.6 Update of guidance on assessing dietary exposure to chemical substances in food (Chapter 6 of EHC 240)

The Committee was informed about activities of an FAO/WHO expert working group established in 2018 to update and extend the guidance on assessing dietary exposure to chemical substances in food. This activity is being undertaken within the context of a joint FAO/WHO project to update various chapters of *Principles and methods for the risk assessment of chemicals in food* (EHC 240) *(5)*.

A revision of the chapter was required to incorporate technological and methodological changes in dietary exposure assessment, including progress in the use of exposure models and more recently available data and databases.

WHO undertook an initial scoping exercise that identified areas of the current chapter that needed to be reviewed and new areas of work to be included and prepared a first draft of an updated chapter. The draft chapter will be reviewed by a number of dietary exposure experts at a consultation in September 2019. A final draft will be prepared and then released for public comment.

2.2.7 Dietary exposure assessment reporting

In 1996, WHO held an expert consultation that introduced dietary exposure assessment in JECFA's risk assessments for food additives and contaminants. At a 2005 expert consultation to prepare a dietary exposure assessment chapter for what would become *Principles and methods for the risk assessment of chemicals in food* (EHC 240) *(5)*, a tiered process for systematically preparing dietary exposure assessments was elucidated. This process includes 1) a budget or other screening method, 2) international and national dietary exposure assessments based on summary food consumption data (e.g. Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme [GEMS/Food] cluster diets, FAO/WHO Chronic Individual Food Consumption database – summary statistics [CIFOCOss], national/regional surveys, published exposure assessments) and 3) refined dietary exposure assessment using food consumption data derived from individual consumers. In this last step, deterministic and probabilistic assessments could be completed as needed and appropriate. Guidance to JECFA monographers was prepared from these consultations.

At the current meeting, the Committee determined that not all steps of the tiered approach are needed in every case to complete the Committee's evaluations. When preparing monographs, JECFA experts comment on each of the steps as appropriate, but in the report of the meeting, only those assessments

where sufficient data were available to produce reliable estimates of dietary exposure are described and used in the safety assessment. The Committee noted that lack of discussion of any of the steps in report items does not reflect a lack of consideration during the overall evaluation.

2.2.8 Framework for developing specifications for steviol glycosides by method of production

Steviol glycosides are constituents of the leaves of the plant *Stevia rebaudiana* Bertoni and have a sweet taste. The functional use of steviol glycosides in food is as a sweetener. They are approximately 100–300 times sweeter than sucrose.

The major glycosides present in the extract of the leaves from the *Stevia rebaudiana* Bertoni plant are stevioside and rebaudioside A. The minor glycosides include rebaudioside M and rebaudioside D and about 40 other steviol glycosides that have been identified to date. Several minor glycosides have more favourable sensory characteristics than the major glycosides, prompting development of technologies that enhance the proportion of minor glycosides to modify the sensory profile of the articles of commerce. These technologies include the following:

a. Extraction: a process of hot water extraction from the leaves of *Stevia rebaudiana* Bertoni.
b. Fermentation: a process in which a genetically modified microorganism is used to produce specific steviol glycosides.
c. Enzymatic modification: a process in which steviol glycosides that have been extracted from the leaves of *Stevia rebaudiana* Bertoni undergo enzymatic conversion of major steviol glycosides to minor ones.
d. Enzymatic glucosylation: a process in which steviol glycosides that have been extracted from the leaves of *Stevia rebaudiana* Bertoni undergo enzyme-catalysed reactions to add glucose units to the steviol glycosides via α-(1-4) linkages.

The microorganisms used in the fermentation or in the production of enzymes used to modify steviol glycosides are of safe lineage. The inserted genes are isolated from non-toxigenic and non-pathogenic sources. Residues from manufacturing processes do not pose any concerns with respect to toxicity or allergenicity.

Steviol glycosides consist of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (e.g. glucose, rhamnose, xylose, fructose, arabinose, galactose, deoxyglucose). Existing specifications for steviol glycosides require that the product consists of ≥95% steviol glycosides on the dried basis.

At the present meeting, the Committee reviewed data on the methods of manufacture, identity and purity of steviol glycosides. The Committee noted that the reviewed products consist of ≥95% steviol glycosides on the dried basis; the remaining 5% or less consists of residues of starting material and food-grade processing aids, depending on the method of production.

A framework was adopted for developing specifications for steviol glycosides by four different methods of production. Specifications for steviol glycosides produced by different production methods were included as annexes, as below:

- Annex 1: Steviol Glycosides from *Stevia rebaudiana* Bertoni (revised from the specifications monograph for Steviol glycosides from *Stevia rebaudiana* Bertoni [INS 960a] prepared at the eighty-fourth meeting of JECFA [Annex 1, reference *236*]).
- Annex 2: Steviol Glycosides from Fermentation (specifications for Rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* [INS 960b(i)] prepared at the eighty-second meeting of JECFA [Annex 1, reference *231*] were revised to include other steviol glycosides from *Saccharomyces cerevisiae* and *Yarrowia lipolytica*).
- Annex 3: Enzyme Modified Steviol Glycosides (new specifications).
- Annex 4: Enzyme Modified Glucosylated Steviol Glycosides (new specifications, tentative pending further information concerning the analytical methods).

At the present meeting, the Committee determined that no safety issues exist for steviol glycosides produced by any one of these methods resulting in products with ≥95% steviol glycosides as per existing specifications. The Committee indicated that the ADI of 0–4 mg/kg bw established at the sixty-ninth meeting of JECFA for steviol glycosides (expressed as steviol) (Annex 1, reference *190*) applies to steviol glycosides produced by the four methods indicated in the annexes of the specifications monograph produced at the current meeting.

The Committee recognized that steviol glycosides could be produced via a new method or the modification or combination of the methods currently described in the annexes of the specifications monograph. If the final product meets the current specification of ≥95% steviol glycosides, the Committee will evaluate possible impurities from the method of manufacture. When appropriate, the modifications will be introduced into the relevant annex; alternatively, a new annex would be added.

2.3 Food additive specifications and analytical methods

2.3.1 Unsulfonated primary aromatic amines in food colours

At the present meeting, the Committee noted that the analytical method for determining unsulfonated primary aromatic amines in certain synthetic food colours (i.e. Allura Red AC, Amaranth, Azorubine, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Fast Green FCF, Fast Red E, Green S, Indigotine, Lithol Rubine BK, Patent Blue V, Ponceau 4R, Quinoline Yellow, Sunset Yellow FCF and Tartrazine) described in Volume 4 of the *Combined compendium of food additive specifications* (Annex 1, reference *180*) is not sufficiently sensitive for determining the impurities at low levels (milligrams per kilogram or below). The Committee also noted that the specification for unsulfonated primary aromatic amines (not more than 0.01%, calculated as aniline) is approximately 100 times higher than equivalent specifications for food colours established by other regulatory authorities. The Committee also noted that more sensitive analytical methods, capable of determining unsulfonated primary aromatic amines at levels of less than 1 mg/kg, had been developed since the publication of Volume 4.

The Committee requests analytical data on unsulfonated primary aromatic amines in the above food colours, along with the analytical methods used, in order to update specifications.

2.3.2 Analytical method for the determination of anthraquinones in cassia gum

At its eighty-second meeting, the Committee made the specifications for cassia gum tentative and requested information on the analytical method for the determination of anthraquinones, including the efficiency of extraction steps and recovery of analytes (Annex 1, reference *230*). At the eighty-sixth meeting, the Committee evaluated the high-performance liquid chromatography (HPLC) method submitted, updated the specifications by including the method received, and removed the tentative status for the specifications of cassia gum (Annex 1, reference *241*). Based on comments received about the method performance, the Committee, at its current meeting, reviewed the method again and noted that additional investigations were required. Therefore, the Committee decided to make the specifications tentative until a suitable analytical method has been identified.

2.3.3 Update on the review of analytical methods for food additives

The Committee was informed of the ongoing FAO initiative to review analytical methods for food additives. The review was initiated to ensure that the analytical methods referenced in the specifications monographs for food additives are fit-for-purpose and up-to-date. *Combined compendium of food additive specifications, Volume 4, Analytical methods, test procedures and laboratory*

solutions used by and referenced in the food additive specifications (FAO JECFA Monographs 1) was published in 2006. Subsequently, several analytical methods associated with the specifications monographs were either included in individual monographs or published separately. The Committee, at previous meetings, noted that advancements in instrumentation technologies since the publication of Volume 4 necessitate a review of analytical methods in individual specifications monographs as well as in Volume 4.

In total, 470 specifications monographs (excluding enzymes) were reviewed, together with different subsections of Volume 4. The initial findings were as follows:

General:

- Approximately 170 specifications monographs are more than 30 years old. Approximately 70 out of 170 specifications monographs were developed between the third and twentieth meetings of JECFA and contain some outdated methods.
- Three specifications monographs include the functional use of fungicidal agents; these products are unlikely to be used as food additives.
- Some functional uses detailed in monographs are not consistent with the functional classes listed in the INS (e.g. yeast food).

Analytical methods:

- Approximately 30 monographs still use obsolete packed column gas chromatographic methods.
- Some analytical techniques (e.g. titrimetric, spectrophotometric, thin-layer chromatographic/paper chromatographic identification techniques) are still in use, although they may no longer be fit-for-purpose and have been superseded by newer approaches.
- Certain limit tests (e.g. nickel, fluoride, iron) still exist, although quantitative analytical methods are available.
- Volume 4 requires considerable updating and inclusion of sophisticated analytical methods and confirmatory methods, such as liquid chromatography with tandem mass spectrometry, inductively coupled plasma mass spectrometry, X-ray fluorescence, etc.
- Many standard and test solutions given in Volume 4 are currently not in use and need thorough revision.
- Potential compatibility issues for monographs were found in some updates for Volume 4 (e.g. replacing packed column gas

chromatographic methods, use of chloroform, replacing methods for subsidiary dyes and organic compounds other than colouring matters, etc.).

In view of above findings, the Committee recommended that:

- A summary of the major findings be compiled for presentation to CCFA.
- A priority list of updates be constructed based on initial findings, paying particular attention to relevance of the proposed update, impact on Volume 4, potential for creating further disconnects between the monographs and Volume 4, and the number of monographs affected. The list should be presented to JECFA and CCFA at a future meeting.
- An outline of the future activities be created, including:
  - A decision on the future role of Volume 4 and its contents (e.g. reproducing technical background about analytical methods).
  - A mechanism for the separate evaluation of enzyme monographs and connected analytical methods, once the process and requirements for enzyme evaluations are concluded.
  - A decision on presentation of the analytical methods in specifications monographs (e.g. full methods, active links, relevant technical details, a database of methods).
  - A decision on the policy to reference other publications by FAO and other standards development organizations.

2.4 Other matters of interest to the Committee

2.4.1 Update on FAO/WHO Global Individual Food consumption data Tool (GIFT)

The FAO/WHO Global Individual Food consumption data Tool (GIFT) is an open-access online platform, hosted by FAO and supported by WHO, providing access to harmonized individual quantitative food consumption data, especially in low- and middle-income countries. The platform is a growing data repository; in 2018, FAO/WHO GIFT received a 4-year grant from the Bill & Melinda Gates Foundation to transform the platform into a robust global tool that will contain at least 50 datasets in 2022.

FAO/WHO GIFT provides sex- and age-disaggregated microdata, which are needed in the field of nutrition and dietary exposure. To facilitate the use of these data by policy-makers, ready-to-use food-based indicators are provided under the form of infographics for a user-friendly overview of key information by population segments and by food groups. The synergy between the FAO/WHO GIFT platform and the dashboards of FAO/WHO FOSCOLLAB (Global platform

for food safety data and information) hosted by WHO has great potential. In fact, in order to enhance the consistency and reliability of nutrient intake and dietary exposure assessments, all datasets available as microdata in FAO/WHO GIFT are harmonized with the food classification and description system FoodEx2. FoodEx2 is also the system used to map all food chemical occurrence microdata available on FAO/WHO FOSCOLLAB. The combination of the two platforms will make it much easier to perform refined dietary exposure assessments for a large variety of food chemicals in all regions of the world. Moreover, all datasets available as microdata in FAO/WHO GIFT are also being made available as summary statistics on FAO/WHO FOSCOLLAB.

For datasets that are not yet available as microdata in FAO/WHO GIFT, the platform provides an up-to-date inventory of individual quantitative food consumption surveys conducted and ongoing in low- and middle-income countries, with detailed survey information on identified studies.

The FAO/WHO GIFT platform is available at http://www.fao.org/gift-individual-food-consumption/en/. The dashboards of FAO/WHO FOSCOLLAB are available at http://apps.who.int/foscollab.

2.4.2 Risk assessments of combined dietary exposure to multiple chemicals

The need to integrate exposure to mixtures of chemicals in the risk assessment framework has long been recognized by FAO/WHO. This work is part of a project entitled "EuroMix" funded by the European Commission, under the Horizon 2020 research programme. In this context, WHO and FAO convened an expert consultation[2] in April 2019 to develop appropriate guidance for risk assessment of combined dietary exposures to multiple chemicals.

The Committee was informed on the key deliberations of the consultation. In particular, it was noted that if a substance under evaluation by JECFA/JMPR has sufficient similarity to an established chemical group previously considered in a risk assessment of combined dietary exposure to multiple chemicals (e.g. organophosphates), the substance should be considered for assessment as part of that group. If a substance under consideration is not part of an established chemical group previously considered, JECFA/JMPR should then determine whether there is a need to include it in a risk assessment of combined dietary exposure to multiple chemicals.

For chemicals that are not part of a previously established group, if the estimated dietary exposure for a single compound under evaluation is more than 10% of the relevant health-based guidance value or the calculated margin of exposure (MOE) is less than 10 times the MOE considered adequate for such a compound for at least one population, the need to include the compound in a

[2] https://www.who.int/foodsafety/areas_work/chemical-risks/Euromix_Report.pdf?ua=1

risk assessment of combined dietary exposure to multiple chemicals should be considered.

The following questions must be answered to determine which substances should be included: *Is there toxicological evidence for combined effects* (using weight of evidence analysis, expert judgement on structural similarities, toxicological profiles, modes of action, etc.) and *Is there potential for co-exposure (from co-occurrence or internal exposure)* (using trial data, monitoring data, use levels in foods, toxicokinetic data, etc.).

For risk characterization, suitable procedures using dose addition can be applied to identify key risk drivers using either deterministic or probabilistic approaches, including the key chemicals contributing to total dietary exposure and/or foods contributing to exposure from each chemical.

The consultation noted that for DNA-reactive mutagens, special consideration will be needed, and they were not included in the approach proposed by the consultation. Furthermore, synergistic interactions between chemicals may need to be considered separately on a case-by-case basis.

3. Specific food additives (other than flavouring agents)

The Committee evaluated two food additives for the first time and re-evaluated three others. In addition, the Committee evaluated the safety of one previously evaluated food additive for use in formula for special medical purposes intended for infants. Four food additives (including one group of food additives) were considered for revision of specifications only. Information on the safety evaluations and specifications is summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are summarized in section 5.

3.1 Safety evaluations[3]

3.1.1 Black carrot extract

Explanation

Black carrot extract (INS 163(vi)) is an anthocyanin-containing food colour obtained by acidic aqueous extraction from the root of black, purple or red carrot. The main colouring components are five cyanidin-based anthocyanins.

Black carrot extract has not been evaluated previously by the Committee. The Committee previously evaluated anthocyanins, including the anthocyanin-containing food colour grape skin extract (INS 163(ii)), at its twenty-sixth meeting (Annex 1, reference *59*). At that meeting, the Committee established an ADI for anthocyanins in grape skin extract of 0–2.5 mg/kg bw, based on a NOAEL of 225 mg/kg bw per day expressed as anthocyanins from a two-generation reproductive toxicity study in rats [1].

Black carrot extract was placed on the agenda of the present meeting for assessment of its safety, dietary exposure and specifications, at the request of the Fiftieth Session of CCFA [2]. In response to the call for data, a submission was received, which included studies identified from the publicly available literature and information on specifications and dietary exposure. A comprehensive literature search retrieved a number of additional studies, primarily on human pharmacokinetics and absorption, distribution, metabolism and excretion (ADME), and one additional genotoxicity study.

Given the similar aglycone structures of anthocyanins, the large number of studies on anthocyanins from various sources published since the previous assessment of grape skin extract and the lack of toxicity data on black carrot extract itself (only one genotoxicity study was submitted), the Committee decided to review the available data on anthocyanins as a whole. The studies described below therefore include previously evaluated studies on grape skin

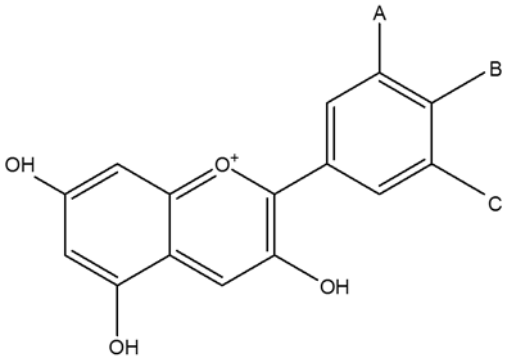
[3] Numbered references cited in the subsections of section 3.1 are provided at the end of each subsection.

extract (published prior to 1982) as well as new studies on materials containing anthocyanins from a range of sources.

Chemical and technical considerations

Anthocyanins are a large group of related compounds consisting of aglycones such as cyanidin or pelargonidin (Fig. 1) combined with sugars such as galactose or glucose and acylating agents such as caffeic acid or *p*-coumaric acid [3].

Fig. 1
General anthocyanin aglycone structure indicating substitution positions



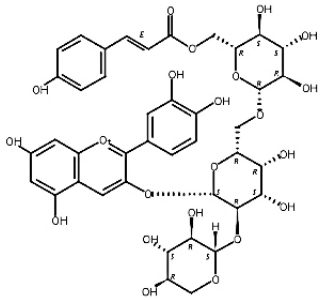
Aglycone	A	B	C
Cyanidin[a,b,c]	-OH	-OH	-H
Pelargonidin[a,b]	-H	-OH	-H
Delphinidin[b]	-OH	-OH	-OH
Peonidin[a,b]	-O-CH_3	-OH	-H
Petunidin[b]	-OH	-OH	-O-CH_3
Malvidin[a,b]	-O-CH_3	-OH	-O-CH_3

[a] Found in black carrot extract.
[b] Found in grape skin extract.
[c] The five main anthocyanins in black carrot extract are formed from this aglycone.

Black carrot extract contains five main anthocyanins formed from the aglycone cyanidin substituted at the central hydroxyl position with a sugar moiety consisting of galactose, glucose and/or xylose. Three of the five anthocyanins are acylated with *p*-coumaric, ferulic or sinapinic acid [4]. One of the five main anthocyanins in black carrot extract is shown in Fig. 2. Anthocyanins in black carrot extract are also formed from other aglycones (malvidin, pelargonidin and peonidin; Fig. 1), which are present in minor amounts along with other polyphenols. Other components include proteins, carbohydrates, lipids, fibres,

minerals and water. In contrast to black carrot extract, the predominant aglycone found in anthocyanins in grape skin extract is malvidin [5].

Fig. 2
Cyanidin 3-*p*-coumaroylxylosylglucosylgalactoside, one of the five main anthocyanins in black carrot extract



Black carrot extract is produced by aqueous acidic extraction of the crushed, ground or milled roots of black, purple or red carrot (*Daucus carota* L., ssp. *sativus*) followed by fermentation to decrease sugars. Methanol or ethanol may be produced during the fermentation step. The anthocyanins may be concentrated by ultrafiltration, reverse osmosis or adsorption onto a polymeric resin followed by desorption with ethanol, isopropyl alcohol and/or water. The commercial product may be a liquid or spray-dried powder.

Black carrot extract is intended for use in colouring dairy-based desserts, processed fruit products, processed vegetable products, confectionery, chewing gum, cereals, pastas and noodles, cereal/starch-based desserts, processed rice and soy products, cakes, cookies, pies, preserved egg products, condiments (vinegar, mustard), sauces and gravies, dietetic foods and dietary supplements, non-alcoholic beverages and alcoholic beverages.

Biochemical aspects

The previous Committee, in its evaluation of grape skin extract, concluded that anthocyanins are not absorbed by humans to any great extent (<2%) and pass through the body unchanged (Annex 1, reference *59*). More recent studies have shown anthocyanins to be absorbed up to about 12% [e.g. 6–8]; therefore, previously evaluated studies on the ADME of anthocyanins have not been included below.

A number of studies have been carried out in humans to investigate the ADME of anthocyanins. Anthocyanins can be absorbed intact or hydrolysed to the

aglycone and then absorbed. They may also be degraded to phenolic compounds by the gut microbiota before absorption. The primary route of metabolism by the microbiota appears to be cleavage of the heterocyclic flavylium ring followed by dihydroxylation or decarboxylation [9, 10]. The rate and extent of absorption are dependent on the size of the molecule, the type of sugar moiety, the degree of acylation and the matrix in which the anthocyanin mixture is consumed [9]. The gut microbiome is likely to be an important site of metabolism of anthocyanins, and changes in the microbiome may have a significant effect on the metabolic products produced following the consumption of anthocyanins [11].

In recent studies in human volunteers using stable ^{13}C-labelled cyanidin-3-*O*-glucoside, an anthocyanin found in grape skin extract and purple corn colour, bioavailability of about 12% (5% in urine and 7% in breath) was reported. Several metabolites were identified, including carbon dioxide in breath and anthocyanin conjugates along with vanillic acid, ferulic acid, hippuric acid and 4-hydroxybenzaldehyde in urine [6, 12].

Toxicological studies

A number of acute and short-term toxicity studies were identified using anthocyanins from a range of sources, including dried fruits and vegetables and extracts of these. In many cases, the anthocyanins in the test material were not identified or quantified [13–17].

In the acute toxicity studies, no effects were observed at oral test substance doses up to 25 000 mg/kg bw [13–17].

No short-term studies were carried out using black carrot extract. A number of short-term studies in a range of species using test substances containing anthocyanins were identified. No treatment-related effects were observed in a 28-day mouse study using dried red cabbage powder [16], two 90-day studies in rats given grape seed extract [14, 18], one 90-day study in rats given an anthocyanin extract [13], one 90-day study in rats given grape skin extract [18] and two 90-day studies in dogs, one using grape colour powder and one grape skin extract [19, 20]. In addition, no effects were observed in a 15-day study in guinea-pigs given anthocyanins in the diet [13].

In a study in rats fed a diet supplemented with grape skin extract at 0, 2000, 10 000 or 50 000 mg/kg feed (equal to 0, 100, 600 and 3300 mg/kg bw per day for males and 0, 100, 700 and 3600 mg/kg bw per day for females, respectively) for 90 days, the anthocyanin content was not characterized, but the test material was said to contain approximately 2% anthocyanins (anthocyanin doses were therefore 0, 2, 12 and 66 mg/kg bw per day for males and 0, 2, 14 and 72 mg/kg bw per day for females, respectively). In this study, calcification of the proximal tubules of the kidney was identified in females in all dose groups, including controls, but the severity was significantly higher in the group receiving 50 000

mg/kg in the diet. A NOAEL of 14 mg/kg bw per day expressed as anthocyanin (10 000 mg/kg feed expressed as grape skin extract, equal to 700 mg/kg bw per day) was identified [21].

In a study by Nabae et al. [22], in which rats were administered purple corn colour (containing 26.4% cyanidin-3-*O*-glucoside) in the diet at 0, 5000, 15 000 or 50 000 mg/kg feed (equal to cyanidin-3-*O*-glucoside doses of 0, 84, 249 and 935 mg/kg bw per day for males and 0, 89, 272 and 1016 mg/kg bw per day for females, respectively) for 90 days, a number of statistically significant findings were observed at the top dose, including effects on haematological and clinical chemistry parameters and relative organ weights. Although the authors concluded that the NOAEL was the highest dose tested, the Committee was of the opinion that the effects observed at 50 000 mg/kg feed were toxicologically relevant and identified a NOAEL of 15 000 mg/kg feed (equal to 249 mg/kg bw per day).

No long-term toxicity or carcinogenicity studies are available.

Eight in vitro and seven in vivo genotoxicity studies are available, but only one assay (an in vitro comet assay in human colon cancer cells) used black carrot extract as the test material [23]. This study showed positive results only at cytotoxic concentrations. No findings were observed for any of the anthocyanin-containing test materials that would raise concerns for genotoxicity [14, 17, 23–29].

Two multigeneration reproductive toxicity studies are available. One of these used grape colour powder administered to rats in the diet at 0, 7500 or 15 000 mg/kg bw per day, but the anthocyanins in the test material were not quantified. There were no treatment-related findings [20]. In a second study, using a grape skin extract preparation (containing 3% anthocyanins; composition of anthocyanins not given) administered to rats in the diet at a concentration of 0, 75 000 or 150 000 mg/kg feed (equivalent to 0, 7500 and 15 000 mg/kg bw per day, respectively, estimated to be 0, 225 and 450 mg/kg bw per day expressed as anthocyanins), decreases in liver, adrenal and thyroid weights were observed in the top-dose group of the first filial (F_1) generation. The NOAEL for this grape skin extract preparation identified by the previous Committee was 75 000 mg/kg feed (equivalent to 7500 mg/kg bw per day and estimated to be 225 mg/kg bw per day expressed as anthocyanins) [1]. The ADI for anthocyanins from grape skin extract established by the previous Committee was based on this study, with application of an uncertainty factor of 100 to the NOAEL and rounding.

The anthocyanin glycosides (an extract from currants, blueberries and elderberries) were reported not to be teratogenic in rats, mice or rabbits when given at a dose of 1500, 3000 or 9000 mg/kg bw per day over three successive generations [13].

Observations in humans

A number of studies have been carried out in humans to identify biological effects of anthocyanins. Although no toxicity issues have been identified from these studies, the study designs limit their suitability for deriving safe levels of anthocyanins.

Assessment of dietary exposure

In the submission to the Committee, the sponsors proposed the use of black carrot extract as a food colour at typical and maximum use levels (expressed as total anthocyanins in milligrams per kilogram) in 77 food categories and subcategories as specified in the Codex GSFA. The anthocyanin content in black carrot extracts reported by the sponsors ranges from 0.8% to 14.5%, with a standardized content of 9%.

The Committee considered the European estimates of dietary exposure to anthocyanins, provided by the sponsors, as being the most representative of actual exposure. The Committee noted that the mean estimated dietary exposures to total anthocyanins ranged from less than 0.1 mg/kg bw per day for the elderly population up to 1.3 mg/kg bw per day for toddlers. The 95th percentile exposure for consumers only ranged from 0.1 mg/kg bw per day for the elderly population up to 6.9 mg/kg bw per day for toddlers in the brand-loyal scenario, whereas the 95th percentile exposure ranged from less than 0.1 mg/kg bw per day for toddlers and children up to 2.4 mg/kg bw per day for toddlers in the non-brand-loyal scenario. The main foods contributing to the overall exposure to anthocyanins were non-alcoholic beverages, flavoured fermented desserts and cider.

The Committee also considered typical exposure to anthocyanins from natural sources. Anthocyanins are naturally present in foods such as fruits, vegetables, nuts, chocolate, tea and wine. The mean dietary exposure to anthocyanins in the USA using National Health and Nutrition Examination Survey (NHANES) 2001–2002 data [30] was 0.2 mg/kg bw per day for a 60 kg adult. In Europe, the mean dietary exposure to anthocyanins using the Comprehensive European Food Consumption Database [31] ranged from 0.05 mg/kg bw per day for adolescents to 1.6 mg/kg bw per day for adults and up to 4 mg/kg bw per day for toddlers.

The Committee noted that the European dietary exposures to anthocyanins from natural sources as described in the current evaluation are higher than the mean dietary exposure of 0.3 mg/kg bw per day that was reported for Europe by EFSA [32], which at that time was based on one national dietary survey from Europe.

With regard to use levels evaluated at this meeting, the Committee noted differences between the sponsors' reported current and proposed use levels of black carrot extract expressed as total anthocyanins and those that were

considered in the EFSA [32] evaluation. The main difference was for the food category processed meat, which is not proposed as a food to which anthocyanins could be added. In the EFSA [32] evaluation, processed meat was the main food contributing to overall exposure to total anthocyanins, contributing up to 30–50% of the average dietary exposures across Europe (0.5–2.4 mg/kg bw per day).

Evaluation

There are no data on the toxicity of black carrot extract, with the exception of one genotoxicity test. Nevertheless, the Committee noted the large number of studies on other sources of anthocyanins published since anthocyanins were last evaluated by JECFA in 1982, including toxicity studies in animals and ADME studies in humans.

The Committee concluded that the effects observed with one anthocyanin-containing test material cannot be extrapolated to another anthocyanin-containing test material based on the available information. This is because the test articles in the metabolism and toxicity studies evaluated at this meeting were very heterogeneous and often not fully described and/or the anthocyanin content of the test material was too low and variable. This agrees with the conclusion of the previous Committee (Annex 1, reference *59*).

Owing to the lack of toxicological data on black carrot extract, the Committee was not able to draw conclusions on its safety. To proceed with the assessment of black carrot extract, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required.

The Committee concluded that the total mean dietary exposure to anthocyanins from naturally occurring sources and added black carrot extract using the non-brand-loyal scenario ranges from 0.1 to 1.9 mg/kg bw per day for the adult population (18+ years old) and from 0.1 to 5.3 mg/kg bw per day for children (<18 years old).

In these estimates, the Committee noted that the use of black carrot extract itself as proposed by the sponsors contributes as much as 25% to the total mean dietary exposure to anthocyanins, including from naturally occurring sources. The Committee noted that the ADI for grape skin extract established by the previous Committee in 1982 was not reconsidered as part of this assessment and remains unchanged.

A consolidated toxicological and dietary exposure monograph was prepared.

At the present meeting, new specifications for the spray-dried powder form of black carrot extract were prepared. The specifications were made tentative pending the submission of further information on the material of commerce (see Recommendations below).

A Chemical and Technical Assessment was prepared.

Recommendations

To proceed with the assessment of black carrot extract, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required.

The specifications were made tentative pending the submission of further information on the material of commerce, including a full characterization of the proteins, carbohydrates, lipids, fibre, minerals and non-anthocyanin polyphenol components in five lots each of the liquid and powder forms of black carrot extract.

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3.1.2 Brilliant Black PN

Explanation

Brilliant Black PN (INS 151; Chemical Abstracts Service [CAS] No. 2519-30-4) is a synthetic disazo dye used as a food colouring agent. JECFA first evaluated Brilliant Black PN at its eighteenth meeting (Annex 1, reference *35*) and established a temporary ADI of 0–2.5 mg/kg bw, based on a NOAEL of 500 mg/kg bw per day obtained from a chronic rat study. An additional uncertainty factor of 2 was applied because the ADI was temporary, pending the submission of metabolic, reproductive and embryotoxicity studies.

At the twenty-second meeting of JECFA (Annex 1, reference *47*), the requested metabolic, reproductive and embryotoxicity studies were not submitted. In addition, the Committee indicated that the etiology and pathology of ileal cysts observed in a 90-day toxicity study in pigs submitted at that meeting should be determined. The Committee maintained the temporary ADI.

At the twenty-fifth meeting of JECFA (Annex 1, reference *56*), multigeneration reproductive toxicity and teratogenicity studies were submitted, both showing no toxicologically relevant effects. A metabolic study was also submitted. No further information on the ileal cysts in pigs was available. Therefore, the Committee established a new ADI of 0–1 mg/kg bw on the basis of the no-effect level of 100 mg/kg bw per day in the pig study.

Brilliant Black PN was placed on the agenda of the present meeting for re-evaluation of its safety, evaluation of its dietary exposure and revision of its specifications, at the request of the Forty-ninth Session of CCFA [1].

Studies on the effects of Brilliant Black PN on enzymes and other biochemical parameters, genotoxicity studies, studies on the toxicity of metabolites and a study on non-allergic hypersensitivity in children were submitted. Additional literature searches in Medline, Toxline, Scopus and SciFinder using the keywords Brilliant Black, clinical, toxicology, genotoxicity, metabolism, absorption, excretion and ADME did not identify any additional

relevant publications. The sponsor submitted use levels of Brilliant Black PN in three main food categories as well as dietary exposure estimates reported in the literature.

Chemical and technical considerations

Brilliant Black PN is intended for use in colouring confectionery, decorations and coatings, desserts including flavoured milk products, edible cheese rind, edible ices, fine bakery wares, fish and fish products, non-alcoholic flavoured drinks, non-dairy beverages, sauces and seasonings, and savoury snacks.

Brilliant Black PN consists mainly of tetrasodium 4-(acetylamino)-5-hydroxy-6-[2-[7-sulfo-4-[2-(4-sulfophenyl)diazenyl]-1-naphthalenyl]diazenyl]-1,7-naphthalenedisulfonate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Brilliant Black PN is manufactured by diazotizing 4-aminobenzenesulfonic acid (sulfanilic acid), coupling with 8-aminonaphthalene-2-sulfonic acid (1,7-Cleve's acid), diazotizing the product and coupling with 4-(acetylamino)-5-hydroxy-1,7-naphthalenedisulfonic acid (*N*-acetyl K acid). The dye is isolated as the tetrasodium salt. Impurities include unreacted starting materials and reaction by-products (≤0.8%), subsidiary colouring matters (≤4%), unsulfonated primary aromatic amines (≤0.01% calculated as aniline) and lead (≤2 mg/kg).

Biochemical aspects

In rats, Brilliant Black PN is poorly absorbed, with 94–98% of administered doses up to 10 mg/kg bw excreted in the faeces within 40 hours and less than 5% detected in the urine within 40 hours [2]. Differences in metabolism following oral and intraperitoneal administration indicate that metabolism by intestinal flora leads to complete azo reduction (cleavage of both azo sites), whereas azoreductases in liver preferentially cleave the azo site between the two naphthalene rings, resulting in sulfonated aromatic amines [3].

In humans, sulfanilic acid was the only metabolite identified in urine following oral administration of a 240 mg dose of Brilliant Black PN, and the amount of metabolite was similar to that observed in rats [3].

In vitro, Brilliant Black PN was shown to induce a dose-dependent decrease in the uptake of radiolabelled *o*-iodohippurate and iodipamide in rat renal cortex slices, which was interpreted as inhibition of the hippurate and liver-like anion transport systems [4].

Brilliant Black PN was identified as a novel allosteric modulator of adenosine receptors using Chinese hamster ovary cells stably transfected with either A_1 or A_3 human receptors [5]. The Committee noted that the effects on adenosine receptors were observed only at high (500 µmol/L) concentrations

of Brilliant Black PN. In view of the poor absorption of Brilliant Black PN, the Committee did not consider this study relevant to the evaluation.

Toxicological studies

In previously evaluated studies, Brilliant Black PN was not acutely toxic by the oral route in mice or rats (median lethal dose [LD_{50}] > 5000 mg/kg bw) [6, 7] and showed no signs of toxicity in mice in a long-term study at doses up to 1300 mg/kg bw per day [8] or in rats in short-term studies with dietary concentrations up to 30 000 mg/kg feed (equivalent to 3000 mg/kg bw per day) and long-term studies with dietary concentrations up to 10 000 mg/kg feed (equal to 360 mg/kg bw per day) [7, 9] and no evidence of carcinogenicity in mice or rats [8, 9]. In rats, there was no reproductive toxicity or teratogenicity at dietary concentrations up to 30 000 mg/kg feed (equivalent to 1500 mg/kg bw per day) [10] and no teratogenicity at doses up to 2500 mg/kg bw per day [11].

The only adverse findings reported previously were cysts containing mucus and fibrin in the ileal mucosa of pigs administered Brilliant Black PN at 300 mg/kg bw per day (one of six pigs) or 900 mg/kg bw per day (four of six pigs) for 90 days. The NOAEL in this study was 100 mg/kg bw per day [12]. The Committee at the twenty-fifth meeting (Annex 1, reference *56*) established an ADI based on this NOAEL. In the current submission, the sponsor reiterated the authors' argument that the cysts might have been due to an irritant effect of local high concentrations of Brilliant Black PN related to the way in which the substance was administered as a bolus in a small amount of feed. The present Committee considered that this explanation lacked plausibility, as the upper parts of the gastrointestinal tract were not affected, as would be anticipated for an irritant effect.

Several new in vitro genotoxicity studies [13–15] and one new in vivo genotoxicity study [13] were available to the Committee and were generally negative. The gene mutation assay in mammalian cells [15] was equivocal in the presence of metabolic activation, which normally would require follow-up, and aneugenicity was not tested for. The positive findings obtained in the in vitro micronucleus test and comet assay are considered to be unreliable due to major shortcomings in study design. Read-across from a structurally related food colour (Allura Red AC) [16] and the lack of genotoxicity of other sulfonated aromatic amines such as those generated by the azoreduction of sulfonated azo dyes [17] were taken into consideration. The Committee concluded that, overall, the data did not indicate concern with respect to the genotoxicity of Brilliant Black PN.

The metabolite sulfanilic acid (the only metabolite found in human urine) did not show genotoxic activity or adverse effects in a 4-week study or in an Organisation for Economic Co-operation and Development (OECD)–

Table 1
Dietary exposure to Brilliant Black PN

	Dietary exposure (mg/kg bw per day)	
Country/region	**Mean**	**High**
Australia[a]		
Mean	0.00–0.001	0.00–0.002[b]
Maximum	0.00–0.004	0.003–0.01[b]
Europe[c]	0.01–0.17	0.02–0.30[d]
Kuwait[e]	0.000 2–0.000 3	–

[a] Exposure only for consumers of foods containing Brilliant Black PN based on mean and maximum analytical concentrations.
[b] Ninetieth percentile of exposure.
[c] Non-brand-loyal scenario based on mean use levels and analytical concentrations.
[d] Ninety-fifth percentile of exposure.
[e] Children; based on analytical concentrations.
Sources: Australia: [22, 23]; Europe: [24]; Kuwait: [25]

compliant reproductive and developmental toxicity study in rats administered doses up to 1000 mg/kg bw per day [18, 19].

Observations in humans

A study in six young patients with moderate to severe chronic urticaria found that one child exhibited immunoglobulin E–independent responses to all tested azo dyes, including Brilliant Black PN [20]. The Committee noted that this study is not informative for the present evaluation.

Assessment of dietary exposure

Brilliant Black PN is proposed by the sponsor for use in 16 food subcategories belonging to three main food categories of the Codex GSFA: "5. Confectionery", "9. Fish and fish products, including mollusks, crustaceans, and echinoderms" and "14. Beverages, excluding dairy products". The typical use levels range from 10 to 300 mg/kg, and the maximum use levels from 10 to 500 mg/kg. Currently, Brilliant Black PN is authorized for use only in food category "01.1.4 Flavoured fluid milk drinks", excluding chocolate milk, at a maximum permitted level of 150 mg/L, as specified in the GSFA [21].

The Committee used only those dietary exposure estimates that were considered to be most representative of actual exposure. These estimates were based on use levels and/or analytical concentrations combined with food consumption data from Australia [22, 23], Europe [24] and Kuwait [25] and are listed in Table 1.

The dietary exposures to Brilliant Black PN in Australia and Kuwait were estimated using analytical concentrations measured in relevant foods, which resulted in low exposure estimates, as Brilliant Black PN was present in only

a limited number of food groups at low levels [22, 23, 25]. The high exposure (90th percentile) to Brilliant Black PN was maximally 0.01 mg/kg bw per day for children up to 16 years of age in Australia, based on the highest levels analysed per food group [23]. This dietary exposure estimate refers to the exposure in persons who had consumed at least one of the foods that contained Brilliant Black PN (consumers only).

For Europe, the dietary exposure was estimated for different age groups using food consumption data from several European countries combined with maximum permitted levels, use levels and/or analytical concentrations, according to three exposure scenarios [24]. Given that the dietary exposure estimates for Australia and Kuwait were so low, the Committee considered the non-brand-loyal scenario to best reflect the dietary exposure to Brilliant Black PN. In this scenario, it is assumed that persons are exposed to a food additive at the typical (mean) reported use level or mean of the analytical concentrations for all relevant food categories and that all foods belonging to an authorized food category contain the food additive at that level. The mean dietary exposure to Brilliant Black PN ranged from 0.01 mg/kg bw per day for adolescents, adults 18–64 years of age and adults 65+ years of age to 0.17 mg/kg bw per day for toddlers in this scenario. The high dietary exposure (95th percentile) ranged from 0.02 mg/kg bw per day for adults 65+ years of age to 0.30 mg/kg bw per day for toddlers. The food categories included in this scenario overlapped largely with those for which use levels are proposed by the sponsor, in addition to other food categories for which no use levels were proposed, such as "Edible ices", "Fine bakery wares", "Seasonings and condiments", "Soup and broths", "Mustard" and "Potato-, cereal-, flour- and starch-based snacks". The Committee noted that the food category "Fine bakery wares" was the most important contributor to the dietary exposure to Brilliant Black PN across all age groups in Europe.

The Committee concluded that the high dietary exposure to Brilliant Black PN of 0.3 mg/kg bw per day, based on European data, is appropriate for use in a risk assessment.

Evaluation

The Committee concluded that the newly available information does not give reason to revise the previously established ADI of 0–1 mg/kg bw based on the short-term toxicity study in pigs. The Committee therefore retained the ADI for Brilliant Black PN.

The Committee noted that the range of estimated dietary exposures for Brilliant Black PN was below the upper end of the ADI and concluded that dietary exposure to Brilliant Black PN does not present a safety concern.

A consolidated toxicological and dietary exposure monograph was prepared.

At the present meeting, the specifications for Brilliant Black PN were revised. Analytical methods for determining subsidiary colouring matters and organic compounds other than colouring matters were replaced with more specific and sensitive HPLC methods. The existing titrimetric method for the assay of Brilliant Black PN was replaced with a visible spectrophotometric method.

A Chemical and Technical Assessment was prepared.

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3.1.3 Carotenoids (provitamin A)

Explanation

β-Carotene (CAS No. 7235-40-7) and β-apo-8′-carotenal (CAS No. 1107-26-2) are provitamin A carotenoids that are used as colours in a wide range of foods and beverages. Currently, both food additives are authorized for use in 79 food categories at maximum permitted levels ranging from 50 mg/kg up to 1200 mg/kg as specified in the Codex GSFA [1].

A group ADI of 0–5 mg/kg bw for β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters was first established at the tenth JECFA meeting (Annex 1, reference *13*). At its eighteenth meeting, the Committee considered additional data and reaffirmed the decision of the tenth

meeting (Annex 1, reference *35*). The group ADI was derived using a four-generation study in rats with a NOAEL for β-carotene of 50 mg/kg bw per day and application of an uncertainty factor of 10 because of the natural occurrence of provitamin A carotenoids in the human diet and the low toxicity observed in animal studies.

β-Carotenes from natural sources were reviewed at the thirty-first, thirty-fifth and forty-first meetings of the Committee (Annex 1, references *77*, *88* and *107*). At the thirty-first meeting, the Committee concluded that the group ADI of 0–5 mg/kg bw established for the sum of the synthetic carotenoids β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters by the eighteenth Committee was not applicable to natural carotenes as they did not comply with the specifications for β-carotene. At the thirty-fifth and forty-first meetings, the Committee considered the available data inadequate to establish an ADI for the dehydrated algal carotene preparations or for the vegetable oil extract of *Dunaliella salina*.

At the fifty-seventh meeting (Annex 1, reference *154*), the Committee undertook a re-evaluation of β-carotene for use as a food colour, but focused its assessment on the production and analytical characteristics of β-carotene produced from *Blakeslea trispora*. The Committee considered that the source organisms, the production process and the composition of β-carotene from *B. trispora* do not raise specific concerns and that the material should be considered toxicologically equivalent to chemically synthesized β-carotene, for which an ADI of 0–5 mg/kg bw was established by the Committee at its tenth meeting. Therefore, the Committee established a group ADI of 0–5 mg/kg bw for synthetic β-carotene and β-carotene derived from *B. trispora*.[4]

β-Carotene-rich extract from *D. salina* was evaluated at the eighty-fourth meeting (Annex 1, reference *234*). The Committee observed that data that had become available since the previous evaluation showed differences in absorption of β-carotene between rodent species and humans. The Committee considered that rodents were inappropriate animal models for establishing an ADI for β-carotene because of the virtual absence of systemic absorption of β-carotene in rodents, but that the non-β-carotene components of *D. salina* d-limonene extract could be evaluated using the results of rodent studies. The Committee recommended that the group ADI for the sum of carotenoids, including β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters, be re-evaluated in light of evidence that shows very low absorption of β-carotene in rodents and rabbits in contrast to humans.

[4] The present Committee was aware that two group ADIs for carotenoids had been established at previous meetings and that synthetic β-carotene had been included in both group ADIs. The Committee speculated that the Committee at the fifty-seventh meeting did not recognize that synthetic β-carotene was already part of a group ADI and included it in a new group ADI.

β-Carotene, β-apo-8′-carotenal, β-carotene from *B. trispora* and β-apo-8′-carotenoic acid methyl and ethyl esters were placed on the agenda of the present meeting for an assessment of their safety, dietary exposure and specifications in response to the recommendation of the eighty-fourth meeting of the Committee. The present Committee considered a submission that comprised a review of information on synthetic β-carotene and β-apo-8′-carotenal that had become available since the eighteenth meeting. A targeted literature search was additionally carried out.

The Committee noted that no data were submitted on β-apo-8′-carotenoic acid methyl and ethyl esters. These food colours were therefore removed from the agenda.

Chemical and technical considerations

Provitamin A and xanthophyll carotenoids are natural pigments that are synthesized by plants and are responsible for the bright colours of various fruits and vegetables. Many different carotenoids are present in foods, and most have antioxidant activity. The most abundant carotenoid, β-carotene, consists of a highly branched, unsaturated chain with identical substituted ring structures at each end. β-Carotene and β-apo-8′-carotenal are provitamin A carotenoids.

β-Carotene, synthetic

Commercially available β-carotene, synthetic (INS 160a(i)) may be synthesized via a double Wittig condensation process or Grignard synthesis with enol ether condensations using a range of vitamin A precursors, including their phosphonium salts. The products of commerce may exist in multiple formulations, including water-dispersible forms, those that are water soluble and microcrystals prepared by spray drying and bound to food-grade carriers and antioxidants. Solvents used in manufacture may include dichloromethane, hexane, methanol, methylcyclohexane, toluene, acetone, ethanol, ethyl acetate, heptane, isobutyl alcohol and isopropyl alcohol [2]. The colouring principle of β-carotene, synthetic consists predominantly of all-*trans*-β-carotene (*E*-isomer) together with minor amounts of other carotenoids. The total colouring matters content is not less than 96% (expressed as β-carotene).

β-Carotene from *Blakeslea trispora*

β-Carotene from *Blakeslea trispora* (INS 160a(iii)) is obtained by co-fermentation using a mixed culture of the two sexual mating types (+) and (−) of natural strains of the fungus that are non-pathogenic and non-toxigenic. The compound is isolated from the fungal biomass by solvent extraction and crystallized. The main articles of commerce are suspensions in food-grade vegetable or plant oil and water-dispersible powders. These formulations are made for ease of use and

in order to improve stability, as carotenes easily oxidize. β-Carotene from *B. trispora* may also contain other carotenoids, of which λ-carotene accounts for the major part, at concentrations up to 3%. As in synthetically produced β-carotene, the colouring principle of β-carotene from *B. trispora* consists predominantly of all-*trans*-β-carotene. The total colouring matters content is not less than 96% (expressed as β-carotene).

β-Apo-8′-carotenal

β-Apo-8′-carotenal (INS 160a(vi)) occurs naturally in various plant materials as an aldehydic carotenoid. The product of commerce is synthetically produced using multiple mechanisms that may include the use of vitamin A precursor molecules and Wittig-type condensation reactions. Sequential chemical reactions are carried out to produce the final material, which exists predominantly as the all-*trans* (*E*) isomer. The articles of commerce may be diluted and stabilized as suspensions in edible fats or oils, emulsions and water-dispersible powders. The total colouring matters content is not less than 96%.

β-Carotene-rich extract from *Dunaliella salina*

β-Carotene-rich extract from *Dunaliella salina* is produced from *D. salina*, an extremely halotolerant alga that inhabits natural and human-made salt lakes and ponds. The carotene-rich alga is harvested and concentrated, and the carotenoids are extracted using an essential oil rich in d-limonene. The resulting extract is saponified, purified, centrifuged, evaporated and finally mixed with a vegetable oil to obtain a commercial product with a carotene content of about 30% by weight. β-Carotene accounts for more than 95% of the carotene content of the extracted material as a mixture of *trans* and *cis* isomers in a ratio of approximately 2:1 by weight. The remainder of the carotene content includes α-carotene, lutein, zeaxanthin and cryptoxanthin. In addition to the colour pigments and vegetable oil used for standardization, d-limonene extracts of *D. salina* contain lipids and other fat-soluble components naturally occurring in the source material, such as fatty acids, long-chain alcohols, alkenes and waxes. The composition of these fat-soluble components is primarily a mixture of fatty acids common to vegetable oils used in foods.

Biochemical aspects

β-Carotene

β-Carotene is absorbed into enterocytes and centrally cleaved to give two retinal molecules. Retinal is reduced to retinol by the enzyme retinaldehyde reductase and then esterified to form retinyl esters by lecithin:retinol acyltransferase and packaged with chylomicrons. Chylomicrons containing retinyl esters are released

into the lymph and then the bloodstream and rapidly taken up into the liver [reviewed in 3, 4]. Although the mechanism of intestinal β-carotene absorption and metabolism appears to be comparable in animal models and humans, marked differences in cleavage rates and consequently bioavailability between species have been shown [5–10].

In a short-term toxicity study in rats administered β-carotene at a dose of 0, 250, 500 or 1000 mg/kg bw per day for 13 weeks, plasma β-carotene concentrations ranged from 0.4 to 0.9 μg/mL [11]. In other studies in rats administered β-carotene at doses up to 1000 mg/kg bw per day for up to 21 weeks, plasma β-carotene concentrations ranged from below the limit of detection to about 0.2 μg/mL [12–14]. More than 95% of radioactivity in plasma and approximately 88–94% of radioactivity in liver were identified as retinol in rats administered 0.5 mg (0.74 MBq) radiolabelled β-carotene. β-Carotene was not detected in plasma [15].

In human subjects, the absorption of β-carotene has been estimated to be in the range of 40–65% [16–18]. In human subjects administered radiolabelled β-carotene, radioactivity in lymph was mainly associated with chylomicrons as retinyl esters, with approximately 20–30% of the absorbed radioactivity recovered as β-carotene [19, 20]. Following the administration of ^{13}C-labelled β-carotene to humans, most of the absorbed dose was converted to vitamin A [21]. Excretion of radioactivity occurred mainly via the faeces, with smaller amounts in the urine [16–18].

A number of studies in human subjects also investigated plasma levels of β-carotene following dosing for up to 12 years with pharmacological amounts of β-carotene. The most informative of these were a number of randomized controlled trials. Mean or median plasma β-carotene levels increased from 0.3 to 1.2 μg/mL in subjects administered 50 mg β-carotene every second day [22]; from 0.17 to 3.0 μg/mL in subjects administered 20 mg β-carotene per day [23]; and from 0.15 to 2.1 μg/mL in subjects administered 30 mg β-carotene per day with 25 000 IU vitamin A [24, 25].

Based on the observed differences in cleavage rates and bioavailability of β-carotene between rats and humans, the Committee reaffirmed the conclusion of the eighty-fourth meeting that this species is not suitable for the evaluation of β-carotene in humans. Absorption and tissue disposition studies with β-carotene in mice or dogs were not available to the Committee.

β-Apo-8′-carotenal

Radiolabelled β-apo-8′-carotenal and its metabolites were at least 25% absorbed from the gastrointestinal tract of rats. Total radioactivity in plasma reached a peak concentration after 10 hours and was eliminated with a half-life of 21 hours. β-Apo-8′-carotenal and its metabolites β-apo-8′-carotenol, β-apo-8′-carotenoic

acid and fatty acid conjugates were identified in the plasma. Radioactivity was recovered in the liver as retinol and fatty acid conjugates of retinol, demonstrating conversion of β-apo-8′-carotenal to vitamin A. Elimination of radioactivity occurred mainly via faeces, with smaller amounts excreted in the urine [26].

A clear sex-related difference was seen in a 13-week toxicity study in which female rats showed higher concentrations of β-apo-8′-carotenal and/or its metabolites in the plasma and liver compared with males [27].

β-Apo-8′-carotenal did not appear in plasma in significant amounts in human male volunteers given a single oral dose of 41 mg β-apo-8′-carotenal. β-Apo-8′-carotenol and β-apo-8′-carotenyl palmitate were identified as the two major metabolites in the plasma and reached their maximum concentrations of 0.29 and 0.23 μmol/L at 11 and 6 hours, respectively. 3-Apo-8′-carotenoic acid was also detected in serum, but the concentrations were not determined [28].

Toxicological studies

β-Carotene

β-Carotene has low acute oral toxicity in rats and dogs [29–32].

No target organ toxicity was observed in short- or long-term studies in rats or dogs administered β-carotene [11, 33–37].

β-Carotene was not carcinogenic in mice or rats [33, 35].

β-Carotene was not genotoxic in vitro or in vivo [32, 38–40].

There was no evidence of reproductive or developmental toxicity in studies in rats or rabbits [41–43].

β-Apo-8′-carotenal

β-Apo-8′-carotenal has low acute oral toxicity in mice [44] and rats [45, 46].

Two new short-term studies in rats were available to the Committee. In a 28-day study, rats were given β-apo-8′-carotenal in the feed at a target dose of 0, 20, 100 or 500 mg/kg bw per day. A NOAEL of 100 mg/kg bw per day was established on the basis of reduced body weight and body weight gain in rats at 500 mg/kg bw per day [47]. The Committee noted the presence of eosinophilic droplets mainly in the kidneys of female rats, but did not consider the finding to be adverse on the basis that the droplets were not linked to other lesions or any other signs of nephropathy.

In a follow-up 90-day study, male and female rats were administered β-apo-8′-carotenal in the feed at a target dose of 0, 10, 30 or 100 mg/kg bw per day. Liver weight and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly increased in female rats at the high dose relative to controls. Upon histopathological examination, a significant increase in the incidence of inflammatory cell foci was seen in the liver of female

rats at 100 mg/kg bw per day. In the kidney, an increase in the incidence of minimal eosinophilic droplets was observed in females at all doses, increasing in severity in high-dose females, but there was no evidence of necrosis or single-cell death at any dose. Findings of tubular injury were generally limited to the occasional tubular epithelial cell containing eosinophilic material appearing to be detached from the tubule and the presence of mitotic figures in the cortex in males and females at 100 mg/kg bw per day [27]. The Committee identified a NOAEL of 30 mg/kg bw per day, on the basis of increased liver weight, serum ALT and AST activities and incidence of inflammatory cell foci in the liver of high-dose female rats and evidence of tubular injury in the kidney of high-dose males and females. The Committee noted that higher plasma and liver concentrations of β-apo-8′-carotenal or its metabolites were achieved in female rats compared with males and considered this to be consistent with the toxicological findings of this study.

No new long-term toxicity or carcinogenicity studies were available to the Committee. In an early study, rats were administered β-apo-8′-carotenal in the diet at 1000 mg/kg feed to (1) a first generation of rats for 2 years, (2) their offspring for 2 years and (3) a third generation of rats for 1 year. The average dose over the course of the study was reported to be 40 mg/kg bw per day. Histopathological examination of the liver and kidneys of treated animals did not identify any adverse effects [48].

β-Apo-8′-carotenal was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA102, TA1535 or TA1537 at concentrations of 8.7–277.9 μg/plate, with or without metabolic activation [49]. In an earlier study, conducted with β-apo-8′-carotenal of low purity (69.2%), a concentration-related increase in the number of revertants was observed only in strain TA100, in the absence and presence of metabolic activation. The study authors noted that this result could be associated with impurities in the test material [50]. In an in vitro mammalian chromosomal aberration test, an increased frequency of chromosomal aberrations was observed in the absence and presence of metabolic activation at concentrations associated with approximately 50% cytotoxicity or greater, but not at lower concentrations, where moderate to low cytotoxicity was observed. The study authors noted that these increases were of questionable biological relevance given that they were observed only at doses associated with significant cytotoxicity [51]. In an in vivo mammalian erythrocyte micronucleus test, treatment with β-apo-8′-carotenal did not induce statistically significant increases in the frequency of micronucleated erythrocytes compared with the concurrent controls at doses up to 800 mg/kg bw per day [52]. The Committee concluded that the weight of evidence suggests that there is no concern for genotoxicity of β-apo-8′-carotenal.

No reproductive toxicity studies were available to the Committee. In a good laboratory practice– and guideline-compliant developmental toxicity study,

rats were administered β-apo-8′-carotenal in the feed from gestation days 6 to 20 at a dose of 0, 20, 100 or 495 mg/kg bw per day. No maternal or developmental toxicity was observed. The NOAEL for maternal toxicity and for embryo and fetal toxicity was 495 mg/kg bw per day, the highest dose tested [53].

Observations in humans

The association between β-carotene intake and cancer risk has been evaluated in a number of observational studies and extensively reviewed [9, 54–56]. It was concluded that intake of β-carotene and fruits and vegetables appears to confer protection against cancers at different sites, with the most consistent effect being a protective effect against lung cancer. Consequently, a number of large, high-quality randomized controlled trials have investigated whether β-carotene supplementation at doses of 20–50 mg/day for durations of up to 12 years reduces cancer risk in human populations.

In the Alpha-Tocopherol, Beta Carotene Cancer Prevention (ATBC) Study, a higher incidence of lung cancer (relative risk [RR] 1.18; 95% confidence interval [CI] 1.03–1.36) and total mortality (RR 1.08; 95% CI 1.01–1.16) was observed among the men who received β-carotene at doses of 20 mg/day for between 5 and 8 years. The elevated risk was related to those who smoked at least one pack of cigarettes per day and was not seen in subjects who smoked less [9, 23]. In the Beta-Carotene and Retinol Efficacy Trial (CARET), participants who were smokers or ex-smokers, or were exposed to asbestos, were given daily doses of 30 mg β-carotene and 25 000 IU vitamin A as retinyl palmitate for 5 years. Lung cancer incidence and total mortality were increased by 28% (RR 1.28; 95% CI 1.04–1.57) and 17% (RR 1.17; 95% CI 1.03–1.33), respectively, in the supplemented group [24, 25].

The Committee noted that the effects observed in heavy smokers and asbestos workers in the ATBC and CARET studies were not seen in population subgroups that were not at increased risk of lung cancer. In the Physicians' Health Study, β-carotene administered to subjects at 50 mg every second day for a period of 12 years did not affect the number of cases of lung cancer, mortality from cancer, all-cause mortality, cardiovascular disease, myocardial infarction or stroke [22]. No effects on cancer incidence or total mortality were seen in a number of other smaller randomized controlled trials in which β-carotene was administered at doses of up to 50 mg/day for durations of up to approximately 9 years [57–62].

Assessment of dietary exposure

β-Carotene and β-apo-8′-carotenal are proposed by the sponsor for use at typical and maximum use levels in 33 and 12 food categories of the Codex GSFA, respectively. For β-carotene, the typical (mean) and maximum use levels ranged

Table 2
Overview of dietary exposure estimates for β-carotene and β-apo-8′-carotenal from their use as food additives in the European population

	Dietary exposure (mg/kg bw per day)			
	β-Carotene		β-Apo-8′-carotenal	
Source	Mean	High	Mean	High
EFSA[a]	0.03–0.22	0.09–0.43[b]	0.01–0.25	0.04–0.49[c]
EFSA FAIM[d]	0.02–0.19	0.03–0.28[c]	–	–
France, Germany, United Kingdom[e,f]	0.009	0.03[g]	–	–

bw: body weight; EFSA: European Food Safety Authority; FAIM: Food Additive Intake Model
[a] All age groups; maximum use levels.
[b] High exposure: 95th or 97.5th percentile.
[c] High exposure: 95th percentile.
[d] All age groups; typical (mean) use levels.
[e] Adults; typical (mean) use levels.
[f] Exposure estimated by the present Committee using a 60 kg adult body weight.
[g] High exposure: 97.5th percentile.
Sources: EFSA: [2, 63]; EFSA FAIM (sponsor submission); France, Germany, United Kingdom: [64]

from 1 to 20 mg/kg and from 2 to 70 mg/kg, respectively. Corresponding ranges for β-apo-8′-carotenal were 0.4–50 mg/kg and 0.4–260 mg/kg. Currently, both food additives are authorized for use in 79 food categories at maximum permitted levels ranging from 50 mg/kg up to 1200 mg/kg, as specified in the GSFA [1].

The Committee used the exposure estimates submitted by the sponsor, which more closely represent actual exposure. These estimates were based on use levels combined with food consumption data from Europe [2, 63] and on a study on dietary exposure to β-carotene based on food consumption data from France, Germany and the United Kingdom [64]. Furthermore, the sponsor also reported on the exposure to β-carotene calculated with the EFSA Food Additive Intake Model (FAIM). The dietary exposure estimates are listed in Table 2.

The Committee concluded that the exposure to β-carotene from its use as a food additive at typical (mean) use levels estimated with EFSA FAIM is appropriate for use in risk assessment. The upper level of 0.28 mg/kg bw per day refers to the exposure in children aged 1–9 years. For adults aged 18 and above, the upper level of exposure to β-carotene equals about 0.1 mg/kg bw per day. The Committee acknowledged that these dietary exposure estimates were overestimations due to the assumption that β-carotene is used in all foods belonging to the relevant food categories.

The Committee considered that the high daily exposure estimate for β-apo-8′-carotenal of 0.49 mg/kg bw per day overestimates the exposure to this additive, owing to the assumption that all foods contained the food additive at the maximum use level. The Committee therefore concluded that the high daily

dietary exposure to β-carotene of 0.28 mg/kg bw per day may also be used for risk assessment of β-apo-8′-carotenal.

Evaluation

The Committee reaffirmed the conclusion from the eighty-fourth meeting that rats are not an appropriate model for deriving an ADI for β-carotene due to the relatively low bioavailability of β-carotene in rats compared with humans. Therefore, the Committee withdrew the two group ADIs of 0–5 mg/kg bw for (1) the sum of the synthetic carotenoids β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters and (2) synthetic β-carotene and β-carotene derived from *Blakeslea trispora*, which were based on a NOAEL from a rat study.

The Committee considered that no adverse health effects were observed in the general population in large, well-conducted human intervention studies in which healthy participants were administered between 20 and 50 mg β-carotene per day for up to 12 years, in addition to the background exposure from the diet.

An additional elevated risk of lung cancer and total mortality was seen in heavy smokers (at least one pack per day) and asbestos workers in intervention studies in which participants were administered 20 mg β-carotene per day for 5–8 years or 30 mg β-carotene per day and 25 000 IU vitamin A for 5 years. The Committee noted that a generally accepted explanation for the cause of these effects has not been identified. The Committee was unable to reach any conclusion about risk from β-carotene exposure in heavy smokers.

For the remainder of the general population, the Committee concluded that the estimated high exposure to β-carotene at 9 mg/day for a 30 kg child and 6 mg/day for a 60 kg adult from its current uses as a food additive, in addition to background exposure from the diet, would not be expected to be a safety concern. This conclusion includes synthetic β-carotene, β-carotene derived from *B. trispora* and β-carotene-rich extract from *Dunaliella salina*.

The Committee was unable to establish a group ADI for synthetic β-carotene, β-carotene derived from *B. trispora*, β-carotene-rich extract from *D. salina*, and β-apo-8′-carotenoic acid methyl and ethyl esters because a group ADI is applicable to the general population, which includes heavy smokers. The Committee noted that it is very unlikely that it will ever be possible to establish a group ADI because further data from the heavy smoker population cannot be gathered ethically.

Because β-apo-8′-carotenoic acid methyl and ethyl esters were previously evaluated on the basis of β-carotene and because no new data were submitted, the Committee was unable to complete an evaluation on β-apo-8′-carotenoic acid methyl and ethyl esters.

The present Committee established an ADI of 0–0.3 mg/kg bw for β-apo-8′-carotenal on the basis of a NOAEL of 30 mg/kg bw per day in a 13-week study in rats and application of an uncertainty factor of 100. An additional uncertainty factor to take into account the short duration of the study was not considered necessary because renal injury and hepatic lesions observed in the 13-week study at 100 mg/kg bw per day were not observed in the 2-year study at 40 mg/kg bw per day, the single dose tested.

Estimated dietary exposure to β-apo-8′-carotenal of 0.28 mg/kg bw per day was at the upper end of the ADI established by the Committee (i.e. 0–0.3 mg/kg bw). The Committee noted that the estimated dietary exposure is overestimated and concluded that the current use of β-apo-8′-carotenal as a food additive will not pose a safety concern.

A toxicological and dietary exposure monograph was prepared.

The specifications for β-carotene, synthetic, β-carotene from *B. trispora* and β-apo-8′-carotenal were revised to replace an identification test for carotenoids with additional spectrophotometric requirements.

β-Carotene-rich extract from *D. salina* was on the agenda of the current meeting at the request of the Fiftieth Session of CCFA [65] to revise the maximum limit on arsenic. The Committee received sufficient analytical data. Based on the arsenic levels from several batches of the product of commerce, the existing specifications were revised from 1 mg/kg to 3 mg/kg. The Chemical and Technical Assessment was revised.

Recommendations

The Committee noted that the use levels of β-carotene and β-apo-8′-carotenal provided by the sponsor were much lower than the corresponding maximum permitted levels as specified in the GSFA, and that the sponsor indicated that the majority of the maximum permitted levels are not justifiable from a technological point of view. Also, use levels were not provided for all authorized food categories. The Committee recommended that the Codex Alimentarius Commission should review current uses of β-carotene (synthetic β-carotene, β-carotene from *B. trispora* and β-carotene-rich extract from *D. salina*) and β-apo-8′-carotenal in the GSFA, including the maximum permitted levels and the food categories in which these food additives may be used.

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3.1.4 Gellan gum

Explanation

Gellan gum (INS 418; CAS No. 71010-52-1) is used as a gelling agent, stabilizer and thickener in a wide range of foods and beverages listed in the Codex GSFA, under the conditions of good manufacturing practice. It is commercially available in three different forms – namely, high-acyl, low-acyl and low-acyl clarified.

Gellan gum was previously evaluated by the Committee at its thirty-seventh meeting, at which an ADI "not specified" was established (Annex 1, reference *95*). This ADI "not specified" was based on the absence of adverse effects in toxicological studies in mice, rats, dogs and prepubertal rhesus monkeys and in a limited study on tolerance of gellan gum in humans. The Committee pointed out that the potential laxative effect of gellan gum at high dietary exposures should be taken into account when gellan gum is used as a food additive (Annex 1, reference *95*).

Gellan gum was evaluated by the Committee at its forty-ninth and seventy-ninth meetings for revision of specifications only (Annex 1, references *124* and *222*). At the seventy-ninth meeting, the Committee evaluated a request to include ethanol as an additional extraction solvent during the processing of gellan gum. The Committee at that meeting included ethanol in the specifications monograph and established a numerical limit of 50 mg/kg for residual ethanol (Annex 1, reference *222*).

At the present meeting, the Committee evaluated gellan gum for use in formulas for special medical purposes for infants (GSFA food category 13.1.3; referred to as "FSMPs" below) and re-evaluated the limit for residual ethanol in the specifications of gellan gum, at the request of the Fiftieth Session of CCFA [1]. Although the request from CCFA included the use of gellan gum in infant formula (GSFA food category 13.1.1) and follow-up formula (GSFA food category 13.1.2), only data supporting the use of gellan gum in FSMPs were received. Therefore, the Committee did not evaluate the use of gellan gum in infant formula or follow-up formula.

The low-acyl clarified form of gellan gum would be added directly to ready-to-feed FSMPs or would be used as a component of concentrated liquid fortification products[5] formulated with hydrolysed protein and/or amino acids (for addition to human milk or infant formula). According to the sponsor, these liquid fortification products also belong to food category 13.1.3. Gellan gum would be used to increase thickness and maintain homogeneity for better delivery of nutrients to the infant. It would also be used as a component of a stabilizer system, which contains octenyl succinic anhydride–modified corn starch (starch sodium octenyl succinate) (INS 1450). The target gellan gum concentration in the fed products (FSMPs, fortified human milk or fortified infant formula) is approximately 40 mg/L. Owing to manufacturing variability, the maximum gellan gum concentration requested is 50 mg/L.

At the present meeting, the Committee considered the submitted data, including new unpublished and published studies. A comprehensive literature search on gellan gum in PubMed did not identify any additional relevant published studies on biochemical or toxicological aspects. Studies from the previously published monograph, new studies that had become available since the thirty-seventh meeting and older studies not previously reviewed by the Committee are described below.

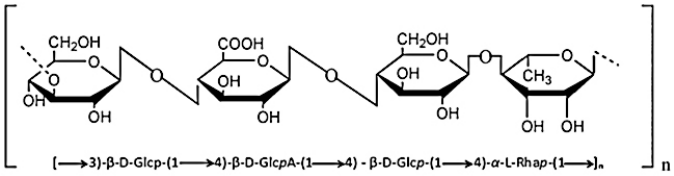
Chemical and technical considerations

Gellan gum is a high-molecular-weight (>500 000 Da) anionic polysaccharide that is produced by a controlled pure culture fermentation of the non-pathogenic Gram-negative bacterium *Pseudomonas elodea* (reclassified as *Sphingomonas elodea*) in the presence of a carbon source, a nitrogen source and inorganic salts. The fermentation broth is pasteurized to kill viable cells, and the gellan gum is recovered via precipitation with food-grade isopropanol or ethanol to obtain the high-acyl form (native gellan gum). Controlled treatment with hot alkali prior to alcohol precipitation results in deacylation and yields gellan gum with varying degrees of acylation, including the low-acyl form. Low-acyl gellan gum can be further filtered to obtain low-acyl clarified gellan gum. The gelling properties of the articles of commerce are controlled by the addition of metal ions such as sodium, potassium and calcium to neutralize the glucuronic acid. By-products of fermentation include polyhydroxybutyrate, enzymes and viable cells of the production organism, which are removed and/or inactivated during processing. The resulting gellan gum is separated, dried and milled.

In its native form, gellan gum is linear; it is composed of β-D-glucopyranosyl, β-D-glucuronopyranosyl and L-rhamnopyranosyl units in molar

[5] Nutritional supplements designed to increase the total energy, protein and micronutrient delivery to preterm infants [2].

Fig. 3
Chemical structure of gellan gum backbone



ratios of 2:1:1 (Fig. 3). Native gellan gum also contains an acetyl and a glyceryl group bound to the glucose adjacent to the glucuronic acid residues.

Different types of gellan gum were used in the toxicological studies evaluated at the thirty-seventh meeting (Annex 1, reference *95*) and in the new studies available for the current meeting. The gellan gum used in the acute toxicity studies [3, 4], the 13-week oral rat study [5] and the genotoxicity studies by Robertson and coworkers [6–9] was the low-acyl form, with greater than 95% polysaccharide content. The gellan gum samples used in the 52-week study in dogs [10], the long-term studies in mice and rats [11, 12], the genotoxicity study by Ivett [13], the special studies by Gordon [14, 15] and the studies in human adults [16, 17] were a blend of five products containing 58.5% polysaccharide (no further information available) with varying degrees of acylation. Low-acyl clarified gellan gum was used in the study in neonatal pigs [18], the clinical trials in infants [19–22] and the commercial products on which the post-marketing surveillance data were available [23–25]. The specific purity of the batches used was not provided. Based on the certificates of analyses of three representative batches submitted, these products are expected to contain greater than 94% polysaccharide. Characterization information on the gellan gum used in the other studies described below was not available.

Biochemical aspects

At its thirty-seventh meeting (Annex 1, reference *95*), the Committee concluded, on the basis of rat studies with radiolabelled gellan gum, that gellan gum is poorly absorbed and primarily excreted in the faeces following oral administration [26].

For the present meeting, data from short-term studies in rats and human volunteer studies were submitted. A study in human volunteers provided no evidence of gellan gum absorption [27]. No statistically significant increases in short-chain fatty acid production were reported in animals or humans following gellan gum exposure, suggesting limited microbial degradation of gellan gum in the gastrointestinal tract. Increases were observed in faecal weights and water content, indicating that gellan gum may be a faecal bulking agent [16, 27–30].

In rats, reduced gastrointestinal transit times were reported after exposure to gellan gum [29–31], whereas in a study in human volunteers, variable effects on gastrointestinal transit times were observed [27].

Toxicological studies

At the thirty-seventh meeting (Annex 1, reference *95*), the Committee noted that gellan gum exhibited low acute oral toxicity, with an LD_{50} value greater than 5000 mg/kg bw in rats [4]. Gellan gum did not cause adverse effects in a 90-day study in rats at doses up to 60 000 mg/kg feed (equivalent to 6000 mg/kg bw per day), a 52-week study in dogs at doses up to 60 000 mg/kg feed (equivalent to 1500 mg/kg bw per day assuming dry laboratory chow diet) and a 28-day study in prepubertal rhesus monkeys at doses up to 3000 mg/kg bw per day via gavage [5, 10, 32].

For the current meeting, an additional series of short-term studies in rats was available, focusing mainly on the gastrointestinal system [28–31, 33]. In all these studies, the animals were given gellan gum at a dietary concentration equivalent to 5000 mg/kg bw per day for 4 weeks. In the study by Tetsuguchi et al. [29], slight morphological changes in the intestinal mucosa were observed microscopically. The variations in the gastrointestinal mucosa were considered by the authors to be related to the gellan gum–induced increase in the viscosity of the intestinal contents, rather than a direct effect of gellan gum, and were not considered to be adverse, and the Committee agreed with this conclusion.

In summary, no adverse effects were reported in any of the short-term studies.

Two additional studies were conducted to specifically assess the potential effects of gellan gum on the gut epithelium and on mineral retention, respectively, using dietary concentrations equivalent to up to 5000 mg/kg bw per day [14, 15]. No adverse effects on intestinal morphology were reported after exposure of rats for 25 days [14]. Gellan gum did not affect growth or mineral retention after exposure of rats for 8 weeks [15].

Both available long-term toxicity and carcinogenicity studies were previously reviewed by the Committee at its thirty-seventh meeting (Annex 1, reference *95*). No treatment-related adverse effects or histopathological changes were reported following administration of gellan gum at dietary concentrations up to 30 000 mg/kg feed (equivalent to 4500 mg/kg bw per day) in mice for up to 98 weeks or 50 000 mg/kg feed (equivalent to 2500 mg/kg bw per day) in rats for 104 weeks [11, 12].

The Committee previously evaluated three in vitro genotoxicity studies on gellan gum, including a bacterial reverse mutation assay, a DNA repair assay (unscheduled DNA synthesis assay) and a gene mutation assay [6, 7, 9]. These studies all showed no evidence of genotoxicity.

Two additional genotoxicity studies were available for the present evaluation. In an alkaline elution assay, gellan gum was found to react with diaminobenzoic acid, forming a fluorescent product that interfered with DNA measurements. The authors therefore concluded that the assay was not valid [8], and the Committee agreed with this conclusion. Gellan gum gave negative results in an in vivo micronucleus assay [13]. However, the Committee noted that this result is not unexpected, given the poor absorption of gellan gum.

Considering the results of all available genotoxicity studies as well as the chemical structure of gellan gum, the Committee concluded that there is no concern for genotoxicity.

No adverse effects were reported in the reproductive and developmental toxicity studies in rats that were evaluated at the previous meeting [34, 35].

For the current meeting, the results of the in utero phase [36] of the long-term toxicity and carcinogenicity study in rats [12] were available. Treatment of the animals with gellan gum started 63 days prior to mating and was continued throughout mating, gestation and lactation. The NOAEL for parental, reproductive and offspring toxicity was 50 000 mg/kg feed (equal to 3520 mg/kg bw per day), the highest dose tested.

Special study in neonatal pigs

To assess the safety of gellan gum specifically as a component of infant formula, a study in neonatal pigs was submitted [18]. These pigs were fed milk replacer with gellan gum (low-acyl clarified product) at a concentration of 0, 41 or 205 mg/L (equal to 0, 19 and 100 mg/kg bw per day for males and 0, 20 and 100 mg/kg bw per day for females, respectively). The neonatal pigs were fed the gellan gum–containing milk replacer during the first 3 weeks of life (starting 2 days after birth) as the sole source of nutrition to model the 0- to 12-week period of development in human infants in which infant formula or (fortified) human milk may be provided as the sole source of nutrition. The aim of this study was to investigate potential effects of gellan gum on growth and development, with emphasis on the gastrointestinal tract and immune system. No gross or microscopic changes were reported in the small or large intestine of the neonatal pigs. The incidence of pelvic dilatation in the kidneys (hydronephrosis) was higher than the background incidence in historical controls. However, as the severity was mild and there were no microscopic correlates, the Committee considered these findings in the kidney to be of no toxicological relevance. Histopathological examination of the non-glandular stomach revealed variable acute inflammation, hyperkeratosis and/or erosion in all groups of animals, including concurrent controls. The author considered the non-glandular stomach lesions likely to be incidental, as no dose–response relationship was observed and as the stomach lesions reported

were recognized as common observations in pigs. The Committee agreed with this conclusion.

The NOAEL for gellan gum was 205 mg/L (equal to 100 mg/kg bw per day), the highest dose tested [18].

Observations in humans

Results from a previously evaluated, limited study on tolerance of gellan gum in adult humans indicated that daily oral doses of up to 200 mg/kg bw administered over a 23-day period did not elicit any adverse reaction, although faecal bulking effects were observed in most subjects. In two males, an increase in the percentage of eosinophils was observed, and the number of eosinophils in one of the subjects was reported to fall outside the normal range [16, 27]. Therefore, a follow-up study was performed to exclude possible sensitizing effects of gellan gum in 20 human volunteers, among whom were the two male volunteers who had elevated eosinophils in the previous study [17, 37]. No allergic reactions were observed among the subjects during or following gellan gum dietary supplementation, and no changes were observed in haematological parameters. Based on the results, the Committee concluded that there are no indications that gellan gum is sensitizing [17, 37].

Four paediatric clinical studies were conducted in preterm infants (gestational age <33 weeks, birth weight <2000 g) with human milk fortification (HMF) products containing gellan gum (low-acyl clarified form) for consumption by preterm and/or very low birth weight infants [19–22]. The products evaluated in these studies included powdered HMF products, acidified liquid HMF (AL-HMF) products (sterilized by acidification) and non-acidified liquid HMF (NAL-HMF) products (sterilized by heat treatment). Gellan gum was an ingredient of the NAL-HMF products. For the four studies taken together, 214 infants received human milk with NAL-HMF products containing gellan gum, and 226 infants received human milk with HMF products without gellan gum. The infants were enterally fed with human milk fortified with HMF products for 29–40 days. The gellan gum concentration in the fortified human milk was approximately 40 mg/L, and the dietary exposure ranged from approximately 3 to 6 mg/kg bw per day. No adverse effects on growth, haematological or biochemical parameters or clinical outcomes were reported with NAL-HMF products containing gellan gum when compared with the other HMF products tested, except for an increase in reticulocyte count in a pilot study by Kumar et al. [21]. The authors indicated that this could possibly be explained by the ferrous sulfate that was given to the NAL-HMF group because of the lower iron content of NAL-HMF products compared with AL-HMF products, but noted that this finding would need confirmation in larger studies. The Committee noted that the HMF products tested differed in several ways (protein content, protein type [hydrolysed vs intact], powder vs

liquid, acidified vs non-acidified, different food additives), so these studies do not provide information specifically about gellan gum. However, these studies did show that the tested NAL-HMF products containing gellan gum were generally well tolerated.

Post-marketing surveillance data over a 2.5-year period showed that the use of gellan gum (low-acyl clarified form) was well tolerated when administered to preterm infants through its use in an HMF product resulting in concentrations in human milk of approximately 40 mg/L [23–25].

Assessment of dietary exposure

At the current meeting, the Committee estimated the dietary exposure to gellan gum from its use in FSMPs and in concentrated liquid fortification products for addition to human milk or infant formula, as proposed by the sponsor. The requested maximum concentration of gellan gum in the fed products (FSMPs, fortified human milk or fortified infant formula) is 50 mg/L. Dietary exposure to gellan gum was assessed using consumption data for infant formula based on enteral feeding volumes of preterm infants, WHO-recommended consumption levels, consumption levels based on estimated energy requirements and actual reported consumption levels.

Based on the different consumption levels, the dietary exposure to gellan gum at the requested maximum concentration of 50 mg/L in fed products was estimated to range from 3.0 to 13 mg/kg bw per day. The dietary exposure of 13 mg/kg bw per day was based on a high level of consumption of infant formula of 260 mL/kg bw per day as derived by the Scientific Committee of EFSA [38]. This high consumption level also covers the potential high consumption of preterm infants on formula feeding [38].

The Committee noted that no dietary exposure assessment was performed for gellan gum for any food uses at the previous meeting (Annex 1, reference *95*).

Evaluation

The Committee previously established an ADI "not specified" for gellan gum (Annex 1, reference *95*). The ADI "not specified" was based on the absence of toxicity in animal studies, including long-term studies in mice and rats and a 52-week study in dogs in which animals were fed gellan gum at doses up to, respectively, 4500 mg/kg bw per day, 2500 mg/kg bw per day and 1500 mg/kg bw per day.

Several additional in vitro studies, animal studies and human data related to the safety of gellan gum have become available since the Committee's last evaluation. Results confirm the absence of any adverse effects arising from exposure to gellan gum. Therefore, the Committee retained the previously established ADI "not specified" for gellan gum.

ADIs established on the basis of the usually provided toxicology data are not applicable to infants up to the age of 12 weeks. The previously evaluated toxicity studies did not include direct oral administration to neonatal animals and thus did not address safety for the young infant age group. At the present meeting, a 21-day neonatal pig study using low-acyl clarified gellan gum, which modelled the 0- to 12-week period of development in human infants, was evaluated. The NOAEL was 100 mg/kg bw per day, the highest dose tested. Based on this NOAEL and the high estimate of dietary exposure of infants to gellan gum of 13 mg/kg bw per day (based on the requested maximum concentration of gellan gum of 50 mg/L and the high level of consumption of infant formula of 260 mL/kg bw per day), an MOE of 7.7 was calculated.

To interpret an MOE related to exposure in infants, the Committee has previously established several considerations that need to be addressed (Annex 1, reference *220*). If these considerations are met, MOEs in the region of 1–10 may indicate low risk for the health of 0- to 12-week-old infants exposed to the food additive through infant formula (Annex 1, reference *220*). The considerations relevant for the current evaluation of gellan gum for use in FSMPs and liquid fortification products for addition to human milk or infant formula are as follows:

- No adverse effects were observed in any of the studies available, indicating that the toxicity of gellan gum is low.
- The NOAEL was the highest dose tested in a study in neonatal pigs, which are considered a relevant animal model for human infants.
- Clinical studies in preterm infants support the tolerability of HMF products containing gellan gum resulting in concentrations of gellan gum in human milk up to approximately 40 mg/L.
- Post-marketing surveillance data over a 2.5-year period showed that the use of gellan gum was well tolerated when administered to preterm infants through its use in an HMF product resulting in concentrations in human milk of approximately 40 mg/L.
- The dietary exposure estimate was based on the requested maximum concentration of gellan gum of 50 mg/L.
- A high level of consumption of infant formula (260 mL/kg bw per day) was used to assess the dietary exposure.

Based on these considerations, the Committee concluded that the MOE of 7.7 calculated for the use of gellan gum in FSMPs and liquid fortification products for addition to human milk or infant formula at a maximum level of 50 mg/L in the fed product indicates low risk for the health of infants, including preterm infants, and that its proposed use is therefore of no safety concern. This conclusion applies only to the use of low-acyl clarified gellan gum. The

Committee recognizes that there is variability in medical conditions among infants requiring these products and that these infants would normally be under medical supervision.

A consolidated toxicological and dietary exposure monograph was prepared.

The Committee discussed the request to revise the limits on residual ethanol. Based on the data submitted, the Committee concluded that the use of ethanol in the manufacturing of gellan gum is not a safety concern when used in accordance with good manufacturing practice. The specification for ethanol was removed, and the existing specifications for gellan gum were revised. The specifications were made tentative, pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021.

A Chemical and Technical Assessment was prepared.

Recommendations

The specifications were made tentative, pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021. Specific information required is as follows:

- A method to differentiate the three commercial forms of gellan gum – i.e. high-acyl, low-acyl and low-acyl clarified.
- A method to determine the degree of acylation.
- Validation data for the above methods, including detailed description of the sample preparation.
- Data from five non-consecutive commercial batches of material using the proposed validated methods for all three forms of gellan gum.

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3.1.5 Potassium polyaspartate

Explanation

Potassium polyaspartate (INS 456) is a food additive intended to be used as a stabilizer to prevent tartrate crystal precipitation in wine at a proposed maximum use level of 300 mg/L. Potassium polyaspartate is produced from L-aspartic acid and potassium hydroxide.

Potassium polyaspartate has not previously been evaluated by the Committee. L-Aspartic acid is a component of the sweetener aspartame, which was evaluated by the Committee at its nineteenth, twentieth, twenty-first, twenty-third, twenty-fourth, twenty-fifth and eighty-second (specifications only) meetings (Annex 1, references *38*, *41*, *44*, *50*, *53*, *56* and *230*), and the use of

L-aspartic acid as a flavouring agent was evaluated by the Committee at its sixty-third meeting (Annex 1, reference *173*). Potassium hydroxide is a food additive (INS 525; CAS No. 1310-58-3) that was evaluated by the Committee at its ninth meeting (Annex 1, reference *11*).

Potassium polyaspartate was placed on the agenda of the present meeting at the request of the Fiftieth Session of CCFA [1] for an assessment of its safety, dietary exposure and specifications. The sponsor submitted unpublished toxicological studies and published papers. Two additional relevant publications were identified in a literature search. The Committee also considered the components of potassium polyaspartate using previous JECFA evaluations and other reviews. The sponsor provided details of typical and maximum use levels in wine and a dietary exposure assessment for Europe. Published estimates of dietary exposure noted by the sponsor were also reviewed. A literature search did not identify any additional estimates of dietary exposure.

Chemical and technical considerations

Potassium polyaspartate is produced from L-aspartic acid in a two-step process. During the first step, heating of solid L-aspartic acid leads to solid-phase polycondensation and production of polysuccinimide. Racemization occurs during this step [2], leading to the occurrence of both D- and L-aspartic acid in the final product. The water-insoluble polysuccinimide obtained is subsequently treated with aqueous potassium hydroxide under controlled conditions, which leads to hydrolysis, opening of the succinimide rings and production of the water-soluble potassium salt. The product contains approximately 70% β-peptide bonds and 30% α-peptide bonds. The final spray-dried potassium polyaspartate is a low-molecular-weight, polydisperse polymer with a weight-average molecular weight of approximately 5000 Da and a number-average molecular weight of about 1000 Da. Up to 20% has a molecular weight of less than 1000 Da.

Biochemical aspects

There are no in vivo data on the absorption of potassium polyaspartate.

In vitro data on Caco-2 monolayers that were used to simulate gastrointestinal absorption [3, 4] suggest that the systemic bioavailability of potassium polyaspartate is low. Other in vitro data obtained with pepsin and pancreatin to simulate gastrointestinal digestion [3] suggest that potassium polyaspartate would not be cleaved in the stomach and the intestine. However, potassium polyaspartate could be digested by microbiota occurring in the human intestine. The Committee noted the absence of information on the extent of fermentation of polyaspartate.

Toxicological studies

No information on the acute toxicity of potassium polyaspartate was available.

A dose range–finding study in rats given potassium polyaspartate by oral gavage at a dose of 0, 60, 125, 250, 500 or 1000 mg/kg bw per day for 14 days showed no treatment-related adverse effects [5, 6].

In a 90-day toxicity study, rats were given potassium polyaspartate by oral gavage at a dose of 0, 250, 500 or 1000 mg/kg bw per day. No treatment-related adverse effects were observed. The NOAEL was 1000 mg/kg bw per day, the highest dose tested [6, 7].

No long-term toxicity or carcinogenicity studies were available.

A bacterial reverse mutation assay and an in vitro micronucleus assay in human lymphocytes gave negative results [6, 8, 9]. The Committee concluded that there is no concern with respect to the genotoxicity of potassium polyaspartate.

No specific studies on reproductive and developmental toxicity, neurotoxicity or immunotoxicity were available. However, the 90-day study described above included additional parameters that provide information on some of these end-points. No effects on the estrous cycle or on weights and histopathology of testes, epididymides, seminal vesicles, uterus or ovaries were observed. There were no signs of neurological dysfunction investigated using a functional observational battery approach. No treatment-related effects indicating an immunotoxic or immunomodulatory potential were observed. In addition, histopathological investigations performed on thyroid and parathyroid and blood concentrations of triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) measured at termination of the treatment found no treatment-related effects that indicated disturbance of thyroid function [6, 7].

Results from an in vitro study in which the human promyelocytic cell line THP-1 was used as a surrogate for monocytes did not provide any indication of an immune response as indicated by CD86 expression and interleukin 8 release [3].

Observations in humans

No information was available.

Studies on L- and D-aspartic acid and potassium

Because the aspartic acid incorporated in the polyaspartate backbone is in an L- and D- configuration, the Committee considered L- and D-aspartic acid resulting from possible breakdown of potassium polyaspartate, as well as potassium.

L-Aspartic acid

L-Aspartic acid is a non-essential amino acid that occurs in food. It is also a component of the intense sweetener aspartame. Because L-aspartic acid results

from the hydrolysis of aspartame, the toxicity of and dietary exposure to L-aspartic acid were considered by the Committee in the course of its evaluations of the use of aspartame. The Committee concluded that L-aspartic acid generated from aspartame was not a safety concern at current dietary exposure to aspartame (Annex 1, reference *54*).

When the use of L-aspartic acid as a flavouring agent was evaluated by the Committee at its sixty-third meeting, the Committee concluded that there was no safety concern at current dietary exposures when used as a flavouring agent (Annex 1, reference *174*).

D-Aspartic acid

D-Aspartic acid is an endogenous amino acid that is involved in the development of the nervous system, plays a role in the neuroendocrine system, including hormone synthesis, has neuronal activities and is implicated in male fertility [10–23]. D-Aspartic acid is present in the human brain and accumulates with age in the central nervous system white matter, but not in grey matter [24, 25].

In a systematic review of 23 animal studies, three of which involved oral exposure of rats, and four human studies, the authors concluded that exogenous D-aspartic acid enhances testosterone levels in male animals at oral doses equivalent to around 130 mg/kg bw per day, whereas studies in humans, in which daily doses ranging from 36 to 70 mg/kg bw per day were consumed as dietary supplements, yielded inconsistent results. The authors noted that the inconsistent results obtained in these human trials could be due to limitations of the study designs, such as short-term supplement duration (12–28 days) and small sample sizes (N = 10–23 in the supplemented groups) [26]. The Committee agreed with this conclusion and noted that no NOAELs could be identified from the oral rat studies, as only single doses were tested.

There is experimental evidence for an L-isomer-selective transport of aspartic acid at the blood–brain barrier in the rat, whereby L-aspartic acid, but not D-aspartic acid, undergoes efflux transport from the brain to the blood; in contrast, the uptake of aspartic acid in brain parenchymal cells is not stereospecific [27, 28]. However, the Committee noted that administration of D-aspartic acid to rats in drinking-water at a dose of 50 mg/kg bw per day for 28 days increased its levels in both liver and blood serum about 5-fold and in kidney homogenates 8-fold, but did not increase the D-aspartate level in brain homogenates [29]. The Committee also noted that while the study did not meet current standards applicable for repeated-dose 28-day oral toxicity studies in rats (OECD Test Guideline 407), no signs of general toxicity were detected, and histopathological evaluation of renal and hepatic tissues did not reveal any treatment-related pathological alterations.

The Committee further noted that free D-aspartic acid can be metabolized by D-amino acid oxidase, which is expressed in brain, spinal cord, liver, renal proximal tubule cells and the proximal and middle small intestine of mice and humans [30].

There are no longer-term (>1 month) oral toxicity studies on D-aspartic acid and no toxicity studies on racemic mixtures of D- and L-aspartic acid.

Potassium

Potassium was evaluated by the Committee in the course of the evaluation of potassium hydroxide as a food additive at its ninth meeting (Annex 1, reference *11*). The result of the evaluation was an ADI "not limited"[6] for potassium hydroxide.

Serum levels of potassium usually rise only moderately in response to potassium intake, even in the case of a short-term (2–24 weeks) high potassium intake of 1755 mg/day, which resulted in an increase in potassium serum levels by only 0.17 mmol/L (6.6 mg/L) [31].

Assessment of dietary exposure

A dietary exposure assessment for potassium polyaspartate was undertaken for the first time by the present Committee. The assessment was based on typical use levels in wine of 100–200 mg/L and a maximum proposed use level of 300 mg/L.

Estimated dietary exposures reviewed were those submitted by the sponsor based on EFSA's FAIM, an EFSA assessment based on the Comprehensive European Food Consumption Database [32] and national dietary survey data for Australia and New Zealand [33]. The Committee also calculated national estimates of dietary exposure based on food consumption data in CIFOCOss for Brazil, China and the USA.

A summary of the national dietary exposure estimates is shown in Table 3.

The estimates of dietary exposure to potassium polyaspartate based on the maximum use level are overestimates; instead, the exposures based on typical use levels provide better estimates of chronic dietary exposures. Mean estimates of dietary exposure based on typical use levels are up to 0.7 mg/kg bw per day, and high exposures are up to 1.6 mg/kg bw per day.

L-Aspartic acid used in the manufacture of potassium polyaspartate also occurs naturally in food and can be consumed via dietary supplements and food additives such as aspartame. The Committee estimated that the dietary exposure for each of L- and D-aspartic acid is up to 0.8 mg/kg bw per day from the typical use of potassium polyaspartate in wine. This represents 50% of the total aspartic acid exposure (for both L- and D-aspartic acid) of 1.6 mg/kg bw per

[6] Now called ADI "not specified".

Table 3
Range of estimated dietary exposures[a] to potassium polyaspartate at typical and maximum food additive use levels

	Estimated dietary exposure (mg/kg bw per day)			
	Typical use level: 100–200 mg/L		Maximum use level: 300 mg/L	
Population group	Mean	High[b]	Mean	High[b]
Children	0–0.06	0–0.14	0–0.09	0–0.22
Adults	0.01–0.68	0–1.52	0.02–1.02	0–2.28
General population	0.09–0.70	0.18–1.58	0.26–1.05	0.54–2.37

bw: body weight
[a] Includes estimates for Europe and national estimates submitted to and calculated by the Committee.
[b] High exposure is the 90th percentile for all estimates other than Europe, for which high exposure is the 95th percentile.

day due to racemization and assumes that potassium polyaspartate is completely fermented in the colon and that the products of the fermentation are absorbed and bioavailable.

Estimated dietary exposure to L-aspartic acid from the food additive use is around 1% of a mean population dietary exposure of 108 mg/kg bw per day (6.5 g/day) for total aspartic acid from the diet (natural and supplemental sources) [34] and less than 1% of a high dietary exposure of 200 mg/kg bw per day (12.0 g/day) for total aspartic acid. The Committee concluded that the amount of additional L-aspartic acid in the diet from potassium polyaspartate is negligible and would be within normal daily variation in dietary exposures.

Dietary exposures to D-aspartic acid from six foods known to contain it (milk, cheese, yoghurt, beer, wine, juice) were estimated. Dietary exposures from the individual foods ranged between 0.001 and 0.07 mg/kg bw per day. The Committee was aware that this is an incomplete list of foods and also noted that food processing (e.g. heat treatment of protein, fermentation) will result in partial conversion of L-aspartic acid to D-aspartic acid for a range of other foods. Therefore, total dietary exposure to D-aspartic acid would be higher than estimated here.

The Committee considered the additional dietary exposure to potassium in the diet from use of the food additive and estimated a dietary exposure to potassium of about 45 mg/day for high consumers of wine. This is well below usual dietary exposures of between 2000 and 3000 mg/day, and the Committee concluded that the additional dietary exposure to potassium from use of the food additive in wine would be within normal daily variation.

Evaluation

In vitro data suggest that the systemic bioavailability of potassium polyaspartate is low and that it would not be cleaved in the stomach or the intestine. The NOAEL

in a 90-day rat study on potassium polyaspartate was 1000 mg/kg bw per day, the highest dose tested. There was no concern for genotoxicity.

Potassium has been evaluated by the Committee in the course of its evaluation of potassium hydroxide (Annex 1, reference *11*), and the result of the evaluation was an ADI "not limited". Exposure to potassium that results from the use of potassium polyaspartate in wine would be within normal daily variation of background potassium exposure from the diet.

The Committee noted that no information on potential microbial fermentation in the human colon is available, but should that occur, there would be potential exposure to L- and D-aspartic acid. L-Aspartic acid is a normal constituent of dietary protein, and systemic exposure to L-aspartic acid from the diet is much higher than potential exposure from the use of potassium polyaspartate in wine.

There are no relevant toxicological data on D-aspartic acid. In three studies, rats exposed to around 130 mg/kg bw per day showed effects on sex hormone levels. However, NOAELs have not been identified in these studies due to the use of single doses. The Committee noted that there is an MOE of more than 100-fold between the potential human exposure to D-aspartic acid of up to 0.8 mg/kg bw per day and the effect level of 130 mg/kg bw per day.

The estimated dietary exposure to D-aspartic acid from typical use of potassium polyaspartate in wine (up to 0.8 mg/kg bw per day) would be expected to be lower than the exposure from non-added sources in the diet. The Committee noted that it had limited data on concentrations of D-aspartic acid in food, but that food processing (e.g. heat treatment of protein, fermentation) will result in partial conversion of L-aspartic acid to D-aspartic acid.

The Committee concluded that the use of potassium polyaspartate in wine at the maximum proposed use level of 300 mg/L is not of safety concern.

A toxicological and dietary exposure monograph was prepared.

New specifications for potassium polyaspartate were prepared.

A Chemical and Technical Assessment was prepared.

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3.1.6 Rosemary extract

Explanation

Rosemary extract (INS 392) is an antioxidant food additive obtained from ground dried leaves of *Rosmarinus officinalis*. The antioxidant properties of rosemary extract are primarily attributed to its phenolic diterpene content – namely,

carnosic acid and carnosol. Rosemary also contains several volatile components that contribute to its characteristic flavour. The rosemary extract for use as an antioxidant has a minimum ratio of total content of carnosic acid and carnosol to total volatile components of 15:1.

The Committee previously evaluated rosemary extract at its eighty-second meeting (Annex 1, reference *230*). At that meeting, the Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid plus carnosol. This ADI was based on a NOAEL of 64 mg/kg bw per day, the highest dose tested in a short-term toxicity study in rats. An uncertainty factor of 200 was used, which includes an uncertainty factor of 100 and an additional uncertainty factor of 2 to account for the temporary designation of the ADI, pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. An additional uncertainty factor to account for the lack of a chronic toxicity study was not considered necessary, based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested. The temporary ADI applies to the rosemary extract that met the specifications prepared at the eighty-second meeting.

Rosemary extract was placed on the agenda of the present meeting at the request of the Fiftieth Session of CCFA [1] for an assessment of its safety, dietary exposure and specifications, including studies to elucidate its potential developmental and reproductive toxicity, information to validate the method of determination of residual solvents and data on typical use levels in food. A study on the reproductive and developmental toxicity of an acetone-based rosemary extract was submitted by the sponsors. In addition, a literature search identified five relevant studies published after the eighty-second meeting of JECFA. The Committee reviewed the data on typical use levels in food that were provided for the present meeting. In addition, updated dietary exposure assessments based on maximum permitted levels were available for review, as were assessments based on typical use levels. A literature search was also undertaken; however, it did not identify any further information on typical use levels or estimates of dietary exposure to rosemary extract.

Chemical and technical considerations

No new manufacturing information was submitted. The Committee received validation data and information on the method for determination of ethanol and acetone used during the manufacturing of rosemary extract.

Biochemical aspects

In a pharmacokinetics study that investigated an ethanol-based extract of dried leaves of rosemary [2], rats were administered rosemary extract at a dose of 240,

820 or 2450 mg/kg bw by oral gavage. Plasma concentrations of carnosic acid and carnosol were determined up to 24 hours after administration. The time at which the maximum concentration (C_{max}) was reached (T_{max}) was approximately 0.5 hour. The C_{max} and area under the plasma concentration versus time curve from time 0 to infinity ($AUC_{0-\infty}$) values showed reasonably good agreement, with a proportional increase with dose. An apparent double-peak phenomenon in the plasma concentration versus time curves, suggesting redistribution and enterohepatic recirculation, was also observed [2]. The Committee noted inconsistencies in C_{max} and T_{max} values between this study and two previously evaluated studies [3, 4].

A study by Seow & Lau [5] using a luciferase reporter gene assay with human (hPXR), mouse (mPXR) and rat (rPXR) pregnane X receptors indicated that carnosol is an activator of all three receptors, whereas carnosic acid is a potent agonist of both hPXR and mPXR, but not rPXR. These new findings provide insight on the molecular basis for the pregnane X receptor–mediated induction of expression of phase 1 and phase 2 enzymes of xenobiotic metabolism and membrane transport proteins.

An in vitro study by Ercan & El [6] showed that a rosemary water extract with 18.7% carnosic acid was a potent inhibitor of pancreatic lipase.

Toxicological studies

A new OECD-compliant (Test Guideline 421) reproductive/developmental toxicity screening study in rats using an acetone extract of rosemary with a high content of carnosic acid was available [7]. Rats were administered rosemary extract in the diet at initial concentrations of 0, 2100, 3600 and 5000 mg/kg feed, which were later reduced in females from gestation day 20 to 0, 1050, 1800 and 2500 mg/kg feed (equal to 0, 130, 219 and 316 mg/kg bw per day for males and 0, 167, 276 and 401 mg/kg bw per day for females, respectively). No adverse effects were observed in parental males or females or in reproductive parameters. Gestation length, litter size and pup body weight on postnatal day 1 and pup survival and body weight gain until postnatal day 13 (termination) were not affected by treatment. A clear dose-related reduction in total-T_4 serum levels in male and female pups was observed on postnatal day 13. Histopathological examination of the thyroid gland (one male and one female pup per litter) showed no abnormality [7]. The Committee noted the high variability in the thyroid hormone measurements in the pups.

A NOAEL of 5000 mg/kg feed (equal to 316 mg/kg bw per day), the highest dose tested, was identified for reproductive and parental toxicity. The Committee noted that it was unclear whether the treatment-related effects on thyroid hormone levels in pups were adverse, and therefore a NOAEL for offspring toxicity could not be identified. The study also did not provide adequate

evidence for the absence of developmental toxicity, given that no fetuses were examined.

One toxicological study on carnosic acid was identified in the literature search. Liu et al. [8] tested carnosic acid in an in vitro screening assay for embryotoxic potential using mouse embryonic stem cells. The embryonic stem cell test is an extensively used screening assay for developmental toxicity that has been validated by the European Union Reference Laboratory for alternatives to animal testing [9]. Studies on the predictivity of the embryonic stem cell assay indicated a significant false-positive rate (approximately 40%), but a very low false-negative rate (approximately 7%) [9]. According to the results from this in vitro assay, carnosic acid is weakly embryotoxic [8].

Observations in humans

A small-scale clinical study (a randomized, double-blinded and placebo-controlled study) investigated the memory-enhancing effects of a combined ethanol extract of three plants, including *Rosmarinus officinalis*. No adverse effects of the combined ethanol extract following administration for 14 days were reported [10]. The Committee noted that this study does not contribute to the evaluation.

Assessment of dietary exposure

The Committee first evaluated dietary exposure to rosemary extract (expressed as carnosic acid plus carnosol) at its eighty-second meeting (Annex 1, reference *230*). At that time, the estimates were based on maximum permitted and proposed levels. The Committee at that meeting noted that the dietary exposure estimates for high consumers of 0.09–0.81 mg/kg bw per day may exceed the upper bound of the temporary ADI by up to 2.7-fold. Based on the conservative nature of the dietary exposure assessments, the Committee requested that data on typical use levels in foods be provided in order to refine the dietary exposure estimates.

At the current meeting, typical use levels of rosemary extract (expressed as carnosic acid plus carnosol) from Europe [11], Australia and New Zealand [12] were available to the Committee for review. Dietary exposure assessments (expressed as carnosic acid plus carnosol) were also available based on typical use levels. These included estimates for Europe based on typical use levels in Europe [11], estimates for Australia and New Zealand based on typical use levels for those countries [12] and an assessment for the USA (from the sponsors) based on concentrations that were between the range of typical use and maximum permitted levels from the European Union, Australia and New Zealand. Although estimates of dietary exposure were also provided based on maximum permitted levels, only estimates of dietary exposure based on typical use levels were used

by the Committee in the evaluation. In addition, only non-brand-loyal results for Europe were used for the purpose of the evaluation.

For children, mean estimates of dietary exposure ranged between <0.01 and 0.14 mg/kg bw per day; high-percentile exposures ranged between <0.01 and 0.30 mg/kg bw per day. For adults, mean estimates of dietary exposure ranged between <0.01 and 0.05 mg/kg bw per day; high-percentile exposures ranged between 0.01 and 0.12 mg/kg bw per day. Estimated dietary exposures based on typical use levels were less than half those estimated by the Committee at the eighty-second meeting, which were based on maximum permitted levels, at the upper ends of the ranges of both mean and high-percentile exposures. Depending on the country, the main contributors to dietary exposure were fine bakery wares, soups and broths, sauces and toppings (including mayonnaise and salad dressings) and processed meat products.

For the present meeting, estimates of dietary exposure from naturally occurring sources were available for Europe (rosemary and other herbs) [11] and Australia and New Zealand (rosemary only) [12]. Estimates included dietary exposures from naturally occurring sources only and in combination with added sources.

For naturally occurring sources only, estimated dietary exposures for children ranged between 0.0 and 0.34 mg/kg bw per day for mean exposures and between 0.0 and 1.66 mg/kg bw per day for high-percentile exposures. Estimated dietary exposures for adults ranged between 0.0 and 0.18 mg/kg bw per day for mean exposures and between 0.0 and 0.52 mg/kg bw per day for high-percentile exposures. When dietary exposures from naturally occurring sources are combined with dietary exposures from added sources at typical use levels, the estimated mean and high-percentile dietary exposures were up to 0.42 mg/kg bw per day for children and up to 0.16 mg/kg bw per day for adults (both estimates from Europe; mean naturally occurring dietary exposure added to mean food additive dietary exposure, eliminating the high dietary exposure of up to 0.52 mg/kg bw per day). The contribution from naturally occurring sources was <1–4% for Australia and New Zealand, based on the distribution of dietary exposures for individuals, and 65–93% for Europe, based on summing mean dietary exposures from added and natural sources.

Evaluation

The Committee concluded that the new studies provided evidence for the absence of reproductive toxicity, but not for the absence of developmental toxicity. The Committee retained the temporary ADI of 0–0.3 mg/kg bw, pending the submission of studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on rodent pup thyroid hormone

levels can be replicated. The temporary ADI will be withdrawn if the requested studies are not submitted by the end of 2021.

Estimated mean and high-percentile dietary exposures to carnosic acid plus carnosol from use of rosemary extract as a food additive for all countries assessed based on typical use levels did not exceed the upper end of the temporary ADI of 0–0.3 mg/kg bw. The Committee noted that when dietary exposures from naturally occurring sources are combined with dietary exposures from added sources at typical use levels, the estimated dietary exposures for children were up to 0.42 mg/kg bw per day, which exceeds the ADI. The Committee noted that the temporary ADI is based on the highest dose tested in a short-term toxicity study in rats and that in the newly submitted reproductive/developmental toxicity screening study, no effects on reproductive toxicity or on parental animals were observed at 316 mg/kg bw per day, the highest dose tested. Therefore, the Committee does not consider the slight exceedance of the ADI to be a safety concern.

An addendum to the toxicological and dietary exposure monograph was prepared.

The Committee removed the specification for ethanol. The specifications monograph for rosemary extract was revised, and the tentative status was removed.

Recommendations

Studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on pup thyroid hormone levels can be replicated were identified as research needs to complete the evaluation. The Committee requests that this information be provided by the end of 2021.

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3.2 Revision of specifications

3.2.1 Citric and fatty acid esters of glycerol

CITREM was on the agenda of the current meeting at the request of the eighty-sixth meeting of JECFA to replace an obsolete packed column gas chromatographic method for the determination of total citric acid content (Annex 1, reference *241*). The Committee received a suitable validated replacement method, along with performance characteristics of the method and data on the total citric acid content in products currently available in commerce, determined using that method. The Committee included the new method in the specifications and deleted the previous method.

The Committee also considered the replacement of the method for glycerol to avoid the use of chloroform. A new HPLC method for the analysis of glycerol, supported by validation data, was provided and included in the revised specifications. The limit for glycerol was maintained.

Data on the use of additional neutralizing salts in CITREM manufacture were received and added to the specifications.

The lead limit for use of CITREM in infant formula was corrected to 0.5 mg/kg according to the previous evaluation.

The limit for sulfated ash was maintained for non-neutralized CITREM, and new limits were set for partially neutralized and for wholly neutralized CITREM.

Data on the sulfated ash levels and the content of minerals in neutralized CITREM products were provided. The Committee noted that although different neutralizing agents were used, this did not affect the current limit for sulfated ash.

The specifications were revised, and the tentative status was removed. The Chemical and Technical Assessment was revised.

3.2.2 Metatartaric acid

Metatartaric acid was on the agenda of the current meeting at the request of the Fiftieth Session of CCFA *(7)* to revise the specifications. The Committee, at its current meeting, received information on optical rotation, infrared identification, free tartaric acid content, degree of esterification and molecular weight distribution together with the analytical methods. The Committee revised the specifications for free tartaric acid, optical rotation, molecular weight and molecular weight distribution and included a specification for polydispersity index.

The specifications for metatartaric acid were revised, and the tentative status was removed. The Chemical and Technical Assessment was revised.

3.2.3 Mannoproteins from yeast cell walls

Yeast extracts containing mannoproteins was on the agenda of the current meeting at the request of the Fiftieth Session of CCFA *(7)* in order to complete the specifications related to the identity and purity of the product of commerce.

Given the additional compositional information received, the Committee revised the specifications monograph and noted that a change in the name of the food additive to "Mannoproteins from yeast cell walls" was appropriate. The Committee noted that all mannoproteins, regardless of the range of molecular weights, were included in the same specifications monograph and therefore that specifying a range of average molecular weight and a method for measuring it was not essential. Data were also received on metallic impurities. The Committee reviewed the information received and decided that only a limit for lead was required.

The specifications were revised, and the tentative status was removed. The Chemical and Technical Assessment was revised.

4. Flavouring agents

4.1 Specifications of identity and purity of flavouring agents

4.1.1 Revised specifications

The Committee received information in support of revision of the full specifications for nine flavouring agents that were on the agenda of the present meeting (JECFA Nos 141, 345, 547, 889, 893, 967, 979, 1029 and 1236).

The Committee revised specifications for methyl propionate (No. 141) and revised the specific gravity to 0.912–0.918 based on data from 20 lots of commercial product.

For ethyl oleate (No. 345), the Committee revised the assay minimum to not less than 75% ethyl oleate based on 29 lots of commercial product. Specifications for the secondary components were also established: ethyl linoleate (3.4–11.5%), ethyl palmitate (0.4–5.1%), ethyl stearate (0.5–2.5%), ethyl laurate (1–2%) and other fatty acid ethyl esters. The secondary components were not considered to pose a safety concern when No. 345 is used as a flavouring agent at current levels of use, as noted in Annex 3.

The Committee revised specifications for alpha-methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide (No. 547) based on data from flavouring agent currently in commerce and revised the refractive index to 1.512–1.522, the specific gravity to 1.040–1.050 and the assay minimum to 95%.

For vanillin (No. 889), the Committee reviewed data from 70 lots of commercial product and revised the melting point to 81–84 °C.

For ethyl vanillin (No. 893), the Committee reviewed data from 45 lots of commercial product and revised the melting point to 76–79 °C.

For 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde (No. 967), the Committee reviewed data from three lots of commercial product and revised the assay minimum to 93%, with a secondary component of up to 2% of gamma-campholenic aldehyde. The secondary component was not considered to pose a safety concern when No. 967 is used as a flavouring agent at current levels of use, as noted in Annex 3.

For alpha- and beta-cyclocitral (50:50 mixture) (No. 979), the Committee revised the specifications to include the CAS numbers for alpha-cyclocitral (CAS No. 432-24-6) and for the mixture of alpha- and beta-cyclocitral (CAS No. 52844-21-0). The Flavis and Council of Europe numbers for alpha- and beta-cyclocitral were also included. The refractive index range was revised to 1.4986–1.4991 based on information provided on the commercial product.

For sodium 2-(4-methoxyphenoxy)propanoate (No. 1029), the Committee revised the CAS number (CAS No. 150436-68-3) and Flavis number

(Flavis No. 08.127) to reflect the salt form. The melting point was revised to 184–190 °C based on information provided on the commercial product. Identifiers and synonyms associated with the free acid were removed.

Based on information provided on 60 lots of commercial product, the Committee revised the specifications for 2,2,6-trimethyl-6-vinyltetrahydropyran (No. 1236) by changing the minimum assay to 95%, the refractive index to 1.442–1.452 and the specific gravity to 0.863–0.873.

5. Future work and recommendations

Unsulfonated primary aromatic amines in food colours

The Committee requests analytical data on unsulfonated primary aromatic amines in the following synthetic food colours – Allura Red AC, Amaranth, Azorubine, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Fast Green FCF, Fast Red E, Green S, Indigotine, Lithol Rubine BK, Patent Blue V, Ponceau 4R, Quinoline Yellow, Sunset Yellow FCF and Tartrazine – along with the analytical methods used, in order to update specifications.

Black carrot extract

To proceed with the assessment of black carrot extract, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required.

In addition, the specifications were made tentative pending the submission of further information on the material of commerce, including a full characterization of the proteins, carbohydrates, lipids, fibre, minerals and non-anthocyanin polyphenol components in five lots each of the liquid and powder forms of black carrot extract.

Carotenoids (provitamin A)

The Committee noted that the use levels of β-carotene and β-apo-8′-carotenal provided by the sponsor were much lower than the corresponding maximum permitted levels as specified in the Codex GSFA, and that the sponsor indicated that the majority of the maximum permitted levels are not justifiable from a technological point of view. Also, use levels were not provided for all authorized food categories. The Committee recommended that the Codex Alimentarius Commission should review current uses of β-carotene (synthetic β-carotene, β-carotene from *Blakeslea trispora* and β-carotene-rich extract from *Dunaliella salina*) and β-apo-8′-carotenal in the GSFA, including the maximum permitted levels and the food categories in which these food additives may be used.

Gellan gum

The specifications were made tentative pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021. Specific information required is as follows:

- A method to differentiate the three commercial forms of gellan gum – i.e. high-acyl, low-acyl and low-acyl clarified.

- A method to determine the degree of acylation.
- Validation data for the above methods, including detailed description of the sample preparation.
- Data from five non-consecutive commercial batches of material using the proposed validated methods for all three forms of gellan gum.

Rosemary extract

Studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on pup thyroid hormone levels can be replicated were identified as research needs to complete the evaluation. The Committee requests that this information be provided by the end of 2021.

Acknowledgements

The Committee wishes to thank Ms M. Sheffer, Ottawa, Canada, for her assistance in the preparation of the report.

FAO and WHO wish to acknowledge the significant contributions of the experts, as well as their institutions (where relevant), to the work of the eighty-seventh meeting of JECFA.

Corrigenda

The following requests for corrections, reported to the JECFA secretariats, were evaluated by the eighty-seventh JECFA meeting and found to be necessary.

- *The following corrections will be made only in the online database for specifications:*

Food additive	Original text	New text	Additional information
Copper sulfate (INS 519)	CAS: 7758-98-7	CAS: 7758-99-8	Original CAS number is for anhydrous form; however, the specifications are for the pentahydrate
Magnesium dihydrogen diphosphate (INS 450(ix))	METHOD OF ASSAY The determination of phosphorus contains the following formula P_2O_5, %w/w = P% × 4.983	METHOD OF ASSAY The determination of phosphorus contains the following formula P_2O_5, %w/w = P% × 2.2921	Original formula did not account for the presence of two phosphorus atoms per molecule
Basic methacrylate copolymer (INS 1205) Will also be applied to anionic methacrylate copolymer (INS 1207) and neutral methacrylate copolymer (INS 1206)	In section Definition: "Basic methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes."	Sentence deleted.	Deletion requested by CCFA51[7]; sentence provided only marginal information
2-Acetyl-1-pyrroline (JECFA No. 1604)	CAS: 99583-29-6	CAS: 85213-22-5	Correction to CAS number

- *The following name was missing from the List of participants in the meeting report of the eighty-sixth meeting of JECFA (WHO Technical Report Series, No. 1014, 2019):*

Dr E. Dessipri, European Directorate for the Quality of Medicines & HealthCare, Council of Europe, Strasbourg, France (*Member*)

- *The following participants were indicated as not attending the eighty-sixth meeting, but actually participated in the meeting by video conference:*

[7] http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-711-51%252FReport%252FREP19_FAe.pdf

Dr M. DiNovi, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*WHO Temporary Adviser*)

Dr J.R. Srinivasan, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*FAO Expert*)

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4. Class names and the International Numbering System for Food Additives. Rome, Italy: Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants, Joint FAO/WHO Food Standards Programme; 2015 (CAC/GL 36-1989; http://www.fao.org/tempref/codex/Meetings/CCFAC/ccfac31/INS_e.pdf).

5. 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, Switzerland: World Health Organization; 2009 (Environmental Health Criteria, No. 240; http://www.inchem.org/documents/ehc/ehc/ehc240_index.htm, accessed 28 June 2016).

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7. Report of the 50th Session of the Codex Committee on Food Additives, Xiamen, China, 26–30 March 2018. Rome, Italy: Food and Agriculture Organization of the United Nations; and Geneva, Switzerland: World Health Organization; Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission; 2018 (REP18/FA).

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).

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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.

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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.

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23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.

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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.

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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, diethylpyrocarbamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.

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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.

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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.

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132. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.

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Annex 2

Toxicological and dietary exposure 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
Black carrot extract	N[a], T[b]	The Committee concluded that the effects observed with one anthocyanin-containing test material cannot be extrapolated to another anthocyanin-containing test material. This is because the test articles used in metabolism and toxicity studies are very heterogeneous and often not fully described and/or the anthocyanin content of the test material is too low and variable. Only one genotoxicity study was available for black carrot extract. **Owing to the lack of toxicological data on black carrot extract, the Committee was not able to draw conclusions on its safety.** To proceed with its assessment, at least a 90-day toxicological study on a well-characterized extract representative of the material of commerce would be required. The Committee concluded that the total mean dietary exposure to anthocyanins from naturally occurring sources and added black carrot extract ranges from 0.1 to 1.9 mg/kg body weight (bw) per day for adults (18+ years) and from 0.1 to 5.3 mg/kg bw per day for children (<18 years). The Committee noted that the contribution of the use of the food colour itself to the total mean dietary exposure to anthocyanins including from naturally occurring sources is as high as 25%. The Committee noted that the ADI for grape skin extract established by the previous Committee in 1982 was not reconsidered as part of this assessment and remains unchanged.
Brilliant Black PN	R[c]	The Committee concluded that the newly available information does not give reason to revise the previously established ADI of 0–1 mg/kg bw based on a short-term toxicity study in pigs. **The Committee therefore retained the ADI for Brilliant Black PN.** The Committee noted that the range of estimated dietary exposures for Brilliant Black PN was below the upper end of the ADI and concluded that **dietary exposure to Brilliant Black PN does not present a safety concern.**
Carotenoids (provitamin A)	R[d]	The Committee reaffirmed the conclusion from the eighty-fourth meeting that rats are not an appropriate model for deriving an ADI for β-carotene due to the relatively low bioavailability of β-carotene in rats compared with humans. Therefore, **the Committee withdrew the two group ADIs of 0–5 mg/kg bw for (1) the sum of the synthetic carotenoids β-carotene, β-apo-8′-carotenal and β-apo-8′-carotenoic acid methyl and ethyl esters and (2) synthetic β-carotene and**

(continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		β-carotene derived from *Blakeslea trispora*[e], which were based on a no-observed-adverse-effect level (NOAEL) from a rat study. The Committee considered that no adverse health effects were observed in the general population in large, well-conducted human intervention studies in which healthy participants were administered 20–50 mg β-carotene per day for up to 12 years, in addition to background exposure from the diet. An additional elevated risk of lung cancer and total mortality was seen in heavy smokers (at least one pack per day) and asbestos workers in intervention studies in which participants were administered 20 mg β-carotene per day for 5–8 years or 30 mg β-carotene per day and 25 000 IU vitamin A for 5 years. The Committee noted that a generally accepted explanation for the cause of these effects has not been identified. **The Committee was unable to reach any conclusion about risk from β-carotene exposure in heavy smokers.** **For the remainder of the general population, the Committee concluded that the estimated high exposure to β-carotene of 9 mg/day for a 30 kg child and 6 mg/day for a 60 kg adult from its current uses as a food additive, in addition to background exposure from the diet, would not be expected to be a safety concern.** This conclusion includes synthetic β-carotene, β-carotene derived from *B. trispora* and β-carotene-rich extract from *Dunaliella salina*. **The Committee was unable to establish a group ADI for synthetic β-carotene, β-carotene derived from *B. trispora*, β-carotene-rich extract from *D. salina*, and β-apo-8′-carotenoic acid methyl and ethyl esters** because a group ADI is applicable to the general population, which includes heavy smokers. The Committee noted that it is very unlikely that it will ever be possible to establish a group ADI because further data from the population of heavy smokers cannot be gathered ethically. Because β-apo-8′-carotenoic acid methyl and ethyl esters were previously evaluated on the basis of β-carotene and because no new data were submitted, **the Committee was unable to complete an evaluation on β-apo-8′-carotenoic acid methyl and ethyl esters**. **The present Committee established an ADI of 0–0.3 mg/kg bw for β-apo-8′-carotenal** on the basis of a NOAEL of 30 mg/kg bw per day in a 13-week study in rats and application of an uncertainty factor of 100. An additional uncertainty factor to take into account the short duration of the study was not considered necessary because kidney and liver effects observed in the 13-week study at 100 mg/kg bw per day were not observed in a 2-year study at 40 mg/kg bw per day, the single dose tested. Estimated dietary exposure to β-apo-8′-carotenal of 0.3 mg/kg bw per day was at the upper end of the ADI established by the Committee (i.e. 0–0.3 mg/kg bw per day). The Committee noted that the estimated dietary exposure is overestimated and concluded that the **current use of β-apo-8′-carotenal as a food additive will not pose a safety concern.**

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Gellan gum	R[f], T[g]	Available studies confirm the absence of any adverse effects arising from exposure to gellan gum. **The Committee retained the previously established ADI "not specified"[h] for gellan gum.** The Committee evaluated low-acyl clarified gellan gum for use in formulas for special medical purposes for infants. Based on a NOAEL of 100 mg/kg bw per day, the highest dose of low-acyl clarified gellan gum tested in a 21-day neonatal pig study, which modelled the 0- to 12-week period of development in human infants, and the high estimate of dietary exposure of infants to gellan gum of 13 mg/kg bw per day (based on the requested maximum concentration of gellan gum of 50 mg/L and the high level of consumption of infant formula of 260 mL/kg bw per day), a margin of exposure of 7.7 was calculated. The Committee concluded on the basis of several considerations (e.g. the low toxicity of gellan gum, the NOAEL being the highest dose tested, clinical studies in preterm infants and post-marketing surveillance data showing that gellan gum is well tolerated) that the margin of exposure of 7.7 calculated for the use of gellan gum in formulas for special medical purposes for infants and liquid fortification products for addition to human milk or infant formula at a maximum level of 50 mg/L in the fed product indicates low risk for the health of infants, including preterm infants, and that **its proposed use is therefore of no safety concern. This conclusion applies only to the use of low-acyl clarified gellan gum.** The Committee recognizes that there is variability in medical conditions among infants requiring these products and that these infants would normally be under medical supervision.
Potassium polyaspartate	N	In vitro data suggest that the systemic bioavailability of potassium polyaspartate is low and that potassium polyaspartate would not be cleaved in the stomach or the intestine. The NOAEL in a 90-day rat study on potassium polyaspartate was 1000 mg/kg bw per day, the highest dose tested. There was no concern for genotoxicity. Potassium has been evaluated by the Committee in the course of its previous evaluation of potassium hydroxide, and the result of the evaluation was an ADI "not limited"[i]. Exposure to potassium that results from the use of potassium polyaspartate in wine would be within normal daily variation of background potassium exposure from the diet. Should microbial fermentation in the human colon occur, there would be potential exposure to L- and D-aspartic acid. L-Aspartic acid is a normal constituent of dietary protein, and systemic exposure to L-aspartic acid from the diet is much higher than potential exposure from the use of potassium polyaspartate in wine. There are no relevant toxicological data on D-aspartic acid. In three studies, rats exposed to around 130 mg/kg bw per day showed effects on sex hormone levels. However, NOAELs have not been identified in these studies due to the use of single doses. The Committee noted that there is a margin of exposure of more than 100-fold between the potential human dietary exposure to D-aspartic acid of up to 0.8 mg/kg bw per day and the effect level of 130 mg/kg bw per day.

(continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
		The estimated dietary exposure to D-aspartic acid from typical use of potassium polyaspartate in wine (up to 0.8 mg/kg bw per day) would be expected to be lower than the exposure from non-added sources in the diet. The Committee noted that it had limited data on concentrations of D-aspartic acid in food, but that food processing (e.g. heat treatment of protein, fermentation) will result in partial conversion of L-aspartic acid to D-aspartic acid. **The Committee concluded that the use of potassium polyaspartate in wine at the maximum proposed use level of 300 mg/L is not of safety concern.**
Rosemary extract	R[j]	The Committee concluded that the new studies provided evidence for the absence of reproductive toxicity, but not for the absence of developmental toxicity. **The Committee retained the temporary ADI of 0–0.3 mg/kg bw, pending the submission of studies on the developmental toxicity of rosemary extract and studies to elucidate whether the effects noted on rodent pup thyroid hormone levels can be replicated.** The temporary ADI will be withdrawn if the requested studies are not submitted by the end of 2021. Estimated mean and high-percentile dietary exposures to carnosic acid plus carnosol from use of rosemary extract as a food additive for all countries assessed based on typical use levels did not exceed the upper end of the temporary ADI (0–0.3 mg/kg bw per day). The Committee noted that when dietary exposures from naturally occurring sources are combined with dietary exposures from added sources at typical use levels, the estimated dietary exposures for children were up to 0.42 mg/kg bw per day, which exceeds the ADI. The Committee also noted that the temporary ADI is based on the highest dose tested in a short-term toxicity study in rats and that in the newly submitted reproductive/developmental toxicity screening study, no effects on reproductive toxicity or on parental animals were observed at 316 mg/kg bw per day, the highest dose tested. **Therefore, the Committee does not consider the slight exceedance of the ADI to be a safety concern.**

N: new specifications; R: existing specifications revised; T: tentative specifications

[a] For the spray-dried powder form of black carrot extract.

[b] The specifications were made tentative pending further information on the material of commerce, including a full characterization of the proteins, carbohydrates, lipids, fibre, minerals and non-anthocyanin polyphenol components in five lots each of the liquid and powder forms of black carrot extract.

[c] Analytical methods for determining subsidiary colouring matters and organic compounds other than colouring matters were replaced with more specific and sensitive high-performance liquid chromatography methods. The existing titrimetric method for the assay of Brilliant Black PN was replaced with a visible spectrophotometric method.

[d] The specifications for synthetic β-carotene, β-carotene from *B. trispora* and β-apo-8′-carotenal were revised to replace an identification test for carotenoids with additional spectrophotometric requirements. Based on the arsenic levels from several batches of the product of commerce for β-carotene-rich extract from *D. salina*, the existing specifications for arsenic were revised from 1 mg/kg to 3 mg/kg.

[e] The Committee was aware that two group ADIs for carotenoids had been established at previous meetings and that synthetic β-carotene had been included in both group ADIs. The Committee speculated that the Committee at the fifty-seventh meeting did not recognize that synthetic β-carotene was already part of a group ADI and included it in a new group ADI.

[f] The Committee concluded that the use of ethanol in the manufacturing of gellan gum is not a safety concern when used according to good manufacturing practice. The specification for ethanol was removed.

[g] The specifications were made tentative, pending submission of new methods for characterizing the three forms of gellan gum in commerce by 2021.

[h] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment

of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect; it should not conceal food of inferior quality or adulterated food; and it should not create a nutritional imbalance.

[i] Now called an ADI "not specified" (see table note h).

[j] The Committee removed the specification for ethanol, and the tentative status of the specifications for rosemary extract was removed.

Food additives considered for specifications only

Food additive	Specifications
Cassia gum	T[a]
Citric and fatty acid esters of glycerol (CITREM)	R[b]
Metatartaric acid	R[c]
Mannoproteins from yeast cell walls	R[d]
Steviol glycosides	See note e

R: existing specifications revised; T: tentative specifications

[a] At the eighty-sixth meeting, the Committee updated the specifications for cassia gum by including the high-performance liquid chromatography method received and removed their tentative status. Based on comments received about the method performance, the present Committee reviewed the method again and noted that additional investigations were required. Therefore, the Committee decided to make the specifications tentative until ongoing investigations are completed.

[b] The Committee received a suitable validated replacement method for an obsolete packed column gas chromatographic 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 products currently available in commerce, determined using that method. The Committee included the new method in the specifications and deleted the previous method. A new high-performance liquid chromatography method for the analysis of glycerol, supported by validation data, was provided and included in the revised specifications. The limit for glycerol was maintained. Data on the use of additional neutralizing salts in CITREM manufacture were received and added to the specifications. The lead limit for use of CITREM in infant formula was corrected to 0.5 mg/kg according to the previous evaluation. Data on the sulfated ash levels and the content of minerals in neutralized CITREM products were provided. The limit for sulfated ash was maintained for non-neutralized CITREM, and new limits were set for partially neutralized and for wholly neutralized CITREM. The tentative status of the specifications was removed.

[c] The Committee received information on optical rotation, infrared identification, free tartaric acid content, degree of esterification and molecular weight distribution, together with the analytical methods. The Committee revised the specifications for free tartaric acid, optical rotation, molecular weight and molecular weight distribution and included a specification for polydispersity index. The tentative status of the specifications for metatartaric acid was removed.

[d] The Committee revised the specifications monograph and noted that a change in the name of the food additive from "Yeast extracts containing mannoproteins" to "Mannoproteins from yeast cell walls" was appropriate. The Committee noted that all mannoproteins, regardless of the range of molecular weights, were included in the same specifications monograph and therefore specifying a range of average molecular weight and a method for measuring it was not essential. Data were also received for metallic impurities. The Committee reviewed the information received and decided that only a limit for lead was required. The tentative status of the specifications was removed.

[e] A framework was adopted for developing specifications for steviol glycosides by four different methods of production. Specifications for steviol glycosides produced by different production methods were included as annexes, as below:

- Annex 1: Steviol Glycosides from *Stevia rebaudiana* Bertoni (**revised** from the specifications monograph for Steviol glycosides from *Stevia rebaudiana* Bertoni prepared at the eighty-fourth meeting of JECFA (INS 960a)).
- Annex 2: Steviol Glycosides from Fermentation (specifications for Rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* (INS 960b(i)) prepared at the eighty-second meeting of JECFA were **revised** to include other steviol glycosides from *Saccharomyces cerevisiae* and *Yarrowia lipolytica*).
- Annex 3: Enzyme Modified Steviol Glycosides (**new** specifications).
- Annex 4: Enzyme Modified Glucosylated Steviol Glycosides (**new** specifications, t**entative** pending further information concerning the analytical methods).

Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications
Methyl propionate	141	R[a]
Ethyl oleate	345	R[b]
alpha-Methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide	547	R[c]
Vanillin	889	R[d]
Ethyl vanillin	893	R[e]
2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	967	R[f]
alpha- and beta-Cyclocitral (50:50 mixture)	979	R[g]

(continued)

Flavouring agent	No.	Specifications
Sodium 2-(4-methoxyphenoxy)propanoate	1029	R[h]
2,2,6-Trimethyl-6-vinyltetrahydropyran	1236	R[i]

R: existing specifications revised

[a] The Committee revised the specific gravity to 0.912–0.918.

[b] The Committee revised the assay minimum to not less than 75% ethyl oleate. Specifications for the secondary components were also established: ethyl linoleate (3.4–11.5%), ethyl palmitate (0.4–5.1%), ethyl stearate (0.5–2.5%), ethyl laurate (1–2%) and other fatty acid ethyl esters.

[c] The Committee revised the refractive index to 1.512–1.522, the specific gravity to 1.040–1.050 and the assay minimum to 95%.

[d] The Committee revised the melting point to 81–84 °C.

[e] The Committee revised the melting point to 76–79 °C.

[f] The Committee revised the assay minimum to 93%, with a secondary component of up to 2% of gamma-campholenic aldehyde.

[g] The Committee revised the specifications to include the Chemical Abstracts Service (CAS) numbers for alpha-cyclocitral (CAS No. 432-24-6) and for the mixture of alpha- and beta-cyclocitral (CAS No. 52844-21-0). The Flavis and Council of Europe (COE) numbers for alpha- and beta-cyclocitral were also included. The refractive index range was revised to 1.4986–1.4991.

[h] The Committee revised the CAS number (150436-68-3) and Flavis number (08.127) to reflect the salt form. The melting point was revised to 184–190 °C. Identifiers and synonyms associated with the free acid were removed.

[i] The Committee changed the minimum assay to 95%, the refractive index to 1.442–1.452 and the specific gravity to 0.863–0.873.

Annex 3

Secondary components for flavouring agents with revised specifications with minimum assay values of less than 95%

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
345	Ethyl oleate	75%	Ethyl linoleate (3.4–11%), ethyl palmitate (0.4–5.1%), ethyl stearate (0.5–2.5%), ethyl laurate (1–2%) and other fatty acid ethyl esters	The impurities are fatty acids with similar structures. As such, there are no safety concerns at current levels when occurring as secondary components in JECFA No. 345 when used as a flavouring agent.
967	2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde	93%	Gamma-campholenic aldehyde (2,2,4-trimethyl-cyclopent-3-en-1-yl acetaldehyde) (2%)	The impurity is a positional isomer of JECFA No. 967, with similar toxicity. As such, there are no safety concerns at current levels when occurring as a secondary component in JECFA No. 967 when used as a flavouring agent.

Annex 4

Meeting agenda

Food and Agriculture Organization of the United Nations

World Health Organization

87th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
FAO Headquarters, Rome, 4 – 13 June 2019

Opening:

Philippine Room (C277) 4 June at 9.30h

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:

- Black carrot extract
- Brilliant Black PN (INS 151)
- Carotenoids (INS 160x):
  - β-carotene
  - β-carotene from *Blakeslea trispora*

  - * β-apo-8′-carotenal
  - * β-apo-8′-carotenoic acid methyl and ethyl esters
- Gellan gum (INS 418)
- Potassium polyaspartate
- Rosemary extract (INS 392)

7.2. Steviol glycosides - Establishment of a framework for safety assessment of steviol glycosides produced by different technologies

7.3. Food additives for revision of specifications and analytical methods:
- β-Carotene-rich extract from *Dunaliella salina*
- Metatartaric acid (INS 353)
- Yeast extracts containing mannoproteins
- Steviol Glycosides (Rebaudioside M manufactured from two strains of yeast from the *Saccharomyces* family)
- Steviol Glycosides (Rebaudioside A and M, respectively, from Multiple Gene Donors Expressed in *Yarrowia lipolytica*) (INS 960)
- Steviol glycosides (Steviol Glycosides, Rebaudioside A, Rebaudioside D, Rebaudioside M; Enzyme Modified Steviol Glycosides, Enzyme Modified Stevia Leaf Extract)

7.4. Establishment of specifications for certain flavouring agents
- Vanillin (JECFA No. 889)
- Ethyl vanillin (JECFA No. 893)
- Methyl propionate (JECFA No. 141)
- 2,6,6-Trimethyl-1&2-cyclohexen-1-carboxaldehyde (JECFA No. 979)
- Sodium 2-(4-methoxyphenoxy)propanoate (JECFA No. 1029)
- 2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde (JECFA No. 967)
- Ethyl oleate (JECFA No. 345)
- 2,2,6-Trimethyl-6-vinyltetrahydropyran (JECFA No. 1236)
- alpha-Methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide (JECFA No. 547)

8. Other matters to be considered (general considerations)
Update of EHC240:
For discussion
- Refinement of criteria for establishing group ADI and ADI not specified
- Proposal for updated guidance on evaluation of enzyme preparations

For consideration
- Proposal for updated guidance on evaluation of genotoxicity studies
- Update of Chapter 5 in EHC240 on dose–response modelling and application of the benchmark-dose approach

9. Errata

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 food additives
Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1014, 2019 (156 pages)

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
Eighty-fourth meeting of the Joint FAO/WHO Expert Committee on 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
Eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives
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)

Safety evaluation of certain food additives
Eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 73, 2017 (493 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)

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 convened to evaluate the safety of various food additives and to prepare specifications for the identity and purity of the food additives, including flavouring agents.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of and assessment of dietary exposure to food additives. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for six food additives or groups of food additives: black carrot extract; Brilliant Black PN; carotenoids (provitamin A); gellan gum; potassium polyaspartate; and rosemary extract.

Specifications for the following food additives were revised: citric and fatty acid esters of glycerol (CITREM); metatartaric acid; mannoproteins from yeast cell walls; and steviol glycosides. Specifications for cassia gum were made tentative.

Specifications for eight flavouring agents were revised: methyl propionate; ethyl oleate; alpha-methyl-beta-hydroxypropyl alpha-methyl-beta-mercaptopropyl sulfide; vanillin; ethyl vanillin; 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde; alpha- and beta-cyclocitral (50:50 mixture); sodium 2-(4-methoxyphenoxy)propanoate; and 2,2,6-trimethyl-6-vinyltetrahydropyran.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to and toxicological evaluations of all of the food additives considered at this meeting as well as the specifications for all of the food additives, including flavouring agents, considered at this meeting.

ISBN 978 92 4 121029 4

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 7.4
Revision Date 10.03.2022
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	:	Ethyl vanillin
Product Number	:	W246409
Brand	:	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.	:	121-32-4

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
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) H319	 Causes serious eye irritation.
Precautionary statement(s) P264 P280 P305 + P351 + P338 P337 + P313	 Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. 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	:	Ethylvanillin 3-Ethoxy-4-hydroxybenzaldehyde
Formula	:	$C_9H_{10}O_3$
Molecular weight	:	166,17 g/mol
CAS-No.	:	121-32-4
EC-No.	:	204-464-7

Component		Classification	Concentration
ethylvanillin			
CAS-No. EC-No.	121-32-4 204-464-7	Eye Irrit. 2; 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
Water Foam Carbon dioxide (CO2) 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

In the event of fire, wear self-contained breathing apparatus.

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.

Light sensitive.

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 entrepeneur 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)	Appearance	Form: powder Color: white
b)	Odor	No data available
c)	Odor Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	Melting point/range: 74 - 77 °C - lit.
f)	Initial boiling point and boiling range	295 °C at 1.013 hPa
g)	Flash point	145 °C - closed cup
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	< 0,01 hPa at 25 °C

l)	Vapor density	No data available
m)	Density	No data available
	Relative density	No data available
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Autoignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: 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:
Alkali metals
Oxidizing agents

10.4 Conditions to avoid
Strong heating.

10.5 Incompatible materials
Aluminum

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 - > 3.160 mg/kg
(OECD Test Guideline 401)
Inhalation: No data available
LD50 Dermal - Rat - > 2.000 mg/kg
(OECD Test Guideline 402)

Skin corrosion/irritation
Skin - Human
Result: slight irritation - 48 h
(Draize Test)
Remarks: (RTECS)

Serious eye damage/eye irritation
Eyes - Rabbit
Result: Eye irritation
(OECD Test Guideline 405)

Respiratory or skin sensitization
- Mouse
Result: negative
(OECD Test Guideline 429)

Germ cell mutagenicity
Test Type: Ames test
Test system: Salmonella typhimurium
Result: negative
Remarks: (National Toxicology Program)
Test Type: Human
Test system: lymphocyte
Remarks: Sister chromatid exchange
Test Type: Mutagenicity (mammal cell test): micronucleus.
Result: negative
Remarks: (National Toxicology Program)
Test Type: Hamster
Test system: fibroblast
Remarks: Cytogenetic analysis

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: CU6125000
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	LC50 - Pimephales promelas (fathead minnow) - 87,6 mg/l - 96 h Remarks: (ECOTOX Database)
Toxicity to daphnia and other aquatic invertebrates	EC50 - Daphnia magna (Water flea) - 130 mg/l - 24 h Remarks: (External MSDS)

12.2 Persistence and degradability

Biodegradability Result: - Readily biodegradable.

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

Product
See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

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

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.

H319 Causes serious eye irritation.

Further information
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of

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