



Toxicological profile for Linalyl acetate

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

3,7-Dimethylocta-1,6-dien-3-yl acetate (PubChem)

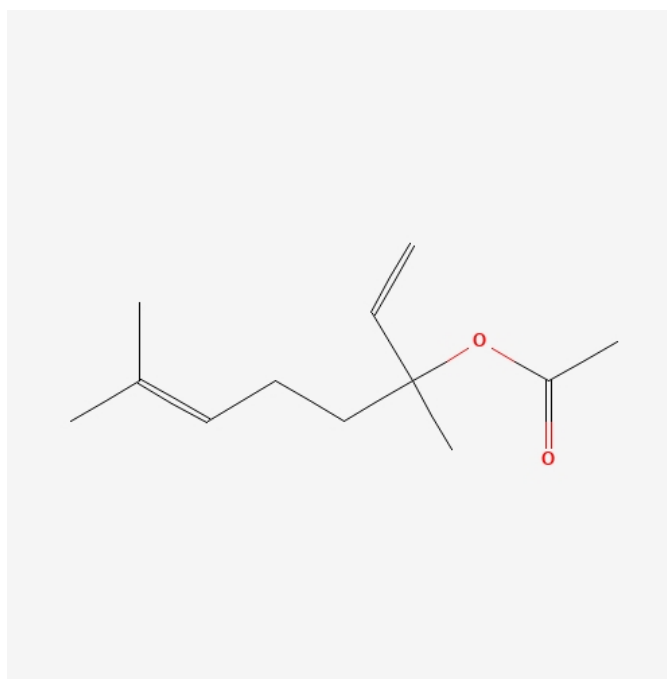
1.2. Synonyms

LINALYL ACETATE; Linalool acetate; 3,7-dimethylocta-1,6-dien-3-yl acetate; Bergamol; Linalol acetate; Licareol acetate; Acetic acid linalool ester; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; 3,7-Dimethyl-1,6-octadien-3-ol acetate; Phanteine; Dehydrolinalool, acetate; 1,6-Octadien-3-ol, 3,7-dimethyl-, 3-acetate; 3,7-Dimethyl-1,6-ctadien-3-ol acetate; Acetic Acid Linalyl Ester; EINECS 204-116-4; EINECS 254-806-4; 3,6-octadien-3-ol acetate; 3,6-octadien-3-yl acetate; FEMA 2636 (PubChem)

1.3. Molecular formula

C₁₂ H₂₀ O₂ (PubChem)

1.4. Structural Formula



(PubChem)

1.5. Molecular weight (g/mol)

196.29 (PubChem)

1.6. CAS registration number

115-95-7

1.7. Properties

1.7.1. Melting point

(°C): <25 (ChemSpider; EPISuite, 2017; PubChem); 85 (ChemSpider)

1.7.2. Boiling point

(°C): 220-221 (ChemSpider; EPISuite, 2017; PubChem); 213 (ChemView, 2023)

1.7.3. Solubility

Insoluble (Merck, 2013; PubChem); solubility in water: poor (PubChem); 30 mg/L at ambient temperature (INERIS, 2010); 140 mg/L at 20°C (ChemView, 2023)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 85 (closed cup) (ChemSpider; Merck, 2013; PubChem); 94 (closed cup) (PubChem)

1.7.6. Flammability limits (vol/vol%)

Combustible liquid. When heated to decomposition it emits acrid smoke and irritating fumes (HSDB, 2017); explosive limits: vol% in air: 0.7-4.3 (PubChem)

1.7.7. (Auto)ignition temperature

(°C): 225 (PubChem)

1.7.8. Decomposition temperature

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

1.7.9. Stability

Above 85°C explosive vapour/air mixtures may be formed (ICSC, 2009); Stable under recommended storage conditions, "combustible liquid", incompatible materials: strong oxidizing agents (PubChem)

1.7.10. Vapor pressure

0.11 mm Hg at 25°C (EPISuite, 2017; PubChem); 0.6 Pa [0.0045 mm Hg] at 20°C or 25°C (INERIS, 2010; PubChem)

1.7.11. log Kow

3.93 (EPISuite, 2017; PubChem); 3.208 (ChemSpider)

2. General information

2.1. Exposure

Cosmetics	Yes (Cosing)	Food	Yes (Burdock GA, 2010)
Environment	Yes (HSDB, 2017)	Pharmaceuticals	Yes (Martindale, 1999)

1 Volume of Use (worldwide band): >1000 metric tons per year (IFRA, 2011) 2 Average Maximum Concentration in Hydroalcohols: 4.60% (IFRA, 2002) 3 97.5th Percentile: 13.00% (IFRA, 2002) 4 Dermal Exposure*: 0.3312 mg/kg/day (IFRA, 2002) 5 Oral Exposure: Not applicable 6 Inhalation Exposures**: 0.03 mg/kg/day (IFRA, 2002) 7 Total Systemic Exposure (Dermal + Inhalation): (0.3312 mg/kg/day × 14.4% absorption) + 0.03 mg/kg/day = 0.078 mg/kg/day

* Calculated using the reported 97.5th percentile concentration based on the levels of the same fragrance ingredient in ten of the most frequently used personal care and cosmetic products (i.e., antiperspirant, bath products, body lotion, eau de toilette, face cream, fragrance cream, hair spray, shampoo, shower gel, and toilet soap) (Cadby et al., 2002; Ford et al., 2000).

** Combined (fine fragrances, hair sprays, antiperspirants/deodorants, candles, aerosol air fresheners, and reed diffusers/heated oil plug-ins) result calculated using RIFM's 2-Box/MPPD in silico models, based on the IFRA survey results for the 97.5th percentile use in hydroalcohols for a 60 kg individual.

As taken from Api et al., 2015

"The assessment of dietary exposure via the consumption of flavoured foods is a key element of the safety evaluation of flavouring substances. Linalyl acetate and linalool are the major flavouring substances in Earl Grey teas; the objective of this study was to determine their transfer rates from the tea leaves into the tea beverage upon preparation of a hot water infusion. Spiking experiments revealed a transfer rate of 66% for linalool. In contrast, the transfer rate for linalyl acetate was only 1.9%; in turn, the hydrolysis product linalool (17.0%) and a spectrum (19.9%) of degradation and rearrangement products (monoterpene alcohols, esters and hydrocarbons) were present in the tea beverage. The transfer rates were shown to be proportional to the length of the infusion. The impact of the hot water treatment on the enantiomeric compositions of linalyl acetate and linalool was determined, and structure-dependent experiments were performed by variation of the acyl and the alcohol moiety of the monoterpene ester. Comparative dietary exposure assessments demonstrated the need to take correction factors based on the experimentally determined transfer rates into account. Based on tea consumption data from the UK National Diet and Nutrition Survey (2000/2001), the exposure to linalyl acetate ranges from 0.2 mg day⁻¹ (average) to 1.8 mg day⁻¹ (high). The corresponding values for linalool are 4.2 mg day⁻¹ (average) and 46.6 mg day⁻¹ (high). The exposure of linalool via consumption of the tea beverage is approximately 26 times higher than that of linalyl acetate, although in the flavoured tea leaves the median content of linalyl acetate is approximately 1.8 times higher than that of linalool." As taken from Orth AM et al. 2014. Food Addit. Contam. Part A. Chem. Anal. Control Expo. Risk Assess. 31(2), 207-17. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24237351>

"Occupational exposure to linalyl acetate may occur through inhalation and dermal contact with this compound at workplaces where linalyl acetate is produced or used. Monitoring data indicate that the general population may be exposed to linalyl acetate via inhalation of ambient air, ingestion of food, and dermal contact with consumer products containing linalyl acetate(SRC)."

"According to the 2012 TSCA Inventory Update Reporting data, 9 reporting facilities estimate the number of persons reasonably likely to be exposed in the manufacturing, processing, or use of

linalyl acetate in the United States may be as low as <10 workers up to the range of 100-499 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 Nov 19, 2015: http://www.epa.gov/cdr/pubs/guidance/cdr_factsheets.html **PEER REVIEWED**"]

As taken from HSDB, 2017

Linalyl acetate (CAS 115-95-7, EC number 204-116-4) is used as a fragrance ingredient.

As taken from CosIng, undated.

Reported uses (ppm): (FEMA, 1994)

Food category	Usual	Max	Food category	Usual	Max
Alcoholic beverages	0.55	1.55	Gravies	0.05	0.10
Baked goods	15.67	23.34	Hard candy	4.04	23.43
Chewing gum	1.91	25.82	Meat products	0.23	0.45
Condiments, relishes	5.00	40.00	Nonalcoholic beverages	5.53	8.46
Frozen dairy	8.67	12.41	Soft candy	9.19	15.28
Gelatins, puddings	11.50	18.07			

Estimated intake from flavouring use: 0.003008 mg/kg bw/day.

As taken from Burdock, 2010.

Annual consumption /of linalyl acetate/ is 3550.00 lb. [Burdock, G.A. (ed.). Fenaroli's Handbook of Flavor Ingredients. 4th ed. Boca Raton, FL 2002, p. 975] **PEER REVIEWED**

As taken from HSDB, 2017

The estimated intake from use as a flavouring in the EU is 1700 µg/person/day (EFSA, 2011).

"It is used as an intermediate and can be found in consumer products as soaps, cleaning products, cosmetics (perfume), oil paint and extracts. Linalyl acetate is a food additive" (OECD-SIDS, 2002).

"Use: Fragrance component in perfumes, cosmetics and soaps; flavoring agent in foods."

As taken from Merck, 2013.

"It is included in some preparations for upper respiratory-tract disorders"

As taken from Martindale, 1999

Linalyl acetate (CAS RN 115-95-7) is listed (at given concentrations, where specified) as an ingredient in auto (1-15%, includes "old" products), commercial/institutional, inside the home (1-5%), personal care (0.1-1%), pesticide and pet care products by the CPID.

"Airborne compounds in the indoor environment arise from a wide variety of sources such as environmental tobacco smoke, heating and cooking, construction materials as well as outdoor sources. To understand the contribution of scented candles to the indoor load of airborne substances and particulate matter, candle emission testing was undertaken in environmentally controlled small and large emission chambers. Candle emission rates, calculated on the basis of measured chamber concentrations of volatile and semi-volatile organic compounds (VOC, SVOC) and particulate matter (PM), were used to predict their respective indoor air concentrations in a standard EU-based dwelling using 2 models: the widely accepted ConsExpo 1-box inhalation model and the recently developed RIFM 2-box indoor air dispersion model. The output from both models has been used to estimate more realistic consumer exposure concentrations of specific chemicals and PM in candle emissions. Potential consumer health risks associated with the candle emissions were characterized by comparing the exposure concentrations with existing indoor or ambient air quality guidelines or, where not existent, to established toxicity thresholds. On the basis of this investigation it was concluded that under normal conditions of use scented candles do not pose known health risks to the consumer." As taken from Petry T et al. 2014. Regul. Toxic. Pharmac. 69, 55-70. Available at: <http://www.sciencedirect.com/science/article/pii/S0273230014000348>

Industrial Processes with risk of exposure:

Australian

"Linalyl acetate has reported domestic use in automotive aftermarket products including car wash soaps, boat wash soaps, polishes and rubbing compounds."

International

"All chemicals in this group, except for (R)-linalyl acetate (CAS No. 16509-46-9) and linalyl octanoate (CAS No. 10024-64-3), have reported cosmetic use in perfumes or as fragrance ingredients.

Linalyl acetate (CAS No. 115-95-7) has been reported as being used in a number of cosmetic products in the Compilation of Ingredients used in Cosmetics in the United States (CIUCUS, 2011).

The chemicals have reported domestic/commercial uses, including in:

- 1. adhesives and sealants;
- 1. cleaning and maintenance products;
- 1. paints, lacquers and varnishes;
- 1. process regulators;
- 1. welding and soldering agents; and
- 1. odour agents.

The chemicals have reported non-industrial uses including:

- 1. as food additives; and
- 1. in non-agricultural pesticides and preservatives.

Linalyl acetate is a substitute for pettigrain oil (HSDB)."

As taken from AICIS, 2018

Linalyl acetate (CAS RN 115-95-7) is used as an oral flavour enhancer and topical fragrance ingredient in non-medicinal natural health products and has an upper limit toxicity restriction of 0.5 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%
beta-myrcene	196996281	5.07	211207074	4.93
cis-ocimene	80830917	2.07	87365036	2.04
trans-beta-ocimene	126377461	3.23	135106552	3.15
linalyl acetate	3287664464	84.09	3542795948	82.07
neryl acetate	64387894	1.647	64935633	1.52
geranyl acetate	120405125	3.08	118335955	2.76
Total ion chromatogram	3909257305	100	4284429942	100

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

Ingredients CAS Number	Chemical Class	Mol. Wt. (M) Bp or Mp (°C)	Max cig Appln Level (ppm)	Purity of sample Pyrolysed (%)	Composition of pyrolysate (Compound %)	Max level in smoke (µg)
Linalyl acetate CAS 115-95-7	Unsaturated ester	M=196 bp 220	7.5	97	Linalyl acetate 78.6 beta-Myrcene 6.5 delta-3-Carene 3.6 beta-Ocimene 3.1 Dihydrolinalool 2.0 alpha-Ocimene 1.1 Limonene 1.1 11 Minor products, 3 unidentified 4	3 0.2 0.1 0.1 0.08 0.04 0.04 0.2

In a pyrolysis study, 61.7% of linalyl acetate added to cigarettes was transferred intact to the smoke (Purkis et al., 2011).

2.3. Ingredient(s) from which it originates

Usually prepared by direct acetylation of linalool, also prepared from myrcene hydrochloride, anhydrous sodium acetate and acetate anhydride in the presence of a catalyst. All synthetic methods tend to avoid the simultaneous formation (due to isomerization) of terenyl and geranyl acetate).

Reportedly found in a number of essential oils, including bergamot, lavender, clary sage and lavandin. Also occurs in citrus peel oils and juices, berries, peach, celery, tomato, cinnamon, clove, nutmeg, pepper, thymus, grape wines, avocado, mushroom, marjoram, mango, cardamom, coriander, gin, origanum, loverage, laurel, myrtle leaf, rosemary, sage and mastic oil gum.

As taken from Burdock, 2010.

Occurs naturally in grapefruit juice at 0.16 mg/kg, other fruits at up to 0.07 mg/kg, tomato at 2.3 mg/kg, lemon oil in trace amounts and nutmeg oil in trace amounts (CoE, 2000).

No evidence of its presence in tobacco naturally.

“Linalyl acetate is a frequently encountered component of plant oils including lavender and citrus leaf oils(1). It is also a primary constituent of oils of orange flower (neroli), lavender and lavandin, bergamot, petitgrain bigarade, and sage (clary)(2). Linalyl acetate is 43.6, 26.8 and 45% of essential oil concentrations of *Lavandula angustifolia*, *Salvia desoleana* Atzei and Bergamot, respectively(3). [(1) Sell CS; Terpenoids. Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2015). New York, NY: John Wiley & Sons. Online Posting Date: 15 Sept 2006. (2)Baser KHC, Demirci F; Essential Oils. Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2015). New York, NY: John Wiley & Sons. Online Posting Date: 14 Jan 2011. (3) OECD; SIDS Initial Assessment Report For SIAM 14, Linalyl Acetate (CAS No. 115-95-7), March 2002. Available from, as of Nov 19, 2015: **PEER REVIEWED**”

“Plant Concentrations:

Linalyl acetate was qualitatively identified as a component of the twig and needle oils of the Balkan pine (*Pinus peuce*)(1). Linalyl acetate is 33% of essential oil of *Skimmia laureola* growing wild in Jammu and Kashmir, India(2). Linalyl acetate is 4.8% of essential oil of *Cuminum cyminum* L collected from Alborz Mountain, Iran(3). *Lavandula angustifolia* essential oil, grown in southeast Spain, contained 21-36% linalyl acetate(4). Linalyl acetate is 50.5% of essential oil of *Skimmia laureola* leaves collected in Patrak, Pakistan(5). Lemon peels (*Citrus limon*), collected from Akure main market, Nigeria contained 1.48% linalyl acetate(6). The essential oil from *Thymus praecox* ssp. *arcticus*, grown in Iceland contained 70% linalyl acetate(7). Linalyl acetate is 43.6, 26.8 and 45% of essential oil concentrations of *Lavandula angustifolia*, *Salvia desoleana* Atzei and Bergamot, respectively(8). [(1) Koukos PK et al; J Agric Food Chem 48: 1266-8 (2000) (2) Shah WA et al; Nat Prod Res 27:1023-7 (2013) (3) Mohammadpour H et al; Jundishapur J Nat Pharm Prod 7:50-5 (2012) (4) Carrasco A et al; Planta Med. Epub PMID: 26441063 Oct 4, 2015 (2015) (5) Barkatullah et al; Molecules 20:4735-45 (2015) (6) Oboh G et al; J Oleo Sci 63:373-81 (2014) (7) Dtahl E; Planta Med 50:157-60 (1984) (8) OECD; SIDS Initial Assessment Report For SIAM 14, Linalyl Acetate (CAS No. 115-95-7), March 2002. Available from, as of Nov 19, 2015: **PEER REVIEWED**”

“Other Environmental Concentrations:

Linalyl acetate is an ingredient in personal and pet care products(1). Linalyl acetate was identified as a volatile organic compound emitted by ten household products used for cleaning and/or conservation (eight waxes or polishes and two detergents)(2). Linalyl acetate was detected at a maximum of 96 ug/cu m after air freshener was sprayed in a closed room(3). The mean concentration of linalyl acetate in 63 snus packets (smokeless tobacco product) was 150.1 ug/pouch, after use, the mean was 121.0 ug/pouch; a mean of 19.2% was extracted during use; experiment was run Aug to Oct 2008 in Sweden(4). [(1) National Library of Medicine Household Products Database. Available from, as of Nov 19, 2015: <http://hpd.nlm.nih.gov/> (2) Knoppel H, Schauenburg H; Environ Int 15: 413-8 (1989) (3) Salthammer T; in Organic Indoor Air Pollutants. Occurrence, Measurement, Evaluation. Salthammer T, ed., Chichester, NY: Wiley-VCH, pp. 219-32 (1999) (4) Digard H et al; Chem Cent J 7: 55 (2013)] **PEER REVIEWED**”

“Linalyl acetate plant concentrations(1).

Genus species	Family	Name(s)	Part	Concn (ppm)
Bursera delpechiana POISS	Burseraceae	Lignallee, Linaloe	Wood	56,400

Cinnamomum camphora (L.) NEES & EBERM	Lauraceae	Camphor, Ho Leaf	Leaf	14,400
Skimmia arborescens T. ANDERSON EX GAMBLE	Rutaceae	Skimmia	Leaf	2,300-16,800
Elettaria cardamomum (L.) MATON	Zingiberaceae	Cardamom	Plant	8,000
Lavandula angustifolia MILLER	Lamiaceae	English Lavender	Flower	7,200
Salvia officinalis L.	Lamiaceae	Sage	Plant	6,048
Citrus aurantium L.	Rutaceae	Bitter Orange, Petitgrain	Leaf	5,500
Thymus vulgaris L.	Lamiaceae	Common Thyme, Garden Thyme, Thyme	Plant	4,680
Monarda clinopodia L.	Lamiaceae	Clinopod Bergamot	Plant	4,050
Lavandula x intermedia EMERIC ex LOIS	Lamiaceae	Dutch Lavender, Lavandin	Plant	3,950
Salvia sclarea L.	Lamiaceae	Clary Sage	Plant	3,750
Pycnanthemum flexuosum (WALT.) BSP	Lamiaceae	Flexuose Mountain Mint	Shoot	612
Monarda russeliana NUTT. ex SIMS	Lamiaceae	Russel's Monarda	Plant	557
Citrus aurantium L.	Rutaceae	Bitter Orange, Petitgrain	Flower	520
Monarda didyma L.	Lamiaceae	Beebalm, Oswego Tea	Plant	505
Monarda punctata L.	Lamiaceae	Horsemint	Plant	378
Jasminum officinale L.	Oleaceae	Jasmine, Poet's Jessamine	Flower	285
Tagetes patula L.	Asteraceae	French Marigold	Plant	262
Ocimum basilicum L.	Lamiaceae	Basil, Cuban Basil, Sweet Basil	Plant	240
Lavandula latifolia MEDIK.	Lamiaceae	Aspic, Broad-Leaved Lavender, Spike Lavender	Plant	198
Origanum vulgare L.	Lamiaceae	Common Turkish Oregano, European Oregano, Oregano, Pot Marjoram, Wild Marjoram, Wild Oregano	Plant	184
Hedeoma pulegioides (L.) PERS.	Lamiaceae	American Pennyroyal	Plant	180
Thymus mastichina L.	Lamiaceae	Spanish Marjoram	Plant	150

Cymbopogon winterianus JOWITT	Poaceae	Java Citronella, Mahapengiri	Plant	140
Tagetes minuta L.	Asteraceae	Aztec Marigold, Dwarf Marigold, Mexican Marigold, Muster John Henry, Stinking-Roger, Wild Marigold	Plant	125
Satureja montana L.	Lamiaceae	Savory, Winter Savory	Plant	3-240
Satureja obovata LAG.	Lamiaceae	Iberian Savory, Savory	Leaf	0-230
Cymbopogon nardus (L.) RENDLE	Poaceae	Ceylon Citronella, Citronella	Plant	96
Micromeria croatica	Lamiaceae	None listed	Leaf	0-140
Monarda media WILLD.	Lamiaceae	Mean Monarda	Plant	69
Tagetes erecta L.	Asteraceae	Aztec Marigold, Marigold	Plant	61
Thymus zygis subsp. Sylvestris	Lamiaceae	'Portuguese' Thyme	Shoot	0-100
Thymus zygis L.	Lamiaceae	Spanish Thyme	Shoot	0-70
Thymus broussonettii BOISS.	Lamiaceae	Moroccan Thyme	Shoot	0-60
Myrtus communis L.	Myrtaceae	Arrayan (Sp.), Mirto (Sp.), Myrte (Ger.), Myrtle	Plant	28
Satureja obovata LAG.	Lamiaceae	Iberian Savory, Savory	Leaf	0-55
Origanum vulgare var. viride (BOISS.) HAYEK	Lamiaceae	Green Turkish Oregano	Plant	0-50
Thymus saturejoides	Lamiaceae	Moroccan Savory Thyme	Shoot	0-50
Monarda citriodora CERV. EX LAGASCA	Lamiaceae	Lemon Mint	Plant	24
Zanthoxylum alatum ROXB.	Rutaceae	Indian Prickly Ash, Wartara Oil Tree	Leaf	0-45
Origanum majorana L.	Lamiaceae	Marjoram, Sweet Marjoram	Plant	17

[(1) USDA; Dr. Duke's Phytochemical and Ethnobotanical Databases. Plants with a chosen chemical. Linalyl acetate. Washington, DC: US Dept Agric, Agric Res Service. Available from, as of Nov 20, 2015: <http://www.ars-grin.gov/duke/> **PEER REVIEWED**"]

As taken from HSDB, 2017

Present in bergamot oil (30–60%), the volatile oil from cardamom, Origanum vulgare and Spanish sage, cassie absolute, clary sage oil, Egyptian dill seed, lavender oil (~40%), spike lavender oil

(~1%), lavandin oil, sweet marjoram oil, bitter orange oil, neroli oil (6-17%), petitgrain oil, Origanum onites essential oil, tagetes oil

As taken from Khan IA and Abourashed EA, 2010.

The lavender EO is chemically composed of camphor, terpinen-4-ol, linalool, linalyl acetate, beta-cimene, and 1,8-cineole. As taken from Elshafie HS and Camele I (2017)

“It forms a principal component of the essential oils from bergamot and lavender.”

As taken from PubChem

3. Status in legislation and other official guidance

Linalyl acetate (CAS RN 115-95-7) is included on the FDA's list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is generally recognized as safe under 21 CFR section 182.60 (Synthetic flavoring substances and adjuvants).

As taken from FDA, 2024.

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives LINALYL ACETATE	
COE No.:	203
FEMA No.:	2636
JECFA No.:	359
Chemical names:	1,5-DIMETHYL-1-ETHENYLHEX-4-ENYL ACETATE
Synonyms:	3,7-DIMETHYLOCTA-1,6-DIEN-3-YL ACETATE; BERGAMOL; LICAREOL ACETATE; LINALOOL ACETATE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1998
ADI:	0-0.5 mg/kg bw (1979)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1979 group ADI of 0-0.5 mg/kg bw for citral, geranyl acetate, citronellol, linalool, and linalyl acetate, expressed as citral, was maintained at the fifty-first (TRS 891/90, 1998) and sixty-first (TRS for JECFA 61 in press) meetings.
Report:	TRS 891-JECFA 51/79
Specifications:	COMPENDIUM ADDENDUM 6/FNP 52 Add.6/180 (1998)
Tox monograph:	FAS 42-JECFA 51/293
Previous status:	1982, FNP 25-JECFA 26/105; COMPENDIUM/851. R 1979, TRS 648-JECFA 23/25, FNP 12-JECFA 23/63. 0-5 (GROUP ADI FOR CITRAL,

	GERANYL ACETATE, CITRONELLOL, LINALOOL, AND LINALYL ACETATE, EXPRESSED AS CITRAL). R 1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/32,33, FAS 68.33/NMRS 44A-JECFA 11/54. 0-0.25 (CONDITIONAL). CO. N

As taken from JECFA, 2004

Linalyl acetate is listed as a fragrance ingredient by IFRA.

Linalyl acetate 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).

Linalyl acetate (CAS RN 115-95-7) is included on the US EPA's list of Safer Chemical Ingredients (US EPA, 2024).

Linalool acetate (CAS RN 115-95-7) is listed in the US EPA InertFinder Database as approved for non-food and fragrance use pesticide products. (US EPA InertFinder)

Linalyl acetate (CAS RN 115-95-7) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR (Chemical Data Reporting) Regulation.

The TSCA inventory and 2020 CDR list.

There is a REACH dossier on linalyl acetate (ECHA, undated).

Linalyl acetate (CAS RN 115-95-7) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2024).

"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. Linalyl acetate 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 October 23, 2015: <http://www.ecfr.gov> **PEER REVIEWED**"

As taken from HSDB, 2017

Linalyl acetate (FEMA no. 2636) has been given GRAS (generally recognized as safe) status by FEMA (Hall and Oser, 1965).

"The chemicals are listed on the following (Galleria Chemica):

as 'Terpenes and terpenoids' in the EU Cosmetic Directive 76/768/EEC Annex III—List of substances which Cosmetic Products must not contain except subject to the restrictions and conditions laid down: Peroxide value less than 10 mmoles/L; and

as 'Terpene hydrocarbons' in the New Zealand Cosmetic Products Group Standard—Schedule 5: Components cosmetic products must not contain except subject to the restrictions and conditions laid down: Peroxide value less than 10 mmoles/L.

The acceptable daily intake (ADI) for linalyl acetate and linalyl formate is 0.5 mg/kg bw (EFSA, 2012)."

"The following exposure standards are identified for terpenes (Galleria Chemica).

A time weighted average (TWA) of 111–150 mg/m³ (20–25 ppm) in Canada (Alberta), Estonia and Sweden.

Short-term exposure limits (STEL) of 300 mg/m³ (50 ppm) in Estonia and Sweden."

As taken from AICIS 2018

INCI Name	LINALYL ACETATE
Description	
CAS #	115-95-7
EC #	204-116-4
Identified INGREDIENTS or substances e.g.	3,7-Dimethyl octa-1,6-diene-3-yl acetate
Cosmetics Regulation provisions	III/337
Functions	FRAGRANCE
Substance	3,7-Dimethyl octa-1,6-diene-3-yl acetate
CAS #	115-95-7
EC #	204-116-4
Name of Common Ingredients Glossary	Linalyl Acetate
INN / ISO / AN	
Regulation	(EU) 2023/1545
Other Directives / Regulations	
Annex / Ref #	III / 337
Product Type, body parts	
Maximum concentration in ready for use preparation	
Other	The presence of the substance shall be indicated in the list of ingredients referred to in Article 19(1), point (g), when its concentration exceeds: — 0,001 % in leave-on products — 0,01 % in rinse-off products.

As taken from CosIng

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Linalool and Dehydrolinalool (analogs) were used as a read across for Linalyl acetate (target) based on:

- The read-across materials are the major metabolite of the target and the analog of the metabolite.
- The target is the acetate form of the linalool. This difference could be mitigated by the fact the target could readily hydrolyzed into the read-across material and acetic acid (Bickers et al., 2003). Besides, the differences between structures do not essentially change the physicochemical properties nor raise any additional structural alerts and therefore, the toxicity profiles are expected to be similar.
- The target and analog show the same skin absorption range.
- The target and analog show similar alerts for Repeated Dose (HESS) Categorization and ER Binding. ER Binding is molecular initiating event analogous to protein binding. ER binding is not

necessarily predictive of endocrine disruption given the complex pre- and post-receptor events that determine activity.

- As per the OECD Toolbox, the target is predicted to metabolize to the analog (metabolite # 2)

As taken from Api et al., 2015

“In neutral gastric juice, linalyl acetate is slowly ($t_{1/2}$ = 121 min) hydrolyzed to a mixture of linalool and the ring closed isomer alpha-terpineol. In acidic artificial gastric juice, linalyl acetate is rapidly hydrolyzed ($t_{1/2}$ < 5min) to yield linalool, which rapidly rearranges into alpha-terpineol (FEMA, 1998; JECFA, 1999).”

“Linalyl acetate was slowly hydrolyzed ($t_{1/2}$ = 153-198 min) in intestinal fluid with or without pancreatin. Linalyl acetate also hydrolyzed in homogenates of rat intestinal mucosal, blood, and liver, but at rates much slower than in acidic gastric juice (rate constant for hydrolysis $k=0.01$ - 0.0055 min^{-1} vs. $> 5 \text{ min}^{-1}$) (FEMA, 1998; JECFA, 1999). Based on these observations it is concluded that linalyl acetate hydrolyzes in gastric juice to yield linalool which, to some extent, is rapidly ring closed to yield alpha-terpineol (FEMA, 1995; JECFA, 1999).”

“Esters are readily hydrolyzed by carboxylesterases or esterases (Sato, 1957) and linalyl acetate would readily be converted to linalool and acetate in vivo. This has been demonstrated in vitro in rat blood and liver. Acetate is a normal constituent of the body. The metabolism of linalool is known and is primarily through glucuronic acid conjugation and excretion (Parke et al., 1974).”

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

“Linalyl acetate is hydrolysed in gastric and pancreatic fluids with mean half-lives of 5.5 and 52.6 minutes respectively. As expected hydrolysis occurs more rapidly at the low pH of gastric fluids. The reaction products are linalool and acetic acid (ester hydrolysis). This is supported by the findings of the hydrolysis study at pH 4, 7 and 9. Therefore it is expected that linalool is the substance that will enter the systemic circulation after oral uptake of linalyl acetate.”

“Linalool is probably converted to geraniol and its metabolites, 1,5-dimethyl-hexadiene-1,6-dicarboxylic acid and 7-carboxy-5-methylocto-6-enoic acid.”

“Linalyl acetate will be hydrolysed to linalool and acetic acid in the gastro-intestinal tract.”

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7).

“... Hydrolysis occurs more rapidly at the low pH of gastric fluids. The reaction products are linalool and acetic acid (ester hydrolysis). This is supported by the findings of the hydrolysis study ... at pH 4, 7 and 9. Therefore it is expected that linalool is the substance that will enter the systemic circulation after oral uptake of linalyl acetate. Linalool is probably converted to geraniol and its metabolites, 1,5-dimethyl-hexadiene-1,6-dicarboxylic acid and 7-carboxy-5-methylocto-6-enoic acid ... [Organization for Economic Cooperation and Development; Screening Information Data Set for LINALYL ACETATE (115-95-7) p.10 (March 2002). Available from, as of July 14, 2008: **PEER REVIEWED**

Linalyl acetate is hydrolysed in gastric and pancreatic fluids with mean half-lives of 5.5 and 52.6 minutes respectively ... [Organization for Economic Cooperation and Development; Screening Information Data Set for LINALYL ACETATE (115-95-7) p.10 (March 2002). Available from, as of July 14, 2008: **PEER REVIEWED**”

As taken from HSDB, 2017

“Two hydrolysis studies on a supporting substance, linalyl acetate [FL-no: 09.013], were found. In an in vitro hydrolysis study, linalyl acetate was easily hydrolysed in water and simulated gastric and pancreatic fluids. The mean half-lives for linalyl acetate hydrolysis were 5.5 and 52.5 min in gastric and pancreatic fluids, respectively (Hall, 1979). In neutral gastric juice, linalyl acetate is slowly ($t_{1/2}$

=121 min) hydrolysed to a mixture of linalool and the ring-closed isomer α -terpineol. In acidic artificial gastric juice, linalyl acetate is rapidly hydrolysed ($t_{1/2} < 5$ min) to yield linalool (Buck & Renwick, 1998). Linalyl acetate was slowly hydrolysed ($t_{1/2} = 153 - 198$ min) in intestinal fluid with or without pancreatin. Linalyl acetate also hydrolyses in homogenates of rat intestinal mucosa, blood and liver, but at rates much slower than in acidic gastric juice (rate constant for hydrolysis, $k = 0.01$ to 0.0055 min^{-1} vs. $> 5 \text{ min}^{-1}$ in gastric juice). Based on these observations it can be concluded that linalyl acetate hydrolyses in gastric juice to yield linalool and acetic acid (Buck & Renwick, 1998). Further, it has been demonstrated that linalyl acetate can be hydrolysed to linalool in *in vitro* studies after incubation with rat caecal flora (Rahman, 1974a)."

"After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep, dihydrolinalool and tetrahydrolinalool are formed as metabolites (Rahman, 1974)."

As taken from EFSA, 2011.

"Linalyl esters readily hydrolyse to linalool and the corresponding carboxylic acids following oral exposure. Hydrolysis is catalysed by carboxylesterases or esterases, in particular beta-esterases in the liver. The hydrolysis products then conjugate with glucuronic acid and are excreted in urine. Based on studies for linalyl acetate, it is expected that linalool is available for systemic circulation following oral administration of linalyl esters (OECD, 2002; Bickers et al., 2003a).

Following an inhalation exposure to linalyl acetate at 5 mg/L for 1 hour in mice, serum levels of linalyl acetate and linalool were reported to be 1–2 ng/mL and 4–5 ng/mL, respectively. In another inhalation study, mice exposed to linalyl acetate for 1 hour had serum levels of 1 ng/mL linalyl acetate and 4 ng/mL linalool. When the mice were exposed to 5 mg/mL lavender oil (37 % linalool and 42 % linalyl acetate), the serum levels were 3 ng/mL for linalool and 11 ng/mL for linalyl acetate (Bickers et al., 2003a).

In vitro studies of linalyl acetate have shown hydrolysis to linalool then, to some extent, rapid rearrangement to the ring-closed form α -terpineol. Linalyl acetate hydrolyses rapidly (half-life ($t_{1/2}$) < 5 min) at the low pH (acidic) of gastric fluids to form linalool and acetic acid. The hydrolysis rate of this chemical is slow in neutral gastric juice ($t_{1/2} = 121$ min) and in intestinal fluid with or without pancreatin ($t_{1/2} = 153$ – 198 min). Hydrolysis of this chemical in homogenates of rat intestinal mucosa, blood and liver is slower than in acidic gastric juice (Bickers et al., 2003a; HSDB)."

As taken from AICIS, 2018

Linalyl acetate was facilely hydrolyzed in water and simulated gastric and pancreatic fluids in an *in vitro* hydrolysis study. The mean half-lives for linalyl acetate hydrolysis were 5.5 and 52.5 min in gastric and pancreatic fluids, respectively (Hall 1979). In neutral gastric juice, linalyl acetate is slowly hydrolyzed ($t_{1/2}=121$ min) to a mixture of linalool and the ring-closed isomer α -terpineol (see Figure 1). In simulated gastric juice, linalyl acetate is rapidly hydrolyzed ($t_{1/2}<5$ min) to yield linalool, and this rapidly rearranges into α -terpineol (Hall 1979; Buck and Renwick 1998a). However, when incubated with intestinal fluid in the presence and absence of pancreatin, linalyl acetate was slowly hydrolyzed ($t_{1/2}=153$ to 198 min) (Buck and Renwick 1998b). Hydrolysis studies have shown that the hydrolysis of linalyl acetate is slower when incubated with homogenates of rat intestinal mucosa, blood, and liver (rate constant $k=0.01$ to $0.0055/\text{min}$) compared to the hydrolysis rate in acidic gastric juice ($k>5/\text{min}$). Based on the *in vitro* hydrolysis data, it can be concluded that linalyl acetate and other linalyl esters are hydrolyzed in gastric juice to yield linalool, which undergoes rapid ring-closure to yield α -terpineol.

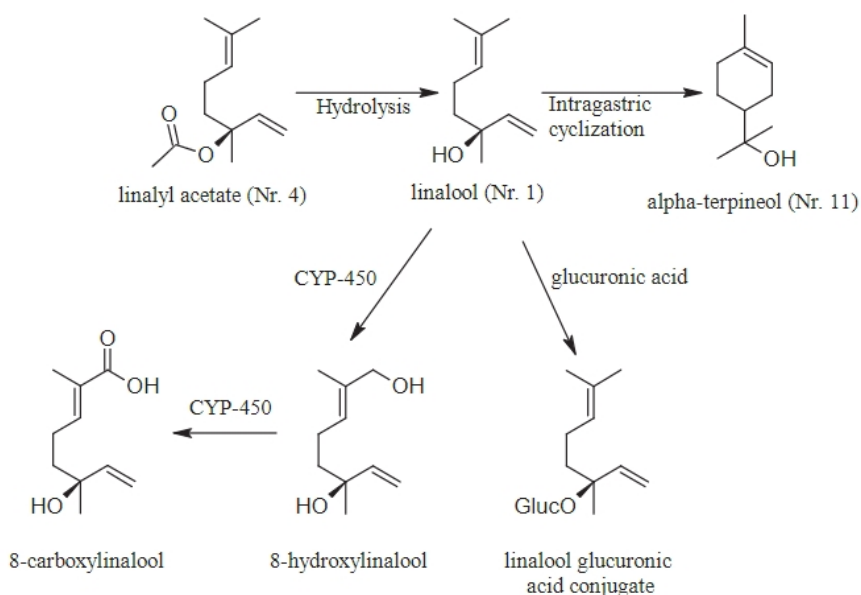


Figure 1–Metabolism of linalool in mammals.

As taken from Marnett LJ et. al. Journal of Food Science (2014). Available at: <https://ift.onlinelibrary.wiley.com/doi/epdf/10.1111/1750-3841.12407>

4.2. Absorption, distribution and excretion

“Linalyl acetate was absorbed quickly and trace amounts could be detected in the blood 5min after finishing” a massage performed on the abdomen of a 60kg male volunteer with 1500mg of lavender oil (containing 24.70% of linalool and 29.59% of linalyl acetate).

“Meyer and Meyer (1959) studied the skin absorption of linalyl acetat in 7 male mice. An area of 2.2 cm² on the shaved abdominal skin was used. Eserine (0.23%) was used as an indicator and the test material was used as a carrier for the Eserine. The latency period between application to the skin and the appearance of the Eserine effec tin the periodically stimulated masticatory muscles was used as a measure of the absorption rate. Linalyl acetate was absorbed in 52min.”

“Linalyl acetate did not enhance skin penetration of Rhodamine B in excised guinea pig skin.”

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

“It was determined that permeation of linalyl acetate through the porcine buccal mucosa is possible in vitro” Test substance: linalyl acetate, 26.8% as constituent of essential oil of *S. desoleana*“.

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

“After a massage performed on the abdomen of a 60kg male volunteer with 1500mg of lavender oil (containing 24.70% of linalool and 29.59% of linalyl acetate), “the peak plasma concentration was reached at 19min with a mean plasma concentration of 121 ng/mL. Most of the linalyl acetate disappeared from the blood 90min with a biological half-life of 14.3 min (Jager et al., 1992).”

“Blood levels of linalyl acetate were measured in mice exposed to either linalyl acetate or lavender oil during an associated pharmacology study. Groups of 4 outbred Swiss mice/group were placed in a light-barrier cage and exposed to air containing 5 mg/L of test material (concentration in cage air) for a 1-h period. Two experimental cages with 4 animals in each were simultaneously used for each experiment, one group inhaled the test compound and the second group remained untreated and acted as a control. Four extracted blood samples from each experiment (4 mice/measurement) were pooled and investigated by GC-FID chromatographic analysis. Blood samples (500 u1) were

taken from the retrobulbar venous plexus after 60 min of exposure. The serum linalyl acetate levels were 1-2 ng/mL after exposure to linalyl acetate. In animals exposed to lavender oil, which contained 37.3% linalool and 41.6% linalyl acetate, the serum linalyl acetate level was found to be 11 ng/mL (Buchbauer et al., 1991; Jirovetz et al., 1990; Jirovetz et al., 1991)."

"... Permeation of *Salvia desoleana* Atzei & Picci essential oil (linalyl acetate, 26.8%) through the porcine buccal mucosa is possible in vitro. /*Salvia desoleana* Atzei & Picci essential oil (linalyl acetate, 26.8%)/ [Organization for Economic Cooperation and Development; Screening Information Data Set for LINALYL ACETATE (115-95-7) p.53 (March 2002). Available from, as of July 14, 2008: **PEER REVIEWED**"

"The percutaneous absorption of a massage oil containing lavender oil was studied following application to the skin of a male subject (age 34 yr). Within 5 min after application, traces of linalool and linalyl acetate, the main constituents of lavender oil, could be detected in the blood. After 20 min, maximum concentrations of 100 ng/mL linalyl acetate and 121 ng/mL linalool were reached. Within 90 min most of the lavender oil was eliminated. It was concluded that lavender oil is rapidly absorbed through the skin and is excreted within 90 minutes. [Jager W et al; Journal of Soc Cosmet Chem 43: 49-54 (1992)] **PEER REVIEWED**"

As taken from HSDB, 2017

"The purpose of this study was to evaluate the in vitro cutaneous penetration of five terpenes--linalool, linalyl acetate, terpinen-4-ol, citronellol and alpha-pinene--applied in pure essential oils or in dermatological formulations (o/w emulsion, oily solution or hydrogel) containing 0.75 % w/w of the essential oils. Different skin absorption was observed depending on the type of the vehicle and terpenes' log P values. Cutaneous accumulation of terpenes is several times higher when they are applied in pure essential oils than in topical vehicles. No penetration of linalyl acetate from topical vehicles into viable skin was observed, but also for this terpene penetration to the upper layers of the stratum corneum was 2-times higher when an oily solution was used....". As taken from Cal K. 2006. *Planta Med.* 72(4), 311-316. PubMed, 2015

"In a percutaneous absorption case study, a massage oil containing lavender oil and peanut oil (2:98 ratio) was applied to the skin of a male subject (age 34 years). The lavender oil contained linalool and linalyl acetate at 24.79 % and 29.59 %, respectively. Following application within 5 minutes, linalool and linalyl acetate were found in trace amounts in the blood. Peak plasma concentrations of 121 ng/mL linalyl acetate and 100 ng/mL linalool were reached after 20 minutes. Most of the lavender oil had disappeared from the blood within 90 minutes with a biological half-life of 14.3 minutes. The authors concluded that lavender oil is rapidly absorbed through the skin and excreted within 90 minutes (Bickers et al., 2003a; HSDB).

The dermal permeability constants are reported to be 0.0746 cm/hour for linalyl acetate (CAS No. 115-95-7), 0.711 cm/hour for linalyl benzoate (CAS No. 126-64-7), 0.860 cm/hour for linalyl isovalerate (CAS No. 1118-27-0), and 0.882 cm/hour for linalyl phenylacetate (CAS No. 7143-69-3) (Galleria Chemica)."

As taken from AICIS, 2018

4.3. Interactions

In Swiss mice (ICR/Ha) dermal coapplication of linalyl acetate (3 mg in 0.1 mL acetone) with /5 ug/ benzo(a)pyrene /3 times weekly for 67 wk/ did slightly increase the number of skin papillomas and carcinomas compared to benzo(a)pyrene controls

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

"The sedative properties of the essential oil of Lavender (*Lavandula angustifolia* Miller) and of its main constituents--linalool and linalyl acetate--were investigated in mice followed up in a series of experimental procedures. The significant decrease in the mobility of female and male laboratory animals under standardized experimental conditions is found to be closely dependent on the exposure time to the drugs. Nevertheless after an injection of caffeine into mice a hyperactivity was observed which was reduced to nearly a normal mobility only by inhalation of these fragrance drugs. ... [Buchbauer G et al; Z Naturforsch (C) 46 (11-12): 1067-72 (1991)] **PEER REVIEWED** PubMed Abstract"

As taken from HSDB, 2017

"BACKGROUND: Smoking is a risk factor for cardiovascular diseases as well as pulmonary dysfunction. In particular, adolescent smoking has been reported to have a higher latent risk for cardiovascular disease. Despite the risk to and vulnerability of adolescents to smoking, the mechanisms underlying the effects of acute nicotine exposure on adolescents remain unknown. This study therefore evaluated the mechanism underlying the effects of linalyl acetate on cardiovascular changes in adolescent rats with acute nicotine exposure. METHODS: Parameters analyzed included heart rate (HR), systolic blood pressure, lactate dehydrogenase (LDH) activity, vascular contractility, and nitric oxide levels. RESULTS: Compared with nicotine alone, those treated with nicotine plus 10 mg/kg ($p=0.036$) and 100 mg/kg ($p=0.023$) linalyl acetate showed significant reductions in HR. Moreover, the addition of 1 mg/kg ($p=0.011$), 10 mg/kg ($p=0.010$), and 100 mg/kg ($p=0.011$) linalyl acetate to nicotine resulted in significantly lower LDH activity. Nicotine also showed a slight relaxation effect, followed by a sustained recontraction phase, whereas nicotine plus linalyl acetate or nifedipine showed a constant relaxation effect on contraction of mouse aorta ($p<0.001$). Furthermore, nicotine-induced increases in nitrite levels were decreased by treatment with linalyl acetate ($p<0.001$). CONCLUSIONS: Taken together, our findings suggest that linalyl acetate treatment resulted in recovery of cell damage and cardiovascular changes caused by acute nicotine-induced cardiovascular disruption. Our evaluation of the influence of acute nicotine provides potential insights into the effects of environmental tobacco smoke and suggests linalyl acetate as an available mitigating agent." As taken from Kim JR et al. 2017. Environ. Health Prev. Med. 22(1), 42. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29165169>

"Olmesartan-associated enteropathy (OAE) is a life-threatening pathological condition, but its underlying mechanisms have not been elucidated. Although intestinal hypermotility is frequently accompanied by chronic diarrhea, there have been no studies of olmesartan-induced hypermotility. Intestinal motility should be well regulated by the enteric nervous system, but degeneration of enteric neurons has been reported in patients with chronic diarrheal diseases, such as irritable bowel syndrome, suggesting a connection between OAE and intestinal hypermotility. In this study, interference with this inhibitory pathway was analyzed in a model of olmesartan-induced intestinal hypermotility (OIH) in rats with nicotine-induced hypertension exposed to chronic immobilizing stress. The effects of the potent inhibitory neurotransmitters norepinephrine (NE) and sodium nitroprusside (SNP), which act via different pathways, were assessed ex vivo, with only NE-modulated frequency and amplitude of spontaneous contractions found to be elevated in OIH rat jejunum. Clinical symptoms frequent in OAE, including atrophy of the intestinal epithelium and weight loss, were observed in these rats. Interestingly, olmesartan significantly elevated heart rate while lowering blood pressure in OIH rats. These abnormal conditions were prevented by adding linalyl acetate (LA), while the blood pressure-lowering effects of olmesartan were maintained. These findings suggest that olmesartan induces intestinal hypermotility by interfering with the sympathetic inhibitory pathway, and reduces epithelial cell size or body weight in hypertensive rats. As LA prevented these effects, combination treatment with olmesartan plus LA may provide better antihypertensive efficacy without inducing OAE." As taken from Kwon S et al. 2018. Biomed. Pharmacother. 102, 362-368. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29571021>

5. Toxicity

5.1. Single dose toxicity

Species	Route	Dose data (LD ₅₀)
Rat	Oral	10 ml/kg bw
Rat	Oral	14550 mg/kg bw
Mouse	Oral	13360 mg/kg bw
Mouse	Oral	13540 mg/kg bw
Rabbit	Dermal	5000 mg/kg bw
Rat	Intraperitoneal	2864 mg/kg bw
Mice	Intraperitoneal	0.8 mL/kg bw

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

“Inhalation mammals LC50: 2740 mg/m³”

“The observed effects upon acute oral toxicity are depression, coma and wet posterior.”

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

Acute oral LD50 in rat was 14.55g/kg and in mice it was 13.36 (Jenner, 1964).

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
mouse	LD50	oral	12gm/kg (12000mg/kg)	BEHAVIORAL: GENERAL ANESTHETIC BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) BEHAVIORAL: ATAXIA	Toksikologicheskii Vestnik. Vol. (5), Pg. 41, 1994.
rat	LD50	oral	13934mg/kg (13934mg/kg)	BEHAVIORAL: GENERAL ANESTHETIC BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) BEHAVIORAL: ATAXIA	Toksikologicheskii Vestnik. Vol. (5), Pg. 41, 1994.

As taken from ChemIDplus, available via PubChem

LD50 Rat oral 14,550 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. 2232]

PEER REVIEWED

LD50 Rat ip 2864 mg/kg (95% C.I. 2414- 3399 mg/kg) [Letizia CS et al; Food Chem Toxicol 41 (7): 965-76 (2003)] **PEER REVIEWED**

LD50 Rat (female) ip 2984 mg/kg (95% C.I. 2293-3884 mg/kg) [Letizia CS et al; Food Chem Toxicol 41 (7): 965-76 (2003)] **PEER REVIEWED**

LD50 Rat (male) ip 2778 mg/kg (95% C.I. 2246-3435 mg/kg) [Letizia CS et al; Food Chem Toxicol 41 (7): 965-76 (2003)] **PEER REVIEWED**

LD50 Mouse oral 13,360 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. 2232] **PEER REVIEWED**

LD50 Rabbit dermal > 5000 mg/kg [USEPA/HPVIS; Detailed Chemical Results on 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate (115-95-7): Acute Toxicity for Mammalian Health Effects SIDS. Available from, as of July 14, 2008: <http://www.epa.gov/hpvis/index.html> **PEER REVIEWED**

"Acute Exposure/ Male and female Sprague-Dawley rats (5/sex/dose) received a single i.p. injection of 1120, 1590, 2240, 3160 or 4470 mg/kg linalyl acetate in corn oil. The initial body weights of the rats ranged from 233 to 297 g. Mortality and/or systemic observations were recorded 1, 3 and 6 h post dose and subsequently once daily for 14 days. Mortality and moribundity were recorded twice daily. Gross necropsy was performed on all animals. No deaths were observed at the three lowest dose levels; 7/10 deaths were observed at 3160 mg/kg and 10/ 10 deaths were observed at 4470 mg/kg. The combined LD50 was calculated to be 2864 mg/kg (95% C.I. 2414-3399 mg/kg; the LD50 in male rats was calculated to be 2778 mg/kg (95% C.I. 2246-3435) and the LD50 in female rats was calculated to be 2984 mg/kg (95% C.I. 2293-3884). Clinical signs observed in all dose groups included depression, ataxia, urine stains, rough coat and soft feces. Labored respiration, wheezing, hunched position, and red stains on the nose and/or eyes were observed at the two highest dose groups; prostration, tremors and lacrimation were also observed at the highest dose level. Necropsy revealed discolored lungs, thickened lobes in the liver and a thick white material adhered to and covering the liver and spleen. In addition, discolored liver, kidneys and adrenals and abnormal contents in the intestines, abdominal cavity and thoracic cavity were also observed in the animals that died during the study. [Letizia CS et al; Food Chem Toxicol 41 (7): 965-76 (2003)] **PEER REVIEWED**"

As taken from HSDB, 2017

Type of Test	Route of Exposure or Administration	Species /Test system	Dose Data	Toxic Effects	Reference
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	13934 mg/kg	Behavioral - general anesthetic Behavioral - somnolence (general depressed activity) Behavioral - ataxia	Toksikologicheskii Vestnik. (18-20 Vadkovskii per. Moscow, 101479, Russia) History Unknown (5),41,1994

LD50 - Lethal dose, 50 percent kill	Oral	Rodent - mouse	12000 mg/kg	Behavioral - general anesthetic Behavioral - somnolence (general depressed activity) Behavioral - ataxia	Toksikologicheskii Vestnik. (18-20 Vadkovskii per. Moscow, 101479, Russia) History Unknown (5),44,1994
TCLo - Lowest published toxic concentration	Inhalation	Rodent - rat	193 mg/m ³ /4H	Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol)	Toksikologicheskii Vestnik. (18-20 Vadkovskii per. Moscow, 101479, Russia) History Unknown (5),44,1994
TDLo - Lowest published toxic dose	Subcutaneous	Rodent - rat	64 mg/kg	Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	Phytomedicine. (Gustav Fischer Verlag, Postfach 720143, D-70577 Stuttgart, Germany) V.1- 1994- 9,721,2002
LD50 - Lethal dose, 50 percent kill	Administration onto the skin	Rodent - rabbit	>5000 mg/kg	Details of toxic effects not reported other than lethal dose value	U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Terpenoid Tertiary Alcohols and Related .pdf http://www.epa.gov/HPV/pubs/summaries/terestr/c12930tc.htm -,2001
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	14550 mg/kg	Behavioral - somnolence (general	U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Terpenoid Tertiary Alcohols and Related .pdf http://www.epa.gov/HPV/pubs/summaries/terestr/c12930tc.htm -,2001

				depressed activity) Behavioral - coma	

As taken from RTECS, 2018

“Based on animal test results, the chemicals have low acute oral toxicity following oral exposure.

The reported median lethal dose (LD50) values are (Bickers et al., 2003b; Galleria Chemica):

linalyl acetate (CAS No. 115-95-7), 10000 mg/kg bw in rats and 13360 mg/kg bw in mice.”

“Based on animal test results, the chemicals have low acute toxicity following dermal exposure.

The reported dermal LD50 values in rabbits are (Bickers et al., 2003b; Galleria Chemica):

linalyl acetate (CAS No. 115-95-7), >5000 mg/kg bw.”

As taken from AICIS, 2018

5.2. Repeated dose toxicity

The margin of exposure for linalyl acetate is adequate for the repeated dose toxicity endpoint at the current level of use... To account for bioavailability following dermal application, the data from an in vitro dermal absorption study (RIFM, 2007a; see Section 4) with linalool were used to revise the NOAEL of 250 mg/kg/day to reflect the systemic dose. At a dermal penetration of 14.4% of the applied dose, the revised repeated dose toxicity NOAEL from the dermal study is 36 mg/kg/day. Therefore, the MOE is equal to the linalool NOAEL in mg/kg/day divided by the total systemic exposure, 36/0.078 or 462. (Api et al., 2015).

The margin of exposure for linalyl acetate is adequate for the respiratory endpoint at the current level of use... The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. There are no inhalation data on linalyl acetate. However, in a 2 week acute inhalation study a NOAEC of 10 ppm (63 mg/m³) for metabolite linalool (CAS # 78-70-6) was reported (RIFM, 2012), which was the highest dose tested. The test substance-related effects were limited to nonadverse microscopic findings in the nasal cavity... Based on the IFRA survey results for hydroalcohols, the 97.5th percentile was reported to be 13%. Assuming the same amount is used in all product types (fine fragrances, hair sprays, antiperspirants/deodorants, candles, aerosol air fresheners, and reed diffusers/heated oil plug-ins), the combined inhalation exposure would be 1.21 mg/day as calculated based on the IFRA survey results for the 97.5th percentile use in hydroalcohols for a 60 kg individual using RIFM's 2-Box/MPPD in silico models. To compare this estimated exposure with that of Randazzo et al. expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew et al., 2009) to give 1.86 mg/kg lung weight/day resulting in an MOE of 1297 (i.e., [2412.5 mg/kg lw/day]/[1.86 mg/kg lung weight/day]). Since the MOE is greater than 100, the material exposure, by inhalation, at 13% in a combination of the products noted above, is deemed to be safe under the most conservative consumer exposure scenario. (Api et al., 2015).

“Linalyl acetate was tested in a 90-day study (RIFM, 1958). The test material was a mixture of materials containing linalyl acetate (24.2 mg/kg), linalyl isobutyrate and geranyl acetate. The materials were blended in the proportion of their use levels and incorporated into the basal diet to provide 100 mg/kg bodyweight of the mixture. Each diet was fed ad libitum to a group of 20 rats

(10/sex) with initial body weights of 50-70g. Records were made of physical appearance and behavior and the efficiency of food utilization (EFU) was calculated for each group. After 13 weeks, urinalysis was conducted on 6 animals (3/sex) and blood hemoglobin levels were determined. All animals were sacrificed after 90 days and gross necropsy was carried out. Blood hemoglobin, urinalysis, body weight, liver and kidney weights, food intake, EFU, behavior and appearance were normal in the males. Growth, food intake and food utilization were slightly depressed in the females.”

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

“Crl:CD/BR rats received 160, 400 or 1000 mg/kg bw linalool during 28 days. One male and one female of the high dose group were found dead. Total protein/albumin were increased in males at 400 mg/kg bw and in both sexes at 1000 mg/kg bw. Calcium was increased at 1000 mg/kg bw in males only. Serum glucose levels were decreased in males at 400 and 1000 mg/kg bw. Liver weight was increased dose related and significantly at 400 and 1000 mg/kg bw. Kidney weight was increased in males at 400 mg/kg bw (relative kidney weight) and in all animals at 1000 mg/kg (absolute). Macroscopically this was accompanied by thickened liver lobes and pale areas on the kidneys. All treated female groups showed hepatocellular cytoplasmic vacuolisation while the highdose males had an increase in degenerative lesions in the renal cortex. Thickening of the stomach mucosa with concomitant lesions in the nonglandular part of the stomach, with some erosion, subacute inflammation and acanthosis were reported in middle- and high-dose animals. The NOAEL derived was 160 mg/kg bw based on effects in liver and kidney.”

“In a study in rats to establish effects of linalool on drug metabolising enzymes, increased liver weight was reported on day 64. Rats received 500 mg/kg bw linalool by gavage during 64 days. Microsomal protein was increased from day 20 onwards. Cytochrome P450 and cytochrome b5 showed biphasic response (initial depression followed by an increase).”

“The ADI for linalyl acetate is of 0.25 mg/kg bw.”

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

Type of Test	Route of Exposure or Administration	Species/ Test system	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	3750 mg/kg/5D (intermittent)	Related to Chronic Data - death	U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Terpenoid Tertiary Alcohols and Related .pdf http://www.epa.gov/HPV/pubs/summaries/tertestr/c12930tc.htm -,2001

As taken from RTECS, 2018

“*Ascaris lumbricoides* is the most prevalent soil-transmitted helminth (STH) infection of human beings worldwide. Chemotherapy with synthetic anthelmintics such as albendazole, mebendazole,

and pyrantel pamoate is the current method of treatment; however, the emergence of anthelmintic resistance could substantially decrease the efficacy of such treatments and the sustainability of STH control programs. Additionally, benzimidazoles are not recommended for pregnant women or children under age one. A blinded, controlled study was conducted to evaluate the efficacy of two microencapsulated, plant-based essential oil blends, TTN1013 (α -pinene, linalyl acetate, p-cymene, and thymol octanoate) and TTN1014 (α -pinene, linalyl acetate, p-cymene, and thymol acetate) as functional foods against *Ascaris suum* infection in pigs, an important pathogen that closely resembles human infections with *A. lumbricoides*. Four groups of 16 female, 21-24 day old, Yorkshire-cross pigs were treated daily with 0.5 or 1.0mg/kg TTN1013, 1.0mg/kg TTN1014, or 1.0mg/kg equivalent of empty capsules, delivered inside a cream-filled sandwich cookie for 14 weeks. All pigs remained clinically normal and showed no signs of reduced intestinal health for the duration of treatment. Based on these results, TTN1013 shows promise as a daily supplement to reduce infection burdens of soil transmitted helminths in both pigs and human beings." As taken from Kaplan RM et al. 2014. *Acta Trop.* 139, 15-22. PubMed, 2015

5.3. Reproduction toxicity

The margin of exposure for linalyl acetate is adequate for the developmental and reproductive toxicity endpoints at the current level of use... There are no developmental toxicity data on linalyl acetate. Metabolite linalool (CAS # 78-70-6) has a gavage developmental toxicity study conducted in rats, which concluded a NOAEL of 1000 mg/kg/day for developmental toxicity, the highest dosage tested (Politano et al., 2008). Therefore, the MOE for developmental toxicity is equal to the linalool NOAEL in mg/kg/day divided by the total systemic exposure, 1000/0.078 or 12821. (Api et al., 2015).

"An essential oil of coriander containing 72.9% of natural linalool was administered to 10 female rats/treatment from day 7 before mating with untreated males until day 4 after parturition. The dosage levels were 250, 500 and 1000 mg/kg bw. No effects on female fertility became apparent. All treated females showed an increased body weight gain and food consumption during gestation compared to control animals. In the high dose group a decrease of body weight and food consumption was seen during the pre-mating period. Salivation was apparent in females at 500 and 1000 mg/kg. One or two females at the high dose group developed ataxia and/or decreased motor activity. At 1000 mg/kg bw females delivered significantly less live pups than in the other dose groups. During the lactation period pup morbidity and mortality was increased. The NOAEL for maternal toxicity based on clinical signs and effects on body weight and food consumption was 500 mg/kg bw (equivalent to 464 mg/kg bw linalyl acetate). The NOAEL on reproduction toxicity and developmental toxicity is 500 mg/kg bw (equivalent to 464 mg/kg bw linalyl acetate), based on the decreased litter size at birth and morbidity/mortality thereafter."

"From the 28-day repeated dose toxicity study with the same essential oil of coriander, no remarkable effects on the primary reproductive organs in both females (ovaries and uteri) and males (testes and epididymides) were noted in any animal from any dosage group up to 1000 mg/kg bw, both macroscopically at dissection and also microscopically during histopathology of every single (10 male, 10 female) high-dose animal."

"The reproductive and developmental toxicity of linalyl acetate has been tested in CrI:CD(SD)BR female rats fed via gavage. "Female rats were mated with untreated stock males (1/1) from the same strain and source. The day of observation of a vaginal plug or vaginal smear was defined as day 0 of gestation. Females were treated daily from 7 days prior to and subsequently through cohabitation, gestation, delivery and a 4-day lactation period. All dams were observed for clinical signs, death and/or delivery of a litter. Body weights were recorded during the whole dosage period. Feed consumption was determined on days 0, 6, 10, 14, 16, 20 and 25 of presumed gestation as well as on days 1 and 4 of lactation. On day 4, all females were subjected to macroscopic examination. All litters were examined for number, viability, body weight, sex ratio and external

anatomy of the pups. Delivered pups were also examined for viability, clinical observations and body weight during the 4-day postpartum period. NOAEL for maternal toxicity is 500 mg/kg/d based on body weight gain, feed consumption and clinical signs (equivalent to ~464 mg/kg bw linalyl acetate). NOAEL for developmental/reproductive toxicity is 500 mg/kg based on increased fetal mortality in utero and after parturition (equivalent to ~464 mg/kg bw linalyl acetate).” As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

5.4. Mutagenicity

Based on the current existing data and use levels, linalyl acetate does not present a concern for genetic toxicity... Additionally, the RIFM Expert Panel has reviewed linalyl acetate and other esters related to linalool and concluded that, based on a weight of evidence, this category of materials does not have significant genotoxic potential (Bickers et al., 2003). Taken together, linalyl acetate does not have potential to be genotoxic. (Api et al., 2015)

“Present letter is aimed at clarifying some critical points highlighted by Api et al. as major limits for the reliability of our data on the genotoxicity of linalyl acetate. Particularly, we explained the choice of the study design and methodology applied for carrying out the Ames test and the micronucleus assay.” As taken from Di Sotto A and Mazzanti G. 2016. Food Chem. Toxicol. 97S, S237-S239. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/26690052>

The Ames test was used to evaluate the mutagenicity of a number of neat complex flavor mixtures. Studies in which linalyl acetate was part of the test mixture include EMT000221 (CD-ROM 1, JTI Submission, 2002). The results show that these mixtures were not mutagenic.

“In an Ames assay (Ames et al., 1975) using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 with and without S9 activation, doses of 100-25,000 nL/plate linalyl acetate in ethanol were not mutagenic. The positive control for the studies conducted with S9 activation was 2-aminoanthracene. For studies conducted without activation, the positive controls were sodium azide (strains TA1535 and TA100), 2-nitrofluorene (strains TA1538 and TA98) and quinacrine mustard (strain TA1537) (RIFM 1987; Heck et al., 1989).”

“In a rec assay using *Bacillus subtilis* strains H17 (rec+) and M45 (rec-), a dose of 18 ug/disk linalyl acetate in dimethyl sulfoxide (DMSO) had no effect (Oda et al., 1979).”

“It was found that linalyl acetate was not clastogenic in human lymphocytes.”

“An in vitro unscheduled DNA synthesis (UDS) assay was conducted in primary rat hepatocytes at doses up to 300 nL/mL. The positive control was DMBA at 3 nL/mL and 10 nL/mL. No increases in net nuclear gain counts were noted. Linalyl acetate did not show genotoxic potential based on the negative results in the in vitro UDS assay (Heck et al., 1989).”

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

“Linalyl acetate does not induce gene mutations or chromosomal effects in vitro.”

“An Ames test was performed with linalyl acetate in *Salmonella* strains TA97, TA98, TA100, TA1535 and TA102 with and without metabolic activation. Linalyl acetate was found to be not mutagenic in this assay”.

“In a chromosome aberration test with human lymphocytes, linalyl acetate did not induce aberrations, both with and without S-9 mix”.

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7).

In vivo					
Species	Test conditions	Endpoint	Result	Reference	
No data were identified specifically on linalyl acetate. However, the following study on linalool (the parent alcohol) can be considered relevant to an assessment of the in vivo genotoxicity of linalyl acetate.					
Mouse, groups of 5 per sex (strain CD-1)	Single gavage administration of up to 1.5 g/kg bw (a dose that produced overt toxicity). Bone marrow cells scored 24 or 48 hr later for micronuclei.	Chromosome damage	negative	RIFM, 2001 (cited in Letizia et al. 2003a)	
[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]					
In vitro					
Test system	Test conditions	Endpoint	Activation	Result	References
Human lymphocytes	Cells treated with linalyl acetate in the absence of S9 (for 3 hr with fixation at 24 hr, or 24- and 48-hr continuous), with up to 130 ug/ml and in the presence of S9 (for 3 hr with fixation at 24- or 48-hr), with up to 180 ug/ml. Metaphase cells (100) examined for chromosomal aberrations.	Chromosome damage	With and without S9	negative [an increase at one low dose with 48-hr continuous exposure was within the historical control range and not considered biologically relevant.]	RIFM, 2000
Rat liver cells	Linalyl acetate tested at up to 300 ug/ml. Cells examined for unscheduled DNA synthesis (an indirect measure of DNA damage).	DNA damage	Not applicable	negative	Heck et al.1989 [additional information given in JECFA, 1999].
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA1538	Linalyl acetate tested at up to 25 mg/plate.	Mutation	With and without S9	negative	Heck et al.1989 [additional information given in JECFA, 1999].
Bacillus subtilis H17 (rec+) and	Linalyl acetate tested in a rec assay (measuring differential toxicity) at up to 18 ug/disc. Toxicity	DNA damage (as indicated by differential	Without	No verdict (as toxicity was not seen in either	Oda et al. 1978

M45 (rec-)	was not achieved in either strain.	toxicity)		strain)	
[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]					

In addition to the above studies on linalyl acetate, the parent alcohol (linalool) has been tested in several in vitro assays. Details are given in the Linalool Ingredient Profile. Briefly, linalool did not induce chromosome aberrations in Chinese hamster ovary cells in the absence of activation (Ishidate et al. 1984), DNA damage in rat liver cells (Heck et al. 1989, with additional data from JECFA, 1999), or Sister Chromatid Exchanges in hamster ovary cells (Sasaki et al. 1989). Linalool also failed to induce mutations in several Ames tests that included a wide variety of *S. typhimurium* bacterial strains, with and without S9 (Heck et al. 1989; JECFA, 1999; NTP) or in *Escherichia coli* (Yoo, 1986). A positive result was obtained in a mouse lymphoma mutation assay when S9 was included (Heck et al. 1989). However, it was suggested that polar substances can induce “false positives” in this assay when S9 is present, as a result of physiological changes in the culture medium (Bickers et al. 2003) and no activity was seen when this assay was repeated, accounting for osmolality, cytotoxicity and pH (RIFM, 1994). A rec assay (measuring differential toxicity) gave indirect evidence of an ability to damage bacterial DNA (Yoo, 1986).

“/GENOTOXICITY/ An in vitro unscheduled DNA synthesis (UDS) assay was conducted in primary rat hepatocytes at doses up to 300 nL/mL. The positive control was dimethylbenz (a) anthracene at 3 nL/mL and 10 nL/mL. No increases in net nuclear grain counts were noted. Linalyl acetate did not show genotoxic potential based on the negative results in the in vitro UDS assay. [Letizia CS et al; Food Chem Toxicol 41 (7): 965-76 (2003)] **PEER REVIEWED**”

“/GENOTOXICITY/ The mutagenic ... effects of ... linalyl acetate ... were evaluated by the bacterial reverse mutation assay on *Salmonella typhimurium* TA98 and TA100, and on *Escherichia coli* WP2uvrA strains. ... Linalyl acetate induced a statistically significant increase in the number of revertant colonies in WP2uvrA, both with and without S-9 mixture. ... [Di Sotto A et al; Mutat Res 653 (1-2): 130-3 (2008)] **PEER REVIEWED** PubMed Abstract”

“/GENOTOXICITY/ An Ames test was performed with linalyl acetate in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA102 with and without metabolic activation. Linalyl acetate was found to be not mutagenic in this assay ... [Organization for Economic Cooperation and Development; Screening Information Data Set for LINALYL ACETATE (115-95-7) p.13 (March 2002). Available from, as of July 14, 2008:**PEER REVIEWED**”

“/GENOTOXICITY/ /In a bacterial reverse mutation assay/ linalyl acetate /at 25,000 ug/plate/ was inactive in *Salmonella* strains TA1535, TA1537, TA1538, TA98 & TA100 both in the presence and absence of metabolic activation system. [USEPA/HPVIS; Detailed Chemical Results on 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate (115-95-7): Genetic Toxicity in vitro for Mammalian Health Effects SIDS. Available from, as of July 14, 2008: <http://www.epa.gov/hpvis/index.html> **PEER REVIEWED**”

“/GENOTOXICITY/ The hepatocytes were isolated from adult male /Sprague-Dawley or Fischer/ rats and incubated for 18-20 hours with /300 ug linalyl acetate/ in serum-free /Williams' medium E/ (WME) medium containing 5-10 uCi/mL (3)H-TdR. Unscheduled DNA synthesis (UDS) was measured by electronically counting nuclear grains and subtracting the average number of grains in 3 adjacent nuclear sized cytoplasmic areas. ... Linalyl acetate was not shown to be clastogenic

under the test conditions. [USEPA/HPVIS; Detailed Chemical Results on 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate (115-95-7): Genetic Toxicity in vitro for Mammalian Health Effects SIDS. Available from, as of July 14, 2008: <http://www.epa.gov/hpvis/index.html> **PEER REVIEWED**

“/GENOTOXICITY/ /In a DNA damage or repair assay/ no evidence of mutagenicity was detected /with 18 ug/disk linalyl acetate in/ Bacillus subtilis H17 Rec+ or M45 Rec-. ... No difference in zones of inhibition for H17 and M45. [USEPA/HPVIS; Detailed Chemical Results on 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate (115-95-7): Genetic Toxicity in vitro for Mammalian Health Effects SIDS. Available from, as of July 14, 2008: <http://www.epa.gov/hpvis/index.html> **PEER REVIEWED**”

As taken from HSDB, 2017

“The potential genotoxicity of lavender essential oil and its major components, linalool, and linalyl acetate, was evaluated in vitro by the micronucleus test on peripheral human lymphocytes. In the range of non-toxic concentrations (0.5-100 µg/ml), linalyl acetate increased the frequency of micronuclei significantly and in concentration-dependent manner; lavender oil did so only at the highest concentration tested, whereas linalool was devoid of genotoxicity. None of the tested substances led to an increase in nucleoplasmic bridges or nuclear buds frequency. These findings suggest that the mutagenic activity of lavender oil can be related to the presence of linalyl acetate, which seems to have a profile of an aneugenic agent”. As taken from Di Sotto A et al. 2011. Environmental and Molecular Mutagenesis 52(1), 69-71. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/20839219>

Type of Test	Route Exposure or Administration	Species/Test System	Dose Data	Reference
Mutation in microorganisms		Bacteria - Escherichia coli	9 mg/plate	MUREAV Mutation Research. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1964- Volume(issue)/page/year: 653,130,2008

As taken from RTECS 2018

“Based on the available in vitro data for the linalyl esters, and the data available for linalool, the chemicals in this group are not considered genotoxic.

In vitro

The following results were reported for linalyl acetate (OECD, 2002; HSDB; REACHa):

negative in bacterial reverse mutation assay (OECD TG 471) with several strains of Salmonella (S.) typhimurium (TA97, TA98, TA100, TA1535, TA1537, TA1538 and TA102) up to 25000 µg/plate, with or without metabolic activation;

negative in Escherichia coli strain WP2uvrA (trp-) at doses of 125–1000 µg/plate, with or without metabolic activation; but positive at doses of 9 mg/plate and above. The authors concluded that linalyl acetate was cytotoxic at doses >9 mg/plate, and exceeded the regulatory requirement of 5 mg/plate;

negative in Bacillus subtilis H17 (rec+) and M45 (rec-) at 18 µg/plate (Marnett et. al., 2014);

no induction of chromosome aberrations (OECD TG 473) in human peripheral blood lymphocytes at doses up to 130 µg/mL without metabolic activation, and up to 180 µg/mL with metabolic activation; and

negative in unscheduled DNA synthesis (UDS) assays in primary rat (Fischer or SD) hepatocytes at doses up to 300 µg/mL, without metabolic activation.”

As taken from AICIS, 2018

“Salmonella typhimurium, TA97, TA98, TA100, TA1535 and TA102. Metabolic activation Male rat liver S9 mix (phenobarbital/β-naphthoflavone induced). Test concentration 5, 15, 50, 150 and 500 µg/plate or tube (based on toxicity), test performed with and without metabolic activation. Linalyl acetate was not mutagenic in this assay.”

As taken from ChemView, 2023

When incubated with *Bacillus subtilis* H17 (rec+) and M45(rec–), linalool, linalyl acetate, and terpinyl acetate were negative in the rec assay at 17, 18, and 19 µg, respectively (Oda and others 1978). (...) No chromosomal aberrations were observed when 0, 10, 33, 56, 100, 130, or 180 µg/mL of linalyl acetate (Nr. 4) were incubated with human peripheral lymphocytes for 3 h with 24- and 48-h fixation times, with and without metabolic activation (Bertens 2000). Linalyl acetate did not induce UDS in Fischer or SD rat hepatocytes at concentrations up to 300 nL/mL (Heck and others 1989).

FLavorin g agent	End point	Test object	Concentratio n	Results	Reference
Linalyl acetate	Reverse mutation	Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100	25000 nL/plate	Negative (a)	(Heck and others 1989)
Linalyl acetate	Unscheduled DNA synthesis	Fischer or SD rat hepatocytes	300 nL/mL	Negative	(Heck and others 1989)
Linalyl acetate	Mutation	<i>Bacillus subtilis</i> H17(rec+) and M45(rec–)	18 µg	Negative	(Oda and others 1978)
Linalyl acetate	Chromosomal aberration	Human peripheral blood lymphocytes	180 µg/mL	Negative	(Bertens 2000)

(a) With and without metabolic bioactivation system, S-9

As taken from Marnett LJ et. al. Journal of Food Science (2014). Available at:

<https://ift.onlinelibrary.wiley.com/doi/epdf/10.1111/1750-3841.12407>

5.5. Cytotoxicity

Lavender (*Lavandula angustifolia*) oil, chiefly composed of linalyl acetate (51%) and linalool (35%), is considered to be one of the mildest of known plant essential oils and has a history in wound healing. Concerns are building about the potential for irritant or allergenic skin reactions with the use of lavender oil. This study has demonstrated that lavender oil is cytotoxic to human skin cells in vitro (endothelial cells and fibroblasts) at a concentration of 0.25% (v/v) in all cell types tested (HMEC-1, HNDF and 153BR). The major components of the oil, linalyl acetate and linalool, were also assayed under similar conditions for their cytotoxicity. The activity of linalool reflected that of the whole oil, indicating that linalool may be the active component of lavender oil. Linalyl acetate cytotoxicity was higher than that of the oil itself, suggesting suppression of its activity by an unknown factor in the oil. Membrane damage is proposed as the possible mechanism of action.

As taken from Prashar et al.; Cytotoxicity of lavender oil and its major components to human skin cells; Cell Prolif. 2004, Jun; 37(3):221-9.

“Bergamot (*Citrus bergamia*, Risso et Poiteau) essential oil (BEO) is a widely used plant extract showing anxiolytic, analgesic and neuroprotective effects in rodents; also, BEO activates multiple death pathways in cancer cells. Despite detailed knowledge of its chemical composition, the constituent/s responsible for these pharmacological activities remain largely unknown. Aim of the present study was to identify the components of BEO implicated in cell death. To this end, limonene, linalyl acetate, linalool, γ-terpinene, β-pinene and bergapten were individually tested in

human SH-SY5Y neuroblastoma cultures at concentrations comparable with those found in cytotoxic dilutions of BEO. None of the tested compounds elicited cell death. However, significant cytotoxicity was observed when cells were cotreated with limonene and linalyl acetate whereas no other associations were effective. Only cotreatment, but not the single exposure to limonene and linalyl acetate, replicated distinctive morphological and biochemical changes induced by BEO, including caspase-3 activation, PARP cleavage, DNA fragmentation, cell shrinkage, cytoskeletal alterations, together with necrotic and apoptotic cell death. Collectively, our findings suggest a major role for a combined action of these monoterpenes in cancer cell death induced by BEO.” As taken from Russo R et al. 2013. *Fitoterapia* 89, 48-57. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23707744>

“Bergamot (*Citrus bergamia*, Risso et Poiteau) essential oil (BEO) is a well characterized, widely used plant extract. BEO exerts anxiolytic, analgesic and neuroprotective activities in rodents through mechanisms that are only partly known and need to be further investigated. To gain more insight into the biological effects of this essential oil, we tested the ability of BEO (0.005-0.03%) to modulate autophagic pathways in human SH-SY5Y neuroblastoma cells. In order to identify the active constituents responsible for these effects, the two most abundant monoterpenes found in the essential oil, d-limonene (125-750 μ M) and linalyl acetate (62.5-375 μ M), were individually tested at concentrations comparable to those found in 0.005-0.03% BEO. Linalyl acetate was ineffective in replicating BEO effects; however, it greatly enhanced LC3 lipidation triggered by D-limonene.” As taken from Russo R et al. 2014. *PLoS One* 9(11), e113682. PubMed

“In Algeria, *Myrtus communis* L. is distributed throughout the Tell Atlas and the coastal regions of Algiers and Constantine. The leaves are used in respiratory disorders, diarrhea and hemorrhoids. The aims of this work were to evaluate the antifungal and anti-inflammatory potential of well characterized essential oils (EO). Since EO can be applied by inhalation, dermal application and oral administration, we used several mammalian cell lines to assess safe bioactive doses. The chemical composition of two samples was investigated by GC-FID, GC-MS and (^{13}C) NMR spectroscopy. Monoterpene derivatives are the main compounds: α -pinene (50.8 and 33.6%), 1,8-cineole (21.9 and 13.3%), linalool (2.7 and 14.8%), and linalyl acetate (0.5 and 9.5%). The antifungal evaluation revealed that the oils were more active against *Cryptococcus neoformans* (yeast) and *Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton rubrum* (dermatophytes). The anti-inflammatory potential was evaluated using an in vitro model of lipopolysaccharide (LPS)-stimulated macrophages. Assessment of cell viability was made through the MTT assay. Both oils were able to significantly inhibit NO production, without affecting cell viability, in concentrations up to 0.64 mg/mL. These promising results, disclose bioactive concentrations of Myrtle essential oils with a safety profile suggesting a potential oral and topical application or use by inhalation.” As taken from Bouzabata A et al. 2015. *Food Chem. Toxicol.* 75, 166-72. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/25446467?dopt=AbstractPlus>

“The composition of the essential oil from leaves of *Skimmia laureola* was determined by GC and GC-MS. Twenty-eight components were identified, accounting for 93.9% of the total oil. The oil is mainly composed of monoterpenes (93.5%), of which monoterpene hydrocarbons and oxygenated monoterpenes represent 11.0% and 82.5%, respectively. Sesquiterpenes constitute only 0.3% of the total oil. Linalyl acetate is the main component (50.5%), with linalool (13.1%), geranyl acetate (8.5%) and cis-p-menth-2-en-1-ol (6.2%) as other principal constituents. The essential oil showed a significant antispasmodic activity, in a dose range of 0.03-10 mg/mL. The essential oil also possesses antibacterial and antifungal activities against some pathogenic strains. The phytotoxic and cytotoxic activities were also assessed.” As taken from Barkatullah et al. 2015. *Molecules* 20(3), 4735-45. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/25786161>

“*Myrtus communis* is a typical plant of the Mediterranean area, which is mainly used as animal and human food and, in folk medicine, for treating some disorders. In the present study, we evaluated in vitro antibacterial and antifungal properties of the essential oils of *Myrtus communis* (McEO), as well as its phytochemical composition. The GC/MS analysis of the essential oil revealed 17

compounds. Myrtenyl acetate (20.75%), 1,8-cineol (16.55%), α -pinene (15.59%), linalool (13.30%), limonene (8.94%), linalyl acetate (3.67%), geranyl acetate (2.99%), and α -terpineol (2.88%) were the major components. The antimicrobial activity of the essential oil was also investigated on several microorganisms. The inhibition zones and minimal inhibitory concentration (MIC) values of bacterial strains were in the range of 16-28 mm and 0.078-2.5 mg/ml, respectively. The inhibitory activity of the McEO against Gram-positive bacteria was significantly higher than against Gram-negative. It also exhibited remarkable activity against several fungal strains. The investigation of the mode of action of the McEO by the time-kill curve against *Listeria monocytogenes* (food isolate) showed a drastic bactericidal effect after 5 min using a concentration of 312 μ g/ml. These results evidence that the McEO possesses antimicrobial properties, and it is, therefore, a potential source for active ingredients for food and pharmaceutical industries." As taken from Ben Hsouna A et al. 2014. Chem. Biodivers. 11(4), 571-80. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24706627>

"*Lavandula angustifolia* is the most widely cultivated *Lavandula* species. The extraction of its flower and leaves has been used as herbal medicine. In this study, the in vitro antitumor activities were tested on human prostate cancer PC-3 and DU145 cell lines. Flow cytometry technology was applied to study apoptosis induction and cell cycle arrest. The PC-3 cell line was used to establish subcutaneous xenograft tumors in nude mice. Paraffin sections from xenograft tumor specimens were used in the TUNEL (terminal deoxynucleotide transferase dUTP nick end labeling) assay and an immunohistochemistry assay to detect cell proliferation markers Ki67 and PCNA. Lavender essential oil, linalool, and linalyl acetate showed stronger inhibitory effect on PC-3 cells than on DU145 cells. The apoptotic cell populations observed in PC-3 cells treated with lavender essential oil, linalool, and linalyl acetate were 74.76%, 67.11%, and 56.14%, respectively. The PC-3 cells were mainly arrested in the G2/M phase. In the xenograft model with PC-3 cell transplantation, essential oil and linalool significantly suppressed tumor growth. The immunosignals of Ki67 and PCNA in the essential oil, linalool, and linalyl acetate treatment groups were significantly lower than that of the control group in xenograft tumor sections. The TUNEL assay indicated that each of the 3 phytochemicals significantly induced apoptosis compared to the control group. This study provides novel insight and evidence on the antiproliferative effect of *L. angustifolia* essential oil and its major constituents on human prostate cancer. The antitumor effect was associated with cell proliferation inhibition and apoptosis induction in xenograft tumors." As taken from Zhao Y et al. 2017. Integr. Cancer Ther. 16(2), 215-226. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27151584>

High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated linalyl acetate 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 linalyl acetate 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.

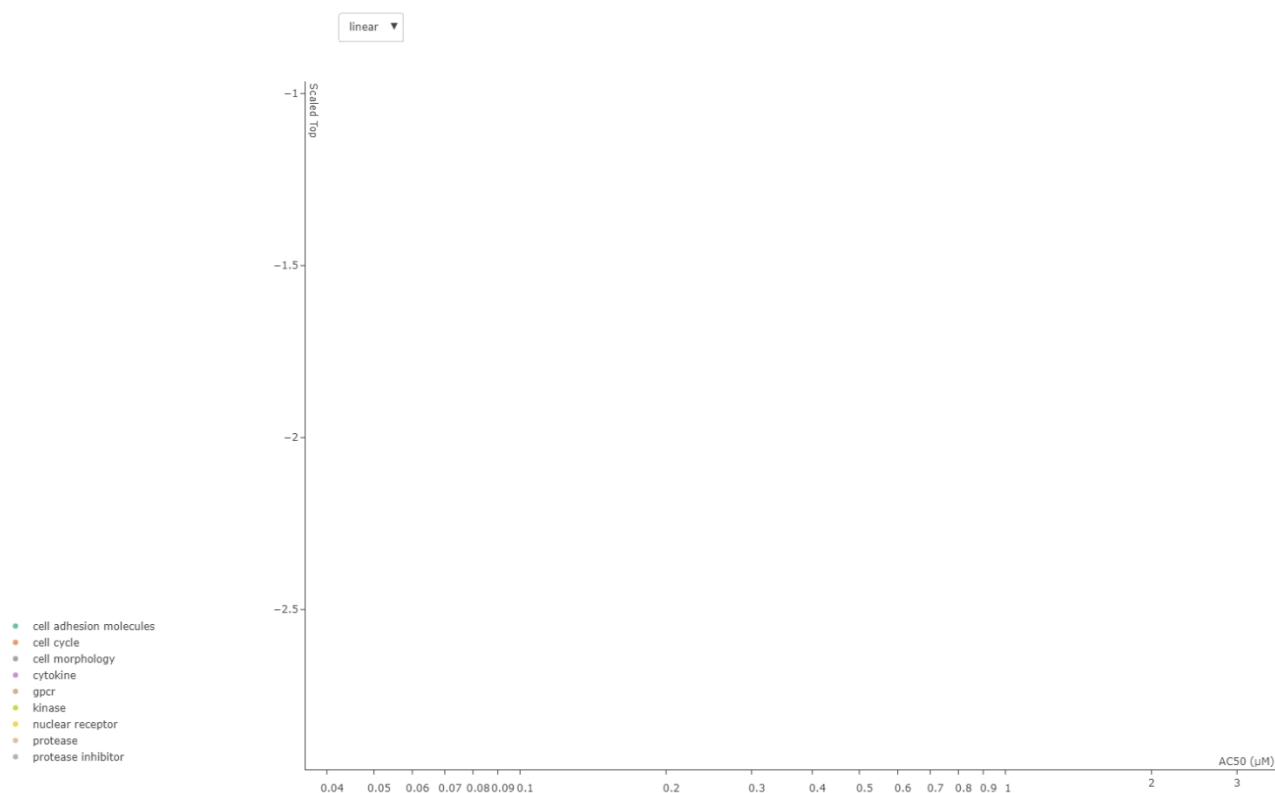


Figure 1

“Recent scientific investigations have reported a number of essential oils to interfere with intracellular signalling pathways and to induce apoptosis in different cancer cell types. In this paper, Lavandin Essential Oil (LEO), a natural sterile hybrid obtained by cross-breeding *L. angustifolia* × *L. latifolia*, was tested on human leukaemia cells (HL60). Based on the MTT results, the reduced cell viability of HL60 cells was further investigated to determine whether cell death was related to the apoptotic process. HL60 cells treated for 24 h with LEO were processed by flow cytometry, and the presence of Annexin V was measured. The activation of caspases-3 was evaluated by western blot and immunofluorescence techniques. Treated cells were also examined by scanning and transmission electron microscopy to establish the possible occurrence of morphological alterations during the apoptotic process. LEO main compounds, such as linalool, linalyl acetate, 1,8-cineole, and terpinen-4-ol, were also investigated by MTT and flow cytometry analysis. The set of obtained results showed that LEO treatments induced apoptosis in a dose-dependent, but not time-dependent, manner on HL60 cells, while among LEO main compounds, both terpinen-4-ol and linalyl acetate were able to induce apoptosis.” As taken from Masci VL et al. 2020. *Molecules* 25(3), 538. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31991893/>

5.6. Carcinogenicity

“To determine co-carcinogenic activity, groups of mice were given dermal applications of linalyl acetate alone or with benzo-a-pyrene (BaP). Linalyl acetate, 3 mg in 0.1 ml acetone, was tested alone and was also tested with simultaneous applications of 1 and 5 ug BaP. Solutions were applied to the clipped backs of 20 female ICR/Ha Swiss mice/group, 3 times a week, for the duration of the study (460 days). Control groups received the carcinogen alone, or the solvent alone or remained untreated. Mice with tumors that appeared grossly to be carcinomas were sacrificed 2 months after the tumor was classified as malignant. All animals were necropsied and tumors were confirmed histologically. No carcinogenic activity was observed when linalyl acetate was administered alone; no co-carcinogenic activity was observed when it was administered with

1ug BaP. Weak co-carcinogenic activity was observed when linalyl acetate was administered with 5 ug BaP (Van Duuren et al., 1971)."

"Linalyl acetate was examined for its ability to induce lung tumors in female A/He mice. The mice were 6-8 weeks old (30/dose group) with an average initial weight of 18-20g. Animals received injections of linalyl acetate in tricapylin 3 times weekly for 8 weeks. Dose levels were set at the maximum tolerated dose (MTD) and a 1:5 dilution of the MTD. A MTD of 1 g/kg had been established in a preliminary toxicity screen. The total cumulative doses were 4.8 g/kg and 24 g/kg. Control groups received 0.1 mL tricapylin alone or they were untreated. The experiments were terminated 24 weeks after the first injection. [...] Six animals died in the low-dose group and 4 animals died in the high-dose group. No significant difference in the incidence of lung tumors was observed between control animals and those treated with linalyl acetate (Stoner et al., 1973)."

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

Species	Test conditions	Evidence of carcinogenicity	Reference
Mouse, groups of 20 females, Swiss ICR/Ha	Skin carcinogenicity and cocarcinogenicity assays. Linalyl acetate was applied (3 mg, thrice weekly, for 67 weeks) to the skin, either alone or concomitantly with an chemical known to induce skin tumours.	No skin tumours were induced by linalyl acetate alone. [A limited study - few animals, short exposure duration, and only skin examined] In the co-carcinogenicity study, linalyl acetate slightly increased the induction of skin tumours by the known carcinogen.	Van Duuren et al. 1971
Mouse, groups of 30 females, strain A/He	Mice (of a strain prone to develop lung tumours) were given intraperitoneal injections of linalyl acetate 24 times over an 8-week period. The total dose given was 4.8 or 24.0 g linalyl acetate/kg body weight. Animals were observed for a further 16 weeks. Lung, liver, kidney, spleen, thymus, intestine and salivary and endocrine glands examined (the lung was the only organ said to be subject to detailed scrutiny). 13-20% of the animals died before the end of the study.	None. A very limited study.	Stoner et al. 1973

In an assessment of linalool and related esters, Bickers et al. (2003) considered it reasonable to conclude that linalool and the related esters have no significant potential for carcinogenicity under the recommended current conditions of use as fragrance ingredients. This conclusion was based on the lack of significant genotoxic potential, weak (if any) tumour promoting activity, the high no-observed-adverse-effect levels observed in subchronic studies, the information on metabolism and detoxification, and the lack of structural alerts for carcinogenicity.

"Lavandula angustifolia is the most widely cultivated Lavandula species. The extraction of its flower and leaves has been used as herbal medicine. In this study, the in vitro antitumor activities were tested on human prostate cancer PC-3 and DU145 cell lines. Flow cytometry technology was applied to study apoptosis induction and cell cycle arrest. The PC-3 cell line was used to establish subcutaneous xenograft tumors in nude mice. Paraffin sections from xenograft tumor specimens were used in the TUNEL (terminal deoxynucleotide transferase dUTP nick end labeling) assay and an immunohistochemistry assay to detect cell proliferation markers Ki67 and PCNA. Lavender essential oil, linalool, and linalyl acetate showed stronger inhibitory effect on PC-3 cells than on DU145 cells. The apoptotic cell populations observed in PC-3 cells treated with lavender essential oil, linalool, and linalyl acetate were 74.76%, 67.11%, and 56.14%, respectively. The PC-3 cells were mainly arrested in the G2/M phase. In the xenograft model with PC-3 cell transplantation, essential oil and linalool significantly suppressed tumor growth. The immunosignals of Ki67 and PCNA in the essential oil, linalool, and linalyl acetate treatment groups were significantly lower than that of the control group in xenograft tumor sections. The TUNEL assay indicated that each of the 3 phytochemicals significantly induced apoptosis compared to the control group. This study provides novel insight and evidence on the antiproliferative effect of L angustifolia essential oil and its major constituents on human prostate cancer. The antitumor effect was associated with cell proliferation inhibition and apoptosis induction in xenograft tumors." As taken from Zhao Y et al. 2017. Integr. Cancer Ther. 16(2), 215-226. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27151584>

"Limited information available for linalyl acetate does not indicate carcinogenic potential. The metabolite linalool is not considered to be a carcinogen (NICNAS). Therefore, the chemicals in this group are not likely to have carcinogenic effects.

In a carcinogenicity assay, linalyl acetate (purity $\geq 85\%$) was administered (intraperitoneally) in mice (A/He) at 200 or 1000 mg/kg bw, 3 times/week for 8 weeks, with observation up to 24 weeks. Histopathological examinations were conducted on the lungs. The other organs (liver, kidney, spleen, thymus, intestine and salivary and endocrine glands) were subjected to macroscopic investigation. There was no evidence of carcinogenic activity (OECD, 2002). It is stated that the duration for this test was too short to develop into possible carcinogenic effects; however, the positive control (urethane) showed a 100 % response.

In a tumour induction dermal study, distilled linalyl acetate was applied topically alone or with a co-carcinogen benzo[a]pyrene (BaP) (at 1 or 5 μg) on female ICR/Ha Swiss mice, 3 times/week for 67 weeks. No carcinogenicity was observed when the chemical was administered alone, or with 1 μg BaP. Weak co-carcinogenic activity (slight increase in the number of skin papillomas and carcinomas compared with BaP alone) was observed when co-administered with 5 μg BaP (OECD, 2002; Bickers et al., 2003a)."

As taken from AICIS, 2018

5.7. Irritation/immunotoxicity

Based on the available data, linalyl acetate does not present a concern for skin sensitization... Linalyl acetate is not predicted to significantly react, directly, to skin proteins (Roberts et al., 2007; OECD toolbox v3.0). However, linalyl acetate is known to undergo autooxidation resulting in degradation products that may be protein reactive (Skold et al., 2008). (Api et al., 2015).

"Linalyl acetate (100%) appeared to be severely irritating to rabbit skin and moderately irritating to the skin of the guinea pig. In a test with miniature swines, application of 0.05 g linalyl acetate under a patch for 48 hours, no irritation was observed".

"Rabbit (5% solution): not irritating."

As taken from OECD-SIDS (2002), Linalyl acetate (115-95-7), p11 available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

“/IMMUNOTOXICITY/ /Linalyl acetate/ was administered intragastrically on a daily basis for 5 days at /3000, 1500, or 750 mg/kg/day in corn oil/ to /female/ B6C3F1 /or CD-1/ mice. A host-resistance assay (*Listeria monocytogenes* bacterial challenge) was conducted to assess cell-mediated immunity. Humoral immunity was measured by the antibody Plaque-Forming cell (PFC) response to sheep erythrocytes. Cyclophosphamide served as an immunosuppressive positive control agent. ... Increased mortality following challenge with *L. monocytogenes* was noted at 750 mg/kg. No significant alteration in the PFC response was noted. The increased mortality observed following challenge with *L. monocytogenes* was not dose-related and therefore considered to be incidental. ... /It was concluded that/ linalyl acetate did not mediate the cell-mediated or humoral immune response. [USEPA/HPVIS; Detailed Chemical Results on 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate (115-95-7): Repeated-Dose Toxicity for Mammalian Health Effects SIDS. Available from, as of July 14, 2008: <http://www.epa.gov/hpvis/index.html> **PEER REVIEWED**”

“/HUMAN EXPOSURE STUDIES/ Application of linalyl acetate in acetone (33%) to the back of male volunteers without known allergies during 48 hours under occlusion did not induce signs of irritation up to 120 hours after removal of the patch [Organization for Economic Cooperation and Development; Screening Information Data Set for LINALYL ACETATE (115-95-7) p.11 (March 2002). Available from, as of July 14, 2008: **PEER REVIEWED**”

“/HUMAN EXPOSURE STUDIES/ ... Linalyl acetate is identified as one of the constituents of lavender oil that may cause allergic reactions. No testing results were available for linalyl acetate, but lavender oil was positive in a human patch test in a hairdresser [Organization for Economic Cooperation and Development; Screening Information Data Set for LINALYL ACETATE (115-95-7) p.11 (March 2002). Available from, as of July 14, 2008: **PEER REVIEWED**”

“/IMMUNOTOXICITY/ ... The aim of the present study was to investigate the autoxidation of linalyl acetate and the influence of oxidation on its sensitizing potency. ... Analyses were performed using gas chromatography, nuclear magnetic resonance spectrometry and mass spectrometry. Sensitizing potencies of compounds were determined using the local lymph node assay (LLNA) in mice. ... : Analyses showed that the content of linalyl acetate decreased over time on air exposure and other compounds were formed. Hydroperoxides, an epoxide and an alcohol were identified as oxidation products from linalyl acetate. In the LLNA, linalyl acetate of high purity showed a weak sensitizing potency (EC3 25%). Autoxidation increased the sensitizing potency of linalyl acetate, and a 10 weeks oxidized sample gave an EC3 value of 3.6%. As for linalool, the hydroperoxides were shown to be the oxidation products with the highest sensitizing potency. ... It is concluded that autoxidation of the weakly allergenic linalyl acetate leads to formation of allergenic oxidation products. [Skold M et al; Contact Dermatitis 58 (1): 9-14 (2008)] **PEER REVIEWED** PubMed Abstract”

As taken from HSDB, 2017

“Respiratory irritation was assessed in CF-1 female mice by recording their respiratory rate using a whole body plethysmograph. Mice were exposed for 1min to linalyl acetate at dose levels of 188-764 ug/L, using a nebulizer for aerosolization, in a 2600 mL Plexiglass inhalation exposure chamber. Mild to moderate respiratory depression was observed. The ECD25 (dose providing a 25% reduction in respiratory rate) was calculated to be 755 ug/L (Troy, 1977).”

“From the animal studies performed, the following results have been obtained: linalyl acetate produced no irritation in swine, a moderate irritation in guinea pigs and a severe irritation in rabbits. In eye irritation studies carried out in rabbits, it has been shown that linalyl acetate produced likely no irritation or in some cases a slight irritation of the eye.”

“Sensitization by linalyl acetate has been tested in human studies. Results showed that the test substance does not produce any sensitization except in a very few cases. No reactions that were

significantly irritant or allergic were observed. The same results have been obtained from guinea pig studies. “

“UV spectrum reveals that linalyl acetate does not absorb UV light at wavelengths in the range of 290-400 nm and therefore would have no potential to elicit photoirritation or photoallergy under the current conditions of use as a fragrance ingredient (Bickers et al., 2003).”

“In a separate experiment, an intact mouse breathing through a tracheal cannula were simultaneously exposed for 1min to 970 ug/L linalyl acetate. Again, a mild to moderate decrease in respiratory rate was observed with nose only inhalation. No significant effects were observed when inhalation was through the tracheal cannula (Troy, 1977).”

“A number of human skin studies assessing the irritation potential of linalyl acetate have been performed. It has been shown that the test substance produced no irritation, or, in a very few cases, contact dermatitis occurred.”

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

“It is reported that repeated skin exposure to Bergamot oil (45% linalyl acetate) leads to nervous and digestive symptoms. Sensitive persons may develop photodermatitis, eczema and pigment changes.”

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

“Cal and Sznitowska (2003) studied skin penetration and elimination of the three acyclic terpenes citronellol, linalool, and linalyl acetate. The pure terpenes were applied onto human skin in vitro, and after 1–4 h their content in the stratum corneum layers and in the epidermis/dermis was determined using gas chromatography. Similarly, the amounts of terpenes in the skin were analyzed during 4 h following a 1-h absorption period. Penetration into all skin layers was demonstrated after 1 h of exposure, with total amounts of linalool and citronellol of 827 and 954 lg/cm², respectively. During the elimination phase, a constant drop in the total amount in the skin was observed only for citronellol, while the total skin content of linalool and linalyl acetate did not change, although diffusion from the stratum corneum into the epidermis/dermis occurred. Permeability coefficients of 5.6 and 6.3 cm/h x 10⁻⁵ were found for carveol and 4-carvomenthenol, respectively, by Williams and Barry (1991a,b). For both linalool and 4-carvomenthenol, penetration was faster from hydrogel formulations as compared to oily solutions or emulsions (Cal, 2006).

Linalool levels in blood of a male volunteer were followed for 90 minutes after the use of 1500 mg of massage oil which contained 2% lavender oil with approximately 25% linalool and 30% linalyl acetate. Trace amounts of both linalool and linalyl acetate were detected in the blood 5 minutes after the massage. Peak plasma concentrations were reached by 19 minutes with a mean plasma concentration of 100 ng/ml for linalool and 121 ng/ml for linalyl acetate.

Most of the linalool and linalyl acetate had disappeared from the blood in 90 minutes with biological half lives of approximately 14 minutes for each (Jäger et al., 1992). Atzl et al. (1972) found menthol in the urine of persons treated dermally with a menthol-containing ointment (no quantitative data available).”

As taken from RIFM EXPERT Panel; A toxicologic and dermatologic assessment of cyclic and non-cyclic terpene alcohols when used as fragrance ingredients; Food and Chemical Toxicology 46 (2008) S1–S71.

Linalyl acetate, a rabbit skin irritant, was found to be non-irritating to skin preparations in vitro using the human 4hr HPT method and the EPISKIN and EPIDERM models. The authors conclude that the results confirm that rabbits overpredict skin effects in humans (Jirova et al 2010).

Linalyl acetate is a fragrance substance of clinical importance known to be a prehapten (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) and to form a sensitising compound by air oxidation (SCCS, 2011 & 2012).

Patch-tests with 6% linalyl acetate (oxidised) were positive in 13/1217 consecutive patients (SCCS, 2012).

“In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. ... In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted 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.”

“A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation.”

As taken from SCCS, 2012. Available at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration onto the skin	Rodent - rabbit	100 mg/24H	Severe	Cosmetics and Toiletries. (Allured Pub. Corp., POB 318, Wheaton, IL 60189) V.91- 1976- 94(8),41,1979
Standard Draize test	Administration onto the skin	Rodent - guinea pig	100 mg/24H	Moderate	Cosmetics and Toiletries. (Allured Pub. Corp., POB 318, Wheaton, IL 60189) V.91- 1976- 94(8),41,1979

As taken from RTECS, 2018

“BiRyuChe-bang (BRC) is a Korean prescription medicine, which has been used to treat allergic rhinitis at Kyung Hee Medical Center. In this work, we investigated the effects of BRC on mast cell-mediated allergic reactions and inflammatory cytokines production, and identified the active component of BRC. Histamine release was measured from rat peritoneal mast cells (RPMCs). Ear swelling and passive cutaneous anaphylaxis (PCA) were examined in mouse models. Phorbol 12-myristate 13-acetate (PMA) plus A23187-induced inflammatory cytokines production was measured using enzyme-linked immunosorbent assay. Reverse transcriptase-polymerase chain reaction was used for the expressions of tumor necrosis factor (TNF)- α , interleukin (IL)-6, and IL-8. Activation of nuclear factor (NF)- κ B was analyzed by Western blotting. BRC significantly inhibited the compound 48/80-induced ear swelling response, histamine release from RPMCs, PCA activated by anti-dinitrophenyl IgE, and PMA plus A23187-induced inflammatory cytokines production ($p < 0.05$). In addition, BRC dose-dependently inhibited the mRNA expressions of TNF- α , IL-6, and IL-8 as well as the activation of NF- κ B in a human mast cell line, HMC-1 cells. BRC inhibited the levels of TNF- α and IL-6 in mice induced with PCA. Several components of BRC, such as 1,8-Cineole, Linalool, Linalyl acetate, α -Pinene, and α -Terpineol, significantly inhibited the release of histamine from RPMCs ($p < 0.05$). Among these components, Linalyl acetate was the most effective for inhibiting histamine release. These results indicate that BRC has a potential

regulatory effect on allergic and inflammatory reactions mediated by mast cells.” As taken from Moon PD et al. 2013. Am. J. Chin. Med. 41(6):1267-82. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24228600>

“Photosensitivity reactions have occurred following the topical use of preparations containing bergamot oil”.

As taken from Martindale, 1999

“BACKGROUND: Linalyl acetate is a fragrance chemical that is prone to autoxidation. Exposure to linalyl acetate occurs through cosmetic products and essential oils, but is difficult to assess, as linalyl acetate is not labelled in the EU. OBJECTIVE: To investigate the frequencies of contact allergy to oxidized linalyl acetate among dermatitis patients, and to investigate the autoxidation of linalyl acetate in terms of hydroperoxide formation and sensitization potency. PATIENTS AND METHODS: Hydroperoxide formation in air-exposed linalyl acetate was determined with high-performance liquid chromatography. The sensitization potencies of hydroperoxides were determined with the local lymph node assay. One thousand seven hundred and seventeen patients were patch tested with oxidized linalyl acetate at 6.0% in petrolatum. RESULTS: Of the patients, 2.2% showed positive reactions to oxidized linalyl acetate. Forty-three per cent of the positive patients also had positive patch test reactions to other fragrance markers. Linalyl acetate hydroperoxides were detected early in the autoxidation process, and accumulated to a concentration of 37% after 42 weeks of air exposure. The linalyl acetate hydroperoxides were classified as moderate sensitizers. CONCLUSIONS: The frequency of positive reactions to oxidized linalyl acetate is comparable to that of previously studied oxidized fragrance terpenes. Oxidized linalyl acetate could thus be a common fragrance contact allergen.” As taken from Hagvall L et al 2015. Contact Dermatitis 72(4), 216-23. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/25645423>

“The analgesic and anti-inflammatory properties of Citrus aurantium L. blossoms essential oil (neroli) were investigated in mice and rats. The analgesic activity of neroli was assessed by acetic acid-induced writhing and Eddy's hot plate methods, while acute and chronic anti-inflammatory effects were investigated by inflammatory paw edema in rat and the cotton pellet-induced granuloma tissue model, respectively. Mechanistic studies were conducted using L-nitro arginine methyl ester (L-NAME), an inhibitor of NO synthase. Neroli significantly decreased the number of acetic acid-induced writhes in mice compared to animals that received vehicle only. Also, it exhibited a central analgesic effect, as evidenced by a significant increase in reaction time in the hot plate method. The oil also significantly reduced carrageenan-induced paw edema in rats. The inhibitory activity of neroli (especially at 40 mg/kg) was found to be very close to the standard drug, diclofenac sodium (50 mg/kg). In cotton pellet-induced granuloma, neroli was effective regarding the transudate and granuloma formation amount. Neroli was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and twenty-three constituents, representing 91.0 % of the oil, were identified. The major components of neroli were characterized as linalool (28.5 %), linalyl acetate (19.6 %), nerolidol (9.1 %), E,E-farnesol (9.1 %), α -terpineol (4.9 %), and limonene (4.6 %), which might be responsible for these observed activities. The results suggest that neroli possesses biologically active constituent(s) that have significant activity against acute and especially chronic inflammation, and have central and peripheral antinociceptive effects which support the ethnomedicinal claims of the use of the plant in the management of pain and inflammation.” As taken from Khodabakhsh P et al. 2015. J. Nat. Med. 69(3), 324-31. PubMed, 2016 available at: <http://www.ncbi.nlm.nih.gov/pubmed/25762161>

“Lavender oil is an essential oil obtained from lavender (*Lavendula angustifolia*). The main components linalool and linalyl acetate have been shown to autoxidize in contact with oxygen in the air, forming sensitizing hydroperoxides. Patients with suspected allergic contact dermatitis were consecutively patch-tested with oxidized lavender oil 6% pet., oxidized linalyl acetate 6% pet., and oxidized linalool 6% pet. to investigate the frequency of contact allergy to oxidized lavender oil, and the pattern of concomitant reactions to oxidized linalool and oxidized linalyl acetate. Positive

reactions to oxidized lavender oil were found in 2.8% of the patients. Among those, 56% reacted to oxidized linalool and/or oxidized linalyl acetate, while 52% reacted to the fragrance markers of the baseline series. Oxidized lavender oil showed among the highest frequencies of contact allergy to studied essential oils. A well-standardized preparation of oxidized lavender oil could be a useful tool for diagnosis of contact allergy to fragrances.” As taken from Hagvall L and Christensson J. 2016. *J. Acta Derm. Venereol.* 96(5), 679-83. PubMed, 2017

“The local lymph node assay (LLNA) is used for assessing sensitizing potential in hazard identification and risk assessment for regulatory purposes. Sensitizing potency on the basis of the LLNA is categorized into extreme (EC3 value of $\leq 0.2\%$), strong ($>0.2\%$ to $\leq 2\%$), and moderate ($>2\%$). To compare the sensitizing potencies of fragrance substances, preservatives, and hair dye substances, which are skin sensitizers that frequently come into contact with the skin of consumers and workers, LLNA results and EC3 values for 72 fragrance substances, 25 preservatives and 107 hair dye substances were obtained from two published compilations of LLNA data and opinions by the Scientific Committee on Consumer Safety and its predecessors. The median EC3 values of fragrances ($n = 61$), preservatives ($n = 19$) and hair dyes ($n = 59$) were 5.9%, 0.9%, and 1.3%, respectively. The majority of sensitizing preservatives and hair dyes are thus strong or extreme sensitizers (EC3 value of $\leq 2\%$), and fragrances are mostly moderate sensitizers. Although fragrances are typically moderate sensitizers, they are among the most frequent causes of contact allergy. This indicates that factors other than potency need to be addressed more rigorously in risk assessment and risk management.” As taken from Lidén C et al. 2016. *Contact Dermatitis* 75(5), 265-275. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/?term=27633650>

“Predictive capacity of the EpiSkin™ model was evaluated on 87 chemicals using the Bottom-Up and the Top-Down testing approaches recommended within Integrated Approach on Testing and Assessment for the identification of both skin irritation and corrosion hazards. Classified (UN GHS Cat. 1 and Cat. 2) chemicals were identified with a very high sensitivity ($\geq 94\%$) and the non-classified (UN GHS Cat. 3 and No Cat.) chemicals with an appropriate specificity (70%). Very high sensitivities were obtained for the identification of Cat. 1 chemicals ($\geq 98\%$), very high specificities for non-Cat. 1 chemicals (93%), and accuracies of $\geq 95\%$ for the identification of skin corrosives vs. non-corrosives by both approaches. Overall accuracies of 72% were found for predicting the single (sub)categories: non-classified, Cat. 2, Subcat. 1B/1C and Subcat. 1A. Results indicated the testing strategies to be more predictive than the individual assays on a conservative safety approach. Finally, no extreme misclassifications (no under-prediction of in vivo Subcat. 1A as non-Cat. 1, and no over-prediction of non-classified chemical as Subcat. 1A) occur. These findings, independently of the approach used, confirm the usefulness of the EpiSkin™ in vitro model for a safe prediction of the skin irritant and corrosive hazards of chemicals.” As taken from Alépée N et al. 2019. *Toxicol. In Vitro* 54, 147-167. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30266437>

“Thymic stromal lymphopoietin (TSLP) is an important factor responsible for the pathogenesis of allergic diseases, such as atopic dermatitis and asthma. Because linalyl acetate (LA) possesses a wide range of pharmacological properties, being antispasmodic, anti-inflammatory, and anti-hyperpigmentation, we hypothesized that LA could inhibit TSLP. Therefore, enzyme-linked immunosorbent assay, quantitative polymerase chain reaction, caspase-1 assay, Western blot analysis, fluorescent analyses of the intracellular calcium levels, and the phorbol myristate acetate (PMA)-induced edema model were used to investigate how LA inhibits the production of TSLP in HMC-1 cells. LA reduced the production and mRNA expression of TSLP in HMC-1 cells. LA also inhibited the activation of nuclear factor- κ B and degradation of I κ B α . PMA plus A23187 stimulation up-regulated caspase-1 activity in HMC-1 cells; however, this up-regulated caspase-1 activity was down-regulated by LA. Finally, LA decreased intracellular calcium levels in HMC-1 cells as well as PMA-induced ear swelling responses in mice. Taken together, these results suggest that LA would be beneficial to treatment of atopic and inflammatory diseases by reducing TSLP.” As taken from Moon PD et al. 2018. *Molecules* 23(7), E1711. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30011850>

“Background Fragrances are a group of substances present in many cosmetic products, which are one of the most common sources of allergic contact dermatitis (ACD) in Europe. Their widespread distribution, the presence of these substances as combinations, their ability to transform into more reactive substances, and the relatively recent regulations regarding these products are the main reasons for this high prevalence. In the last few years, advances on knowledge about haptens in fragrances have made possible to discover new allergens, to state that current fragrance markers are not enough to detect most of fragrance ACD cases and to know the products that should be patched and their concentrations. In this review, we revise the mechanisms of sensitization to allergens that are not represented in baseline series and the concentrations at which they should be used, which should be included in specific series and the usefulness of baseline and fragrance specific series. Purpose of review To revise the most recent advances in knowledge about fragrance contact dermatitis, especially about sensitization mechanisms and effectivity of fragrance markers in baseline series and fragrances in essential oils. Recent findings Fragrance allergic contact dermatitis (ACD) is one of the most common sources of ACD only preceded by nickel sulfate. Many studies have shown that fragrance mix (FM)I, FMII, and colophonium, fragrance markers in baseline series, are not enough to screen for cases of ACD to fragrances. Some fragrances use limonene and linalool hydroperoxides, which have shown a high prevalence of sensitization and have been proposed as new allergens to include in the European Baseline Series, while other fragrances like Evernia furfuracea, which have also shown a high prevalence of sensitization, will not be included. Essential oils are obtained by distillation process and are widely used. In some cases, their allergenic components are not known. Co-sensitization to other fragrance components is frequent and they should be patch tested if allergic contact dermatitis to fragrances or cosmetics is suspected. Summary A more extensive group of fragrance markers should be included in the European Baseline Series, with at least limonene and linalool hydroperoxides and Evernia furfuracea. The use of products containing fragrances is very common and people sensitized to one of these substances should be suspected of multiple co-sensitizations.” As taken from Schneller-Pavelescu L et al. 2019. Current Treatment Options in Allergy 6(1), 92-102. Available at <https://link.springer.com/article/10.1007/s40521-019-0196-6>

“Skin irritation

Results for linalyl acetate appeared to be species-specific. Moderate skin irritation effects were reported in a rabbit study but not observed in miniature swine (pig) study. No effects were observed in humans up to a concentration of 32 %. However, it should be noted that concentrations less than 100 % of linalyl acetate were used for human patch test studies.”

“In a skin irritation study conducted according to the OECD Test Guideline (TG) 404, 0.5 mL of undiluted linalyl acetate was applied (semi-occlusively) on the skin of rabbits for 4 hours, with observation up to 7 days. Two test runs were conducted, using a total of 7 rabbits (3 in the first test, 4 in the second test). The mean erythema scores (24, 48, 72 hours) for test 1 and test 2 were 1.88 and 1.91, respectively. The mean oedema scores were 1.78 and 1.00, respectively. Slight to marked desquamation was observed in 6/7 animals at the end of the observation period. In another skin irritation study (BASF test), 2 rabbits were applied undiluted linalyl acetate under occlusive patches for 5 minutes, 15 minutes and 20 hours, with observation period up to 8 days. Desquamation was observed and the mean erythema score (24, 48 hours) was ≥ 2 . Based on the above studies, the chemical was determined to be a skin irritant (REACHa).

In several skin irritation studies in rabbits, reactions to linalyl acetate (undiluted) ranged from slight to severe. Severe irritation was observed in a 24-hour open patch test on the dorsal skin of albino Angora rabbits (n = 6). Minimal irritation was observed in a 24-hour closed patch test on New Zealand female rabbits (n = 9). Undiluted linalyl acetate was moderately irritating on male Hartley guinea pigs and not irritating when applied at 0.05 g on miniature swines for 48 hours (OECD, 2002; Bickers et al., 2003a).

Linalyl esters (acetate, benzoate, butyrate, formate, isobutyrate, isovalerate, phenylacetate and propionate) tested at 5 % in diethyl phthalate were slightly irritating in rabbits. Effects generally cleared by 72 hours.”

“Linalyl esters were tested in guinea pigs and rats for photoirritation or photoallergy and showed no potential to elicit these effects (Bickers et al., 2003a).”

“Eye irritation

Based on the available information, the chemicals are not considered to be eye irritants.

In several eye irritation studies, the results for six undiluted linalyl esters (acetate, benzoate, butyrate, formate, isobutyrate, and propionate) ranged from very slight to slight irritation effects in rabbits.”

“When tested at a concentration of 5 % in diethyl phthalate, linalyl esters (acetate, benzoate, butyrate, formate, isobutyrate, isovalerate, phenylacetate and propionate) caused very slight irritation in rabbits (Bickers et al., 2003a).

In an eye irritation study, 0.05 mL of linalyl acetate was administered to one eye of each of 2 Vienna White rabbits, with observation for 8 days. Slight conjunctival redness was observed in both animals, but this was reversible by day 2 (REACHa).”

“Observation in humans

In human patch-test studies in approximately 380 healthy male and female volunteers, no irritation was observed with linalyl esters tested up to a concentration of 32 % (Bickers et al., 2003a). No signs of irritation were observed up to 120 hours after exposure (OECD, 2002).”

“Skin Sensitisation

Based on the available data, linalyl acetate in its highly purified form is a weak skin sensitiser. However when exposed to air, the chemical is auto-oxidised into a potent contact allergen. Exposure to linalyl acetate will primarily be as a constituent in natural oils (lavender oil), which also involves co-exposure to linalool, a known skin sensitiser. Given that public exposure to linalyl acetate will be through products exposed to air, linalyl acetate is expected to undergo auto-oxidation.”

“In a skin painting study, female white guinea pigs were topically induced with linalyl acetate (95–99% purity) at concentrations of 10 and 50 %. The animals were challenged at day 28 and day 31 at concentrations of 5 and 10 % linalyl acetate, respectively. No positive responses were reported. However, when repeatedly applied to the clipped flank skin, strong desquamation was observed within the area of application (REACHa).

The skin sensitising effects for auto-oxidised linalyl acetate were investigated in a local lymph node assay (LLNA) (OECD TG 429) with female CBA/Ca mice. The hydroperoxide oxidation products of linalyl acetate were also tested. The EC3 values for linalyl acetate as unchanged (97 % purity), distilled, or air-exposed over 10 weeks were reported to be 20 %, 25.4 % and 3.6 %, respectively. For the air-exposed sample, 74 % of the original test substance remained after 10 weeks. The EC3 value for the identified hydroperoxide derivatives was 3.6 %, similar to that of air-exposed linalyl acetate. The authors concluded that the hydroperoxides were responsible for the sensitising potency of the chemical (Skold et. al., 2008; REACHa).

In guinea pig maximisation tests, linalyl acetate was weak to moderately sensitising at a concentration of 10 %, and non-sensitising when tested at 5 %. The purity of linalyl acetate was not stated. In a Freund's complete adjuvant test (FCAT), female albino Dunkin-Hartley guinea pigs (n = 30) were intradermally induced with linalyl acetate at 10 % in Freund's complete adjuvant and topically at 10 % in acetone. The animals were challenged topically with 5, 10 and 20 % in acetone. Skin reactions were observed in 0/10, 2/10 and 4/10 animals, respectively (Bickers et al., 2003a; REACHa).”

“The Scientific Committee on Consumer Safety (SCCS) has categorised linalyl acetate as an ‘established contact allergen in humans’. This is based on up to 10 positive test reactions reported in humans (SCCS, 2012).”

In a number of human maximisation tests, linalyl acetate was tested at concentrations of 10 or 20 % in petrolatum, or at 12 % in an unspecified vehicle. Reactions were observed in a total of 3 out of 131 subjects. No reactions were observed when the same samples were retested, or purified then retested (Bickers et al., 2003a).”

“In a multicentre study, a total of 1855 patients with a history of allergy to fragrances were patch-tested with 10 % linalyl acetate (95–99 % purity) in petrolatum for 48 hours. Positive reactions were reported in 4 patients, and ‘doubtful positive reactions’ (only erythema) were observed in 8 patients (REACHa).”

As taken from AICIS, 2018

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing linalyl acetate 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 linalyl acetate 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	1.3	JTI KB Study Report(s)
In vitro cytotoxicity	1.3	JTI KB Study Report(s)

“Twelve rats (per steam temperature level) were exposed to an atmosphere saturated with steam at 20 °C or 100 °C that had been passed through a 5-cm layer of linalyl acetate. Animals were observed over an 8-h period. No mortalities were observed at either exposure level. There were no toxic signs observed at 100 °C. Gross observations at necropsy were normal (RIFM, 1969).”

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

A sedative effect was found when female mice were exposed by inhalation at an undisclosed concentration for 1 hr (Buchbauer et al. 1993).

“The mitogen-activated protein kinase (MAPK) family, including extracellular signal-regulated kinase (ERK)1/2, c-Jun N-terminal kinase (JNK)1/2 and p38 MAPK, is known to be activated by ultraviolet (UV) radiation in melanocytes to regulate melanin production. Reactive oxygen species (ROS) play important roles in the pathway of ERK and JNK activation. It has been established that the essential oil of *Achillea millefolium* L. (AM-EO) has activities that suppress the oxidative stress and inflammatory responses. Thus, we analyzed the effects of AM-EO on melanogenesis in melanocyte stimulating hormone (α -MSH) treated melanoma cells. The results demonstrated that AM-EO suppresses melanin production by decreasing tyrosinase activity through the regulation of the JNK and ERK signaling pathways. This effect might be associated with the AM-EO activity leading to the suppression of ROS, and linalyl acetate is its major functional component. Therefore, we propose that AM-EO has the potential to treat hyperpigmentation in the future.” As taken from Peng HY et al. 2014. PLoS One 9(4), e95186. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24743745>

High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated linalyl acetate 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 linalyl acetate 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.

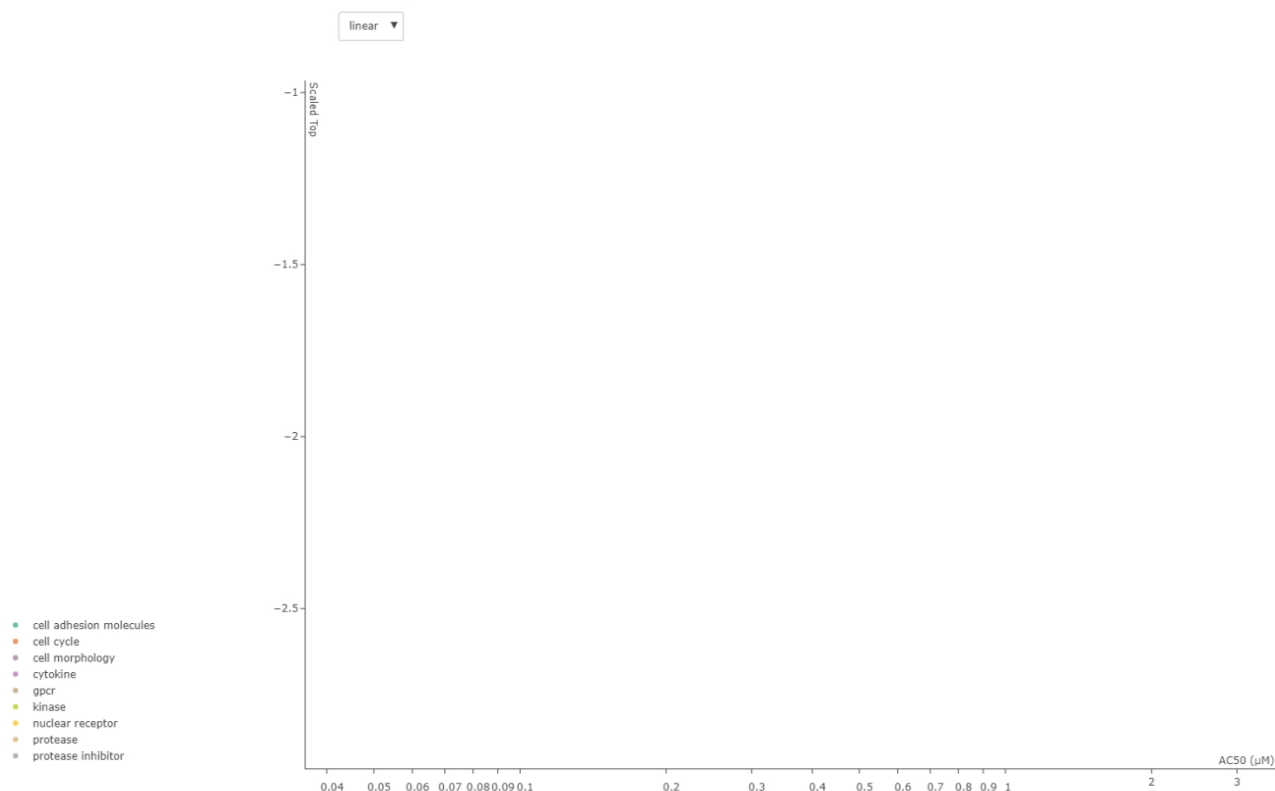


Figure 1

“Daidai (bitter orange, *Citrus aurantium*) is characterized by its fresh citrus scent. In Japanese cuisine, its juice is an important ingredient. As tons of industrial waste is obtained while processing the daidai juice, additional utilization of this waste has great social value. In our study, we prepared the essential oil from the waste obtained during daidai juice processing and demonstrated that the oil activates human TRPA1 (hTRPA1). This oil contains 10 types of terpenes, all of which activated hTRPA1 with an EC50 value of 6-167 μM. To our knowledge, this study is the first to show a hTRPA1 activation by five terpenes: linalyl acetate, geranyl acetate, osthole, geranyl propionate, and neryl acetate. Because physiological benefits of TRPA1 agonists, such as enhancement of energy metabolism and promotion of skin barrier recovery, have been reported, the oil could be a promising ingredient for anti-obesity food products and cosmetics.” As taken from Terada Y et al. 2019. Biosci. Biotechnol. Biochem. 83(9), 1721–1728. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31072263/>

6. Functional effects on

6.1. Broncho/pulmonary system

“Rabbits were exposed by inhalation of steam containing 9 and 27 mg/kg H₂O: effects at 27 mg/kg H₂O consisted of weak bronchomucotropic action (some augmentation of volume output of respiratory tract fluid).”

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7).

6.2. Cardiovascular system

“In this study, we compared the effect of the essential oil of Citrus bergamia Risso [bergamot, bergamot essential oil (BEO)] on the intracellular Ca levels in vascular endothelial (EA) and mouse vascular smooth muscle (MOVAS) cells, using the fura-2 fluorescence technique. BEO caused an initial transient increase in intracellular Ca concentration ([Ca]_i) in EA cells, followed by a decrease, whereas it induced a sustained increase in [Ca]_i in MOVAS cells. Linalyl acetate (LA) as a major component of BEO-induced [Ca]_i mobilization was similar to BEO in EA cells. The increase of [Ca]_i by LA was higher in EA cells than in MOVAS cells. [Ca]_i rise induced by extracellular Ca application was significantly blocked by BEO or LA in EA cells but not in MOVAS cells, suggesting that BEO and LA block Ca influx in EA cells. The present results suggest that BEO and LA differentially modulate intracellular Ca levels in vascular endothelial and smooth muscle cells. In addition, blockade of Ca influx by BEO and LA in EA cells may explain the protective effects of BEO on endothelial dysfunction associated with cardiovascular disease.” As taken from You JH et al. 2013. J. Cardiovasc. Pharmacol. 61(4), 324-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23288200>

“Pain and urinary symptoms following colorectal cancer (CRC) surgery are frequent and carry a poor recovery. This study tested the effects of inhalation of Lavandula angustifolia Mill. (lavender) oil or linalyl acetate on pain relief and lower urinary tract symptoms (LUTS) following the removal of indwelling urinary catheters from patients after CRC surgery. This randomised control study recruited 66 subjects with indwelling urinary catheters after undergoing CRC surgery who later underwent catheter removal. Patients inhaled 1% lavender, 1% linalyl acetate, or vehicle (control group) for 20 minutes. Systolic and diastolic blood pressure (BP), heart rate, LUTS, and visual analog scales of pain magnitude and quality of life (QoL) regarding urinary symptoms were measured before and after inhalation. Systolic BP, diastolic BP, heart rate, LUTS, and QoL satisfaction with urinary symptoms were similar in the three groups. Significant differences in pain magnitude and urinary residual sense of indwelling catheters were observed among the three groups, with inhalation of linalyl acetate being significantly more effective than inhalation of lavender or vehicle. Inhalation of linalyl acetate is an effective nursing intervention to relieve pain and urinary residual sense of indwelling urinary catheters following their removal from patients who underwent CRC surgery.” As taken from Yu SH and Seol GH. 2017. Evid. Based. Complement. Alternat. Med. 2017, 3954181. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28154606>

“Ischemic stroke remains an important cause of disability and mortality. Hypertension is a critical risk factor for the development of ischemic stroke. Control of risk factors, including hypertension, is therefore important for the prevention of ischemic stroke. Linalyl acetate (LA) has been reported to have therapeutic effects in ischemic stroke by modulating intracellular Ca²⁺ concentration and having anti-oxidative properties. The preventive efficacy of LA has not yet been determined. This study therefore investigated the preventive efficacy of LA in rat aortas exposed to hypertension related-ischemic injury, and the mechanism of action of LA. Hypertension was induced in vivo following ischemic injury to the aorta induced by oxygen-glucose deprivation and reoxygenation in vitro. Effects of LA were assayed by western blotting, by determining concentrations of lactate dehydrogenase (LDH) and reactive oxygen species (ROS) and by vascular contractility assays. LA significantly reduced systolic blood pressure in vivo. In vitro, LA suppressed ischemic injury-

induced expression of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit p47phox, as well as ROS production, LDH release, and ROS-induced endothelial nitric oxide synthase suppression. These findings indicate that LA has anti-hypertensive properties that can prevent hypertension-related ischemic injury and can prevent NADPH oxidase-induced production of ROS." As taken from Hsieh YS et al. 2018. PLoS One 13(5), e0198082. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29799836>

6.3. Nervous system

"There is no evidence from the subchronic studies on linalool or its esters that linalyl acetate has any significant potential for neurotoxic effects, but these endpoints have not been specifically examined."

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

"The local anaesthetic activity of the essential oil of *Lavandula angustifolia* is evaluated. 1. This essential oil and pure linalyl acetate (0.01-10µg/ml) were able to drastically reduce, in a dose dependent manner, the electrically evoked contractions of rat phrenic-hemidiaphragm. This finding excludes antimuscarinic activity of linalyl acetate. 2. A rabbit conjunctival reflex test treatment with a solution of the same test substances (30-2500 µg/ml administered in the conjunctival sac) results in a dose-dependent increase in the number of stimuli necessary to provoke the reflex (i.e. suppression of the reflex). The local anaesthetic activity is not caused by antimuscarinic activity of these compounds, but is suggested to be related to effects on the Na⁺ and /or Ca⁺⁺ channels."

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

"/SIGNS AND SYMPTOMS/ Nervous and digestive symptoms develop from repeated skin exposure ... persons sensitive to this oil may develop photodermatitis, eczema, and pigment changes. /Oil of bergamot/ [Gosselin, R.E., H.C. Hodge, R.P. Smith, and M.N. Gleason. Clinical Toxicology of Commercial Products. 4th ed. Baltimore: Williams and Wilkins, 1976., p. II-33] **PEER REVIEWED**"

"/CASE REPORTS/ An 18-month-old boy ingested a small amount of homemade lavandin extract. The child developed a central nervous system depression and a confused state three hours after ingestion. The electroencephalogram showed fast rhythm disorders consistent with a toxic etiology. The outcome was favorable. Poisoning was confirmed by headspace-gas chromatography-mass spectrometry. Linalyl acetate, linalyl formate, and acetone were identified in pure lavandin extract and in the child's blood and urine. We report the only case of lavandin extract poisoning confirmed by toxicological analysis. [Landelle C et al; Clin Toxicol (Phila) 46 (4): 279-81 (2008)] **PEER REVIEWED** PubMed Abstract"

As taken from HSDB, 2017

"Inhalation exposure of Swiss mice to 2.74 mg linalyl acetate/L air during 90 minutes led to reduced motor activity compared to untreated controls. The effect was more severe in mice of aged 6-8 weeks (up to 100% reduction) than in mice of 6 months (up to 81% reduction). A relationship with dose was suspected, based on the (not reported) results of a separate test with a double dose in old mice (ref. 16)."

As taken from OECD-SIDS (2002), Linalyl acetate (115-95-7) p10 & p 44. Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

"Citrus aurantium L. blossoms are an important medicinal plant part in Iran and some other countries. It is used in traditional medicine as an antiseizure and anticonvulsant natural agent. Early in vitro research of the anticonvulsant activity of the blossom extracts were done but there has

been no investigation focused on the blossom essential oil and its anticonvulsant activity. The anticonvulsant activity of the essential oil of *C. aurantium* blossoms (neroli) was investigated. The anticonvulsant activity of neroli was assessed in pentylenetetrazole (PTZ)-induced convulsion by i.v. and i.p. methods and maximal electroshock (MES) in mice, with diazepam as the standard drug. While mechanistic studies were conducted using flumazenil, a GABA A-benzodiazepine receptor complex site antagonist. Neroli produced protection against clonic by i.v administration of PTZ at 20 and 40 mg/kg, compared with protection with benzodiazepine. The mean onset and percentage protection against convulsion in neroli-treated mice were reduced by flumazenil. Intraperitoneal PTZ also decreased the latency of clonic seizure in the neroli (40 mg/kg) treated group. We also showed that neroli (20 and 40 mg/kg), exhibited inhibition of the tonic convulsion induced by MES and decreased the mortality rate. Neroli was analyzed by GC and GC-MS and twenty three constituents, representing 91.0 % of the chromatographical oil were identified. The major components of neroli were characterized as linalool (28.5%), linalyl acetate (19.6%), nerolidol (9.1%) E,E-farnesol (9.1%), α -terpineol (4.9%) and limonene (4.6%) which might be responsible for the anticonvulsant activity. The results suggest that neroli possesses biologically active constituent(s) that have anticonvulsant activity which supports the ethnomedicinal claims of the use of the plant in the management of seizure.” As taken from Azanchi T et al. 2014. Nat. Prod. Commun. 9(11), 1615-8. PubMed, 2015

“The analgesic and anti-inflammatory properties of *Citrus aurantium* L. blossoms essential oil (neroli) were investigated in mice and rats. The analgesic activity of neroli was assessed by acetic acid-induced writhing and Eddy's hot plate methods, while acute and chronic anti-inflammatory effects were investigated by inflammatory paw edema in rat and the cotton pellet-induced granuloma tissue model, respectively. Mechanistic studies were conducted using L-nitro arginine methyl ester (L-NAME), an inhibitor of NO synthase. Neroli significantly decreased the number of acetic acid-induced writhes in mice compared to animals that received vehicle only. Also, it exhibited a central analgesic effect, as evidenced by a significant increase in reaction time in the hot plate method. The oil also significantly reduced carrageenan-induced paw edema in rats. The inhibitory activity of neroli (especially at 40 mg/kg) was found to be very close to the standard drug, diclofenac sodium (50 mg/kg). In cotton pellet-induced granuloma, neroli was effective regarding the transudate and granuloma formation amount. Neroli was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and twenty-three constituents, representing 91.0 % of the oil, were identified. The major components of neroli were characterized as linalool (28.5 %), linalyl acetate (19.6 %), nerolidol (9.1 %), E,E-farnesol (9.1 %), α -terpineol (4.9 %), and limonene (4.6 %), which might be responsible for these observed activities. The results suggest that neroli possesses biologically active constituent(s) that have significant activity against acute and especially chronic inflammation, and have central and peripheral antinociceptive effects which support the ethnomedicinal claims of the use of the plant in the management of pain and inflammation.” As taken from Khodabakhsh P et al. 2015. J. Nat. Med. 69(3), 324-31. PubMed, 2016 available at: <http://www.ncbi.nlm.nih.gov/pubmed/25762161>

“This study sought to investigate the effects of essential oil from lemon (*Citrus limoni*) peels on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in vitro. The essential oil was extracted by hydrodistillation, dried with anhydrous Na₂SO₄ and characterized using gas chromatography. Antioxidant properties of the oil and inhibition of pro-oxidant-induced lipid peroxidation in rats brain homogenate were also assessed. The essential oil inhibited AChE and BChE activities in a concentration-dependent manner. GC analysis revealed the presence of sabinene, limonene, α -pinene, β -pinene, neral, geranial, 1,8-cineole, linalool, borneol, α -terpineol, terpinen-4-ol, linalyl acetate and β -caryophyllene. Furthermore, the essential oil exhibited antioxidant activities as typified by ferric reducing property, Fe(2+)-chelation and radicals [DPPH, ABTS, OH, NO] scavenging abilities. The inhibition of AChE and BChE activities, inhibition of pro-oxidant induced lipid peroxidation and antioxidant activities could be possible mechanisms for the use of the essential oil in the management and prevention of oxidative stress-induced

neurodegeneration.” As taken from Oboh G et al. 2014. J. Oleo. Sci. 63(4), 373-81. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24599102>

“Olfactory stimuli are used in aromatherapy to enhance mood, well-being and work efficiency. Nevertheless, the impact of fragrances on cognitive performance in humans is not well understood. The present investigation aimed to evaluate the effects of 1,8-cineol, jasmine absolute ether, linalyl acetate and peppermint essential oil on human vigilance performance. The odorants were administered by means of inhalation and, except for peppermint essential oil, were tested at 2 different dosages. Performance in a standard visual vigilance task was measured in terms of speed and accuracy and subjective ratings of the odorants were assessed in terms of pleasantness, intensity, arousal and stress. We hypothesized that 1,8-cineol, jasmine absolute ether and peppermint essential oil would improve vigilance performance, whereas linalyl acetate would impair such performance. Comparison of the performances of the seven independent experimental groups with that of a control group did not show any of the expected effects. In contrast, inhalation of linalyl acetate decreased reaction times. Within-group analyses, however, revealed significant interactions between subjective ratings of the odorants and task performance. The results of the present investigation emphasize the high impact of subjective factors on the modulation of attentional functions by olfactory stimuli in humans. [Heuberger E, Ilmberger J; Nat Prod Commun 5 (9): 1441-6 (2010)] **PEER REVIEWED** PubMed Abstract”

“Terpenoids are major subcomponents in aroma substances which harbor sedative physiological potential. We have demonstrated that various monoterpenoids such as the acyclic linalool enhance GABAergic currents in an allosteric manner in vitro upon overexpression of inhibitory $\alpha 1\beta 2$ GABA_A receptors in various expression systems. However, in plants or humans, i.e., following intake via inhalation or ingestion, linalool undergoes metabolic modifications including oxygenation and acetylation, which may affect the modulatory efficacy of the generated linalool derivatives. Here, we analyzed the modulatory potential of linalool derivatives at $\alpha 1\beta 2\gamma 2$ GABA_A receptors upon transient overexpression. Following receptor expression control, electrophysiological recordings in a whole cell configuration were used to determine the chloride influx upon co-application of GABA EC₁₀₋₃₀ together with the modulatory substance. Our results show that only oxygenated linalool metabolites at carbon 8 positively affect GABAergic currents whereas derivatives hydroxylated or carboxylated at carbon 8 were rather ineffective. Acetylated linalool derivatives resulted in non-significant changes of GABAergic currents. We can conclude that metabolism of linalool reduces its positive allosteric potential at GABA_A receptors compared to the significant potentiation effects of the parent molecule linalool itself.” As taken from Milanos S et al. 2017. Food Chem. 5, 46. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28680877>

linalool and linalyl acetate showed a sedative effect and marked narcotic actions, respectively. Elshafie HS and Camele I (2017)

“In an inhalation study, a decrease in motor activity was observed in Swiss mice exposed to 2.74 mg linalyl acetate/L air for 90 minutes. A 100 % reduction in motor activity was reported in mice aged 6–8 weeks, and up to 81 % reduction in mice aged 6 months old (HSDB).”

As taken from AICIS, 2018

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

“The ability of linalyl acetate to induce hepatic enzymes was investigated in female Sprague-Dawley rats. Rats (8/group) were dosed by gavage with 782 mg/kg or 1563 mg/kg linalyl acetate in corn oil for 4 consecutive days. Control animals received corn oil alone. The animals were sacrificed and the livers removed and weighed. The livers of each animal were homogenized and centrifuged. The supernatant was then assayed for P-450 and P-448 activity, utilizing para-nitroanisole O-demethylase (PNAS), 7-ethoxycoumarin O-demethylase (7EC) and 7-ethoxyresorufin O-demethylase (ETR) as substrates. Liver weights were increased in the high-dose

group as compared to the controls; liver to body weight ratios were significantly increased in the high-dose group as compared to the low-dose group and the controls. PNAS and 7EC activities were significantly greater in both the low- and high-dose groups as compared to the controls; ETR activity was not increased in treated animals as compared to controls. Based on these results it was concluded that linalyl acetate is an inducer of P-450 but not of P-448 (RIFM, 1986b)."

As taken from Letizia C S et al (2003b). Fragrance material review on linalyl acetate. Food and Chemical Toxicology, 41, 965-976.

"Linalool and linalyl acetate are the principal components of many essential oils known to possess several biological activities, attributable to these monoterpene compounds. In this work, we evaluated individually the anti-inflammatory properties of (-) linalool, that is, the natural occurring enantiomer, and its racemate form, present in various amounts in distilled or extracted essential oils. Because in the linalool-containing essential oils, linalyl acetate, is frequently present, we also examined the anti-inflammatory action of this monoterpene ester. Carrageenin-induced edema in rats was used as a model of inflammation. The experimental data indicate that both the pure enantiomer and its racemate induced, after systemic administration, a reduction of edema. Moreover, the pure enantiomer, at a dose of 25 mg/kg, elicited a delayed and more prolonged effect, while the racemate form induced a significant reduction of the edema only one hour after carrageenin administration. At higher doses, no differences were observed between the (-) enantiomer and the racemate; a further increase in the dose of both forms did not result in an increased effect at any time of observation. The effects of equi-molar doses of linalyl acetate on local edema were less relevant and more delayed than that of the corresponding alcohol. These findings suggest a typical pro-drug behavior of linalyl acetate. The results obtained indicate that linalool and the corresponding acetate play a major role in the anti-inflammatory activity displayed by the essential oils containing them, and provide further evidence suggesting that linalool and linalyl acetate-producing species are potentially anti-inflammatory agents." As taken from Peana AT et al. Phytomedicine. 2002 Dec; 9(8):721-6. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/12587692?dopt=Abstract>

"/ALTERNATIVE and IN VITRO TESTS/ In a preliminary experiment, ... lavender essential oil relaxed vascular smooth muscle. Thus, /these/ ... experiments were designed to investigate the relaxation mechanism of linalyl acetate as the major ingredient of lavender essential oil in rabbit carotid artery specimens. Linalyl acetate produced sustained and progressive relaxation during the contraction caused by phenylephrine. The relaxation effect of linalyl acetate at a concentration near the EC50 was partially but significantly attenuated by nitroarginine as an inhibitor of nitric oxide synthase, 1H-(1,2,4)oxadiazolo(4,3-a)quinoxaline-1-one as an inhibitor of guanylyl cyclase, or by the denudation of endothelial cells. In specimens without endothelium, the phenylephrine-induced contraction and phosphorylation of myosin light chain (MLC) were significantly attenuated after the pretreatment with linalyl acetate. The relaxation caused by linalyl acetate in the endothelium-denuded specimens was clearly inhibited by calyculin A as an inhibitor of MLC phosphatase, although not by ML-9 as an inhibitor of MLC kinase. Furthermore, suppression of the phenylephrine-induced contraction and MLC phosphorylation with linalyl acetate was canceled by the pretreatment with calyculin A. These results suggest that linalyl acetate relaxes the vascular smooth muscle through partially through activation of nitric oxide/cyclic guanosine monophosphate pathway, and partially MLC dephosphorylation via activating MLC phosphatase. [Koto R et al; J Cardiovasc Pharmacol 48 (1): 850-6 (2006)] **PEER REVIEWED** PubMed Abstract"

"The sedative properties of the essential oil of Lavender (*Lavandula angustifolia* Miller) and of its main constituents--linalool and linalyl acetate--were investigated in mice followed up in a series of experimental procedures. The significant decrease in the mobility of female and male laboratory animals under standardized experimental conditions is found to be closely dependent on the exposure time to the drugs. Nevertheless after an injection of caffeine into mice a hyperactivity was observed which was reduced to nearly a normal mobility only by inhalation of these fragrance

drugs. ... [Buchbauer G et al; Z Naturforsch (C) 46 (11-12): 1067-72 (1991)] **PEER REVIEWED** PubMed Abstract”

“Acute Exposure/ Male and female Sprague-Dawley rats (5/sex/dose) received a single i.p. injection of 1120, 1590, 2240, 3160 or 4470 mg/kg linalyl acetate in corn oil. The initial body weights of the rats ranged from 233 to 297 g. Mortality and/or systemic observations were recorded 1, 3 and 6 h post dose and subsequently once daily for 14 days. Mortality and moribundity were recorded twice daily. Gross necropsy was performed on all animals. No deaths were observed at the three lowest dose levels; 7/10 deaths were observed at 3160 mg/kg and 10/ 10 deaths were observed at 4470 mg/kg. The combined LD50 was calculated to be 2864 mg/kg (95% C.I. 2414-3399 mg/kg; the LD50 in male rats was calculated to be 2778 mg/kg (95% C.I. 2246-3435) and the LD50 in female rats was calculated to be 2984 mg/kg (95% C.I. 2293-3884). Clinical signs observed in all dose groups included depression, ataxia, urine stains, rough coat and soft feces. Labored respiration, wheezing, hunched position, and red stains on the nose and/or eyes were observed at the two highest dose groups; prostration, tremors and lacrimation were also observed at the highest dose level. Necropsy revealed discolored lungs, thickened lobes in the liver and a thick white material adhered to and covering the liver and spleen. In addition, discolored liver, kidneys and adrenals and abnormal contents in the intestines, abdominal cavity and thoracic cavity were also observed in the animals that died during the study. [Letizia CS et al; Food Chem Toxicol 41 (7): 965-76 (2003)] **PEER REVIEWED**”

As taken from HSDB, 2017

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 linalyl acetate 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 linalyl acetate. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	<1	Carmines, 2002 & Rustemeier et al., 2002
	12	Baker et al., 2004a
	0.65	JTI KB Study Report(s)
	104	
	90	Roemer et al., 2014
In vitro genotoxicity	<1	Carmines, 2002 & Roemer et al., 2002
	12	Baker et al., 2004c
	0.65	Renne et al., 2006

	0.65 104	JTI KB Study Report(s)
	30.8 90	fGLH Study Report (2010) Roemer et al., 2014
In vitro cytotoxicity	<1 12	Carmines, 2002 & Roemer et al., 2002 Baker et al., 2004c
	0.65 104	JTI KB Study Report(s)
	30.8 90	fGLH Study Report (2010) Roemer et al., 2014
	<1 12	Carmines, 2002 & Vanscheeuwijck et al., 2002 Baker et al., 2004c
Inhalation study	0.65 104	Renne et al., 2006 JTI KB Study Report(s)
	90	Schramke et al., 2014
Skin painting	0.65 104	JTI KB Study Report(s)
In vivo genotoxicity	90	Schramke et al., 2014

Transfer studies:

In a pyrolysis study, 61.7% of linalyl acetate 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 linalyl acetate 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 linalyl acetate 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	1.3	JTI KB Study Report(s)
In vitro cytotoxicity	1.3	JTI KB Study Report(s)

Aerosol from heated tobacco stick(s) containing Linalyl acetate 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	0.038	Labstat International Inc. (2021a) Labstat International Inc. (2023a)

In vitro genotoxicity	0.038	Labstat International Inc. (2021b) Labstat International Inc. (2023b)
In vitro cytotoxicity	0.038	Labstat International Inc. (2021b) Labstat International Inc. (2023b)

10. Ecotoxicity

10.1. Environmental fate

"Linalyl acetate's production and use in extracts, perfumery, as a flavoring agent, and as a substitute for petitgrain oil may result in its release to the environment through various waste streams. If released to air, a vapor pressure of 0.111 mm Hg at 25 deg C indicates linalyl acetate will exist solely as a vapor in the atmosphere. Vapor-phase linalyl acetate will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals and ozone; the half-life for these reactions in air are estimated to be 200 and 38 minutes, respectively. Linalyl acetate may also react with nitrate radicals in the atmosphere. Linalyl acetate does not contain chromophores that absorb at wavelengths >290 nm and, therefore, is not expected to be susceptible to direct photolysis by sunlight. If released to soil, linalyl acetate is expected to have moderate mobility based upon an estimated Koc of 430. Volatilization from moist soil surfaces is expected to be an important fate process based upon an estimated Henry's Law constant of 1.7×10^{-3} atm-cu m/mole. Linalyl acetate may volatilize from dry soil surfaces based on its vapor pressure and that it exists as a liquid under environmental conditions. Utilizing the Japanese MITI test, 75% of the Theoretical BOD was reached in 4 weeks indicating that biodegradation is an important environmental fate process. If released into water, linalyl acetate is expected to adsorb to suspended solids and sediment based upon the estimated Koc. Volatilization from water surfaces is expected to be an important fate process based upon this compound's estimated Henry's Law constant. Estimated volatilization half-lives for a model river and model lake are 4.8 hours and 5.8 days, respectively. An estimated BCF of 180 suggests the potential for bioconcentration in aquatic organisms is high. The hydrolysis half-life is <24 hours at 20 deg C, with expected end products of linalool and acetic acid. Occupational exposure to linalyl acetate may occur through inhalation and dermal contact with this compound at workplaces where linalyl acetate is produced or used. Monitoring data indicate that the general population may be exposed to linalyl acetate via inhalation of product vapors, ingestion of food, and dermal contact with consumer products containing linalyl acetate. (SRC) **PEER REVIEWED**"

"AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 430(SRC), determined from a structure estimation method(2), indicates that linalyl acetate is expected to adsorb to suspended solids and sediment(SRC). Volatilization from water surfaces is expected(3) based upon an estimated Henry's Law constant of 1.7×10^{-3} atm-cu m/mole(SRC), developed using a fragment constant estimation method(2). Using this Henry's Law constant and an estimation method(3), volatilization half-lives for a model river and model lake are 4.8 hours and 5.8 days, respectively(SRC). The hydrolysis half-life is <24 hours at 20 deg C, with expected end products of linalool and acetic acid(4). According to a classification scheme(5), an estimated BCF of 180(SRC), from its log Kow of 3.93(6) and a regression-derived equation(2), suggests the potential for bioconcentration in aquatic organisms is high(SRC). A 75% of Theoretical BOD using activated sludge in the Japanese MITI test(4) suggests that biodegradation is an important environmental fate process in water(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Nov 19, 2015: <http://www2.epa.gov/tsca-screening-tools> (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (4) OECD; SIDS Initial Assessment Report For SIAM 14, Linalyl Acetate (CAS No. 115-95-7), March 2002. Available from, as of Nov 19, 2015: (5) Franke C et al; Chemosphere 29: 1501-14 (1994) (6) Griffin S et al; Environ Health Perspect 107: 481-7 (1999)] **PEER REVIEWED**"

Volatilization from Water/Soil:

The Henry's Law constant for linalyl acetate is estimated as 1.7×10^{-3} atm-cu m/mole(SRC) using a fragment constant estimation method(1). This Henry's Law constant indicates that linalyl acetate is expected to volatilize from water surfaces(2). Based on this Henry's Law constant, the volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 3 m/sec)(2) is estimated as 4.8 hours(SRC). The volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec)(2) is estimated as 5.8 days(SRC). Linalyl acetate's Henry's Law constant indicates that volatilization from moist soil surfaces may occur(SRC). Linalyl acetate has a reported vapor pressure of 0.111 mm Hg(3) and exists as a liquid under environmental conditions; therefore, linalyl acetate may volatilize from dry soil(SRC). [(1) Meylan WM, Howard PH; Environ Toxicol Chem 10: 1283-93 (1991) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (3) Perry RH, Green D; Perry's Chemical Handbook. 6th ed. New York, NY: McGraw Hill p. 3-57 (1984)] **PEER REVIEWED**

Environmental Water Concentrations:

SURFACE WATER: Linalyl acetate was identified at an unreported concentration in water samples taken from Stockacher Aach, an industrially polluted river in southwest Germany(1). [(1) Juettner F; Wat Sci Tech 25: 155-64 (1992)] **PEER REVIEWED**

TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 430(SRC), determined from a structure estimation method(2), indicates that linalyl acetate is expected to have moderate mobility in soil(SRC). Volatilization of linalyl acetate from moist soil surfaces is expected to be an important fate process(SRC) given an estimated Henry's Law constant of 1.7×10^{-4} atm-cu m/mole(SRC), using a fragment constant estimation method(2). Linalyl acetate has a reported vapor pressure of 0.111 mm Hg(3) and exists as a liquid under environmental conditions; therefore, linalyl acetate may volatilize from dry soil(SRC). A 75% of Theoretical BOD using activated sludge in the Japanese MITI test(4) suggests that biodegradation is an important environmental fate process in soil(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Nov 19, 2015: <http://www2.epa.gov/tsca-screening-tools> (3) Griffin S et al; Environ Health Perspect 107: 481-7 (1999) (4) OECD; SIDS Initial Assessment Report For SIAM 14, Linalyl Acetate (CAS No. 115-95-7), March 2002. Available from, as of Nov 19, 2015: **PEER REVIEWED**

Soil Adsorption/Mobility:

Using a structure estimation method based on molecular connectivity indices(1), the Koc of linalyl acetate can be estimated to be 430(SRC). According to a classification scheme(2), this estimated Koc value suggests that linalyl acetate is expected to have moderate mobility in soil. [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Nov 19, 2015: <http://www2.epa.gov/tsca-screening-tools> (2) Swann RL et al; Res Rev 85: 17-28 (1983)] **PEER REVIEWED**

Artificial Pollution Sources:

Linalyl acetate's production and use in extracts, perfumery, as a flavoring agent, and as a substitute for petitgrain oil(1) may result in its release to the environment through various waste streams(SRC). [(1) Lewis RJ Sr; Hawley's Condensed Chemical Dictionary. 15th ed. New York, NY: Van Nostrand Reinhold Co., p. 757-8 (2007)] **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), linalyl acetate, which has a vapor pressure of 0.111 mm Hg at 25 deg C(2), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase linalyl acetate is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals and ozone(SRC); the half-lives for these reactions in air are estimated to be 200 and 38 minutes, respectively(SRC), calculated from respective rate constants of 1.2×10^{-10} and 4.3×10^{-16} cu

cm/molecule-sec at 25 deg C(SRC) that were derived using structure estimation methods(3). Linalyl acetate may also react with nitrate radicals in the atmosphere(4). Linalyl acetate does not contain chromophores that absorb at wavelengths >290 nm(5) and, therefore, is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Perry RH, Green D; Perry's Chemical Handbook. 6th ed. New York, NY: McGraw Hill p. 3-57 (1984) (3) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Nov 19, 2015: <http://www2.epa.gov/tsca-screening-tools> (4) Atkinson R; J Phys Chem Ref Data, Monogr 2: 1-216 (1994) (5) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 8-12 (1990)] **PEER REVIEWED**

As taken from HSDB, 2017

EPI Suite provides the following information:

Henry's Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	1.74E-003 atm-m3/mole (1.76E+002 Pa-m3/mole)
Group Method:	Incomplete
Henry's LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.682E-003 atm-m3/mole (1.704E+002 Pa-m3/mole) VP: 0.131 mm Hg (source: MPBPVP) WS: 20.1 mg/L (source: WSKOWWIN)

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

Log Kow used:	3.93 (exp database)
Log Kaw used:	-1.148 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	5.078
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):	0.6443 0.9294 2.6935 (weeks-months) 3.6401 (days-weeks) 0.6648 0.6802 0.1133
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	14.8 Pa (0.111 mm Hg)
Log Koa (Koawin est):	5.078
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	2.03E-007 2.94E-008

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	7.32E-006
Mackay model:	1.62E-005
Octanol/air (Koa) model:	2.35E-006

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

OVERALL OH Rate Constant =	116.2433 E-12 cm3/molecule-sec
Half-Life =	0.092 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	1.104 Hrs

Ozone Reaction:

OVERALL Ozone Rate Constant =	43.174999 E-17 cm3/molecule-sec
Half-Life =	0.027 Days (at 7E11 mol/cm3)
Half-Life =	38.222 Min
Reaction With Nitrate Radicals May Be Important!	
Fraction sorbed to airborne particulates (phi): 1.18E-005 (Junge-Pankow, Mackay avg) 2.35E-006 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	432.4 L/kg (MCI method)
Log Koc:	2.636 (MCI method)
Koc :	1080 L/kg (Kow method)
Log Koc:	3.033 (Kow method)

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

Total Kb for pH > 8 at 25 deg C:	4.610E-002 L/mol-sec
Kb Half-Life at pH 8:	174.013 days
Kb Half-Life at pH 7:	4.764 years

(Total Kb applies only to esters, carbmates, alkyl halides)

Volatilization from Water: Henry LC: 0.00174 atm-m³/mole (estimated by Bond SAR Method)

Half-Life from Model River:	1.901 hours
Half-Life from Model Lake:	138.2 hours (5.759 days)

Removal In Wastewater Treatment:

Total removal:	54.06 percent
Total biodegradation:	0.21 percent
Total sludge adsorption:	22.37 percent
Total to Air:	31.48 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.13	0.494	1000
Water	21.6	900	1000
Soil	77.8	1.8e+003	1000
Sediment	0.518	8.1e+003	0

Persistence Time: 398 hr

"Linalyl acetate will partition primarily to water (Mackay level III modelling). In a hydrolysis study linalyl acetate was found to disappear from the test medium within 2.4 hours at pH 4, 7 and 9 (at 50°C) (t_{1/2} < 24 hours at 20 degreeC). Hydrolysis products are linalool and acetic acid." As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

The Ecological Categorization Results from the Canadian Domestic Substances List state that 1,6-octadien-3-ol, 3,7-dimethyl-, acetate (CAS RN 115-95-7) is not persistent in the environment:

Media of concern leading to Categorization	Air-Soil
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	37.5

MITI probability of biodegradation	0.6802
TOPKAT probability of biodegradation	0.984
EPI Predicted hydrolysis half-life (days)	1.74E+003
EPI Predicted Ozone reaction half-life (days)	0.02654
EPI Predicted Atmospheric Oxidation half-life (days)	0.09201

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

10.2. Aquatic toxicity

Based on current VoU (2011), linalyl acetate presents a risk to the aquatic compartment in the screening level assessment (Api et al., 2015)

“Fish and Invertebrates In a 96-hour flow-through test with carps (*Cyprinus carpio*), fish were exposed to 10, 18, 32, 56 and 100 mg/L (nominal concentrations). Mean measured concentrations were 7.9, 12.3, 20.1, 27.3 and 27.2 mg/L. At the highest concentrations (32 mg/L and above) all fish died and a precipitate was observed. At all concentrations hypoactive swimming behaviour, loss of equilibrium and/or immobility was observed. A 96-hour LC50 of 11 mg/L was derived based on measured concentrations....

A calculation with ECOSAR yields a 96-hour LC50 value of 2.86 mg/L

For *Daphnia magna* a 48-hour EC50 was calculated by the reviewer with the Spearman-Kärber method. The EC50 derived, based on nominal concentrations in a static test, was 15 mg/L (mean measured 6.2 mg/L). In accordance with the expectation of hydrolysis, the measured concentration after 48 hours was below the detection limit (except in the highest concentration). As the EC50 for linalool is reported to be 59 mg/L (SIAR on linalool), the toxicity observed can not be attributed to the hydrolysis product only. The EC50 value based on mean measured concentrations is not very reliable, since this mean concentration overestimates exposure to linalyl acetate ($t_{1/2}$ hydrolysis <24 hours). However, the alternative of using the nominal concentrations to derive an EC50 is even less realistic.... A calculation with ECOSAR yields a 48-hour EC50 value of 2.91 mg/L Conclusions: Linalyl acetate is moderately toxic to fish and toxic to daphnia. All values are based on measurements of the parent compound, which is expected to hydrolyse very quickly. The ECOSAR prediction provides an indication for a possible higher toxicity than found in the described tests. The 96-hour LC50 in fish is 11 mg/L. The 48-hour EC50 for daphnia is 6.2 mg/L (based on mean measured concentrations).”

“Algae In a test with algae (*Scenedesmus subspicatus*, 72-hours exposure), a reduction of biomass was seen at nominal concentrations of 4.4 mg/L and above. The 72-hour EbC50 was 16 mg/L and the 72-hour ErC50 was 62 mg/L, both based on nominal concentrations. Analyses showed test concentrations below the limit of detection for all treatments after 72 hours, which is again expected in view of hydrolytic instability of the compound. The EC50 for linalool is >1000 mg/L (SIAR linalool). Based on the considerations mentioned regarding the daphnia study (see above), the EC50 were based on mean measured concentrations, EbC50 4.2 mg/L and ErC50 16 mg/L ... The NOEC for effects on algal growth based on the area under the growth curve is <4.4 mg/L (measured concentration <1.2 mg/L) A calculation with ECOSAR yields a 96-hour EC50 value of 0.244 mg/L”. Conclusions: Based on the information available linalyl acetate is toxic to algae (72-hour EC50 4.2 mg/L). The ECOSAR prediction provides an indication for a possible higher toxicity than found in the described test.”

“Microorganisms

In a test with activated sludge (according to OECD guideline 209) the EC50 for the inhibition of micro-organisms was determined to be 415 mg/L. The substance was dissolved in ethanol, which explains that the EC50 value is above water solubility of the EC50

Conclusion:

Linalyl acetate is of low toxicity towards aquatic micro-organisms.”

“Determination of PNEC aqua

Data are available from short term tests at 3 trophic levels. Based on the lowest value (calculated for algae EbC50 4.2 mg/L) and applying an assessment factor of 100 in accordance with the OECD guidance the resultant PNECaqua is 0.042 mg/L. The same assessment factor would be recommended using the EU TGD as no significant difference in sensitivity between species tested appears to be present.

Conclusions: Linalylacetate is of moderate hazard to the aquatic environment with a PNECaqua of 0.042 mg/L.”

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7). Available at <http://www.inchem.org/documents/sids/sids/115957.pdf>

The Ecological Categorization Results from the Canadian Domestic Substances List state that 1,6-octadien-3-ol, 3,7-dimethyl-, acetate (CAS RN 115-95-7) is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	1.3329
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	1.564
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	1.3329
Toxicity to fish (LC50 in mg/l) as predicted by Aster	2.36841
Toxicity to fish (LC50 in mg/l) as predicted by PNN	4.19406
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	1.042
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	8.25E-003

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

“Twenty acyclic monoterpenes with different functional groups (acetoxyl, hydroxyl, carbonyl and carboxyl) bearing a variable number of carbon double bonds were assayed as repellent and larvicidal agents against the West Nile vector Culex pipiens. Seven of them were derivatives that were synthesized through either hydrogenation or oxidation procedures. All repellent compounds were tested at the dose of 1mgcm(-2) and only neral and geranial were also tested at a 4-fold lower dose (0.25mgcm(-2)). Repellency results revealed that geranial, neral, nerol, citronellol, geranyl acetate and three more derivatives dihydrolinalool (3), dihydrocitronellol (5) and dihydrocitronellyl

acetate (6) resulted in no landings. Based on the LC50 values the derivative dihydrocitronellyl acetate (6) was the most active of all, resulting in an LC50 value of 17.9mgL(-1). Linalyl acetate, citronellyl acetate, neryl acetate, geranyl acetate, dihydrocitronellol (5), dihydrocitronellal (7), citronellol, dihydrolinalyl acetate (2), citronellic acid and tetrahydrolinalyl acetate (1) were also toxic with LC50 values ranging from 23 to 45mgL(-1). Factors modulating toxicity have been identified, thus providing information on structural requirements for the selected acyclic monoterpenes. The acetoxyl group enhanced toxicity, without being significantly affected by the unsaturation degree. Within esters, reduction of the vinyl group appears to decrease potency. Presence of a hydroxyl or carbonyl group resulted in increased activity but only in correlation to saturation degree. Branched alcohols proved ineffective compared to the corresponding linear isomers. Finally, as it concerns acids, data do not allow generalizations or correlations to be made." As taken from Michaelakis A et al. 2014. Chemosphere 96, 74-80. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23938144>

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

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 115-95-7:

Values used to Generate ECOSAR Profile: Log Kow: 4.387 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 39.51 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations Esters

Vinyl/Allyl Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)	
Esters :	Fish	96-hr	LC50	1.060	
Esters :	Daphnid	48-hr	LC50	1.706	
Esters :	Green Algae	96-hr	EC50	0.497	
Esters :	Fish		ChV	0.048	
Esters :	Daphnid		ChV	0.587	

Esters :	Green Algae		ChV	0.277	
Esters :	Fish (SW)	96-hr	LC50	1.393	
Esters :	Mysid	96-hr	LC50	0.423	
Esters :	Fish (SW)		ChV	0.307	
Esters :	Mysid (SW)		ChV	0.240	
Vinyl/Allyl Esters :	Fish	96-hr	LC50	0.572	
Vinyl/Allyl Esters :	Daphnid	48-hr	LC50	1.997	
Vinyl/Allyl Esters :	Green Algae	96-hr	EC50	0.437	
Vinyl/Allyl Esters :	Fish		ChV	0.004 !	
Vinyl/Allyl Esters :	Daphnid		ChV	0.031 !	
Vinyl/Allyl Esters :	Green Algae		ChV	0.110	
Neutral Organic SAR :	Fish	96-hr	LC50	1.159	
(Baseline Toxicity) :	Daphnid	48-hr	LC50	0.820	
	Green Algae	96-hr	EC50	1.520	
	Fish		ChV	0.147	
	Daphnid		ChV	0.148	
	Green Algae		ChV	0.650	

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.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Record for 3,7-dimethyl-1,6-octadien-3-ol acetate:

Spec. Sci. Name =====	Resp. Site =====	Media Type =====	Exp. Type =====	Dose# =====	Endpoint =====	Effect =====	Signif. =====	Dose =====
Spec.	Exp.		Chem.	Res. Sample	BAF/BCF	Effect	Sig. Level	Dose Stat.

Common Name	Dur. (Days)	Test Loc.	Anal.	Unit		Meas.		Meth.
Trichoderma harzianum Fungus	1	CUL LAB	CM U		LOEL	MOR MORT	SIG <0.05	F (8.0-11.0) ul/ml
Trichoderma harzianum Fungus	1	CUL LAB	CM U		LOEL	MOR MORT	SIG <0.05	F (8.0-11.0) ul/ml
Trichoderma harzianum Fungus	1	CUL LAB	CM U		LOEL	MOR MORT	SIG <0.05	F (8.0-11.0) ul/ml
Verticillium fungicola Fungus	1	CUL LAB	CM U		LOEL	MOR MORT	SIG <0.05	F (8.0-11.0) ul/ml
Trichoderma harzianum Fungus	MYC 21	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F (6.0-11.0) ul/ml
Trichoderma harzianum Fungus	MYC 21	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F (6.0-11.0) ul/ml
Trichoderma harzianum Fungus	MYC 21	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F (6.0-11.0) ul/ml
Verticillium fungicola Fungus	MYC 21	CUL LAB	CM U		LOEL	POP ABND	SIG <0.05	F (6.0-11.0) ul/ml
Trichoderma harzianum Fungus	MYC 21	FLT LAB	DA U		LOEL	POP ABND	SIG <0.05	F (> 5- < 13) ul/eu

Trichoderma harzianum Fungus	MYC 21	FLT LAB	DA U	_____	LOEL _____	POP ABND	SIG <0.05	F (> 5- < 13) ul/eu _____
Trichoderma harzianum Fungus	MYC 21	FLT LAB	DA U	_____	LOEL _____	POP ABND	SIG <0.05	F (> 5- < 13) ul/eu _____
Verticillium fungicola Fungus	MYC 21	FLT LAB	DA U	_____	LOEL _____	POP ABND	SIG <0.05	F (> 5- < 13) ul/eu _____
Trichoderma harzianum Fungus	MYC 1	CUL LAB	CM U	_____	LOEL _____	POP ABND	SIG <0.05	F (7.5- 10.5) ul/ml _____
Trichoderma harzianum Fungus	MYC 1	CUL LAB	CM U	_____	LOEL _____	POP ABND	SIG <0.05	F (7.5- 10.5) ul/ml _____
Trichoderma harzianum Fungus	MYC 1	CUL LAB	CM U	_____	LOEL _____	POP ABND	SIG <0.05	F (7.5- 10.5) ul/ml _____
Verticillium fungicola Fungus	MYC 1	CUL LAB	CM U	_____	LOEL _____	POP ABND	SIG <0.05	F (7.5- 10.5) ul/ml _____
Linepithema humile Argentine Ant	_____7	NONE FIELDN	EN U	2 _____	LOEL _____	POP ABND	ASIG 0.05	F 2 g _____
Linepithema humile Argentine Ant	_____14	NONE FIELDN	EN U	2 _____	NOEL _____	POP ABND	ANOSIG 0.05	F 2 g _____
Linepithema		NONE	EN	2	NOEL	POP	ANOSIG	F 2 g

ma humile Argentine Ant	21	FIELDN	U			ABND	0.05	

As taken from US EPA ECOTOX database

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 115-95-7:

Values used to Generate ECOSAR Profile: Log Kow: 4.387 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 39.51 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	435.141 *

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

“Plant extracts, particularly plant oils, had been used and were still in use as repellents against mosquitoes. Some of them (e.g., lavender, geraniol, and citriodiol) have been notified by the European Commission as active substances to be used in repellents, which are categorized as biocides in product type 19. In the literature, it is known that these substances must be added to repellent products in high concentrations (e.g., 20% and more) in order to reach repellent efficacy. Therefore, the question arose whether they also have repellent effects if they were added as fragrances at low concentrations of 0.25 or 1% to registered active substances in order to obtain a better scent of this product. In the present study, the repellent effects of 0.25 and 1% additions of 15 plant extracts (citronellol, cinerol, citral, menthol, linalyl acetate, Eucalyptus citriodora, Eucalyptus globulus, Cymbopogon nardus, lilac, sandalwood, Vitex agnus castus, rosewood, lavender, geraniol, and paramenthan diol) when exposed on skin to hungry Aedes aegypti mosquitoes. These experiments showed that there was no repellent effect in any of these compounds even when the test was done already 10 min after distributing any of the compounds onto the hands of volunteers. These experiments have proven that these 15 compounds do not produce repellent effects as long as they are used in low concentrations of 0.25 or 1% as fragrances to ameliorate the odor of a notified repellent that is brought onto the skin.” As taken from Semmler M et al. 2014. Parasitol. Res. 113(1), 185-8. PubMed, 2015 available at: <http://www.ncbi.nlm.nih.gov/pubmed/24142288?dopt=AbstractPlus>

10.5. All other relevant types of ecotoxicity

Environmental Biodegradation:

AEROBIC: Linalyl acetate, present at 100 mg/L, reached 75% of its Theoretical BOD in 4 weeks using an activated sludge inoculum at 30 mg/L and the Japanese MITI test(1). [(1) OECD; SIDS Initial Assessment Report For SIAM 14, Linalyl Acetate (CAS No. 115-95-7), March 2002. Available from, as of Nov 19, 2015: **PEER REVIEWED**

The rate constant for the vapor-phase reaction of linalyl acetate with photochemically-produced hydroxyl radicals has been estimated as 1.2×10^{-10} cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 3.3 hours at an atmospheric concentration of 5×10^5 hydroxyl radicals per cu cm(1). The rate constant for the vapor-phase reaction of linalyl acetate with ozone has been estimated as 4.3×10^{-16} cu cm/molecule-sec at 25 deg C(SRC) that was derived using a structure estimation method(1). This corresponds to an atmospheric half-life of about 38 minutes at an atmospheric concentration of 7×10^{11} ozone molecules per cu cm(2). Linalyl acetate may also react with nitrate radicals in the atmosphere(3). The hydrolysis half-life is <24 hours at 20 deg C, with expected end products of linalool and acetic acid(4). Linalyl acetate does not contain chromophores that absorb at wavelengths >290 nm(5) and, therefore, is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Nov 19, 2015: <http://www2.epa.gov/tsca-screening-tools> (2) Atkinson R, Carter WPL; Chem Rev 84: 437-70 (1984) (3) Atkinson R; J Phys Chem Ref Data, Monogr 2: 1-216 (1994) (4) OECD; SIDS Initial Assessment Report For SIAM 14, Linalyl Acetate (CAS No. 115-95-7), March 2002. Available from, as of Nov 19, 2015: (5) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 8-12, 8-13 (1990)] **PEER REVIEWED**

Environmental Bioconcentration:

An estimated BCF of 180 was calculated in fish for linalyl acetate(SRC), using a log Kow of 3.93(1) and a regression-derived equation(2). According to a classification scheme(3), this BCF suggests the potential for bioconcentration in aquatic organisms is high(SRC). [(1) Griffin S et al; Environ Health Perspect 107: 481-7 (1999) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Nov 19, 2015: <http://www2.epa.gov/tsca-screening-tools> (3) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

As taken from HSDB, 2017

EPISuite provides the following information: **Bioaccumulation Estimates (BCFBAF v3.01):**

Log BCF from regression-based method:	2.260 (BCF = 182 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-0.4673 days (HL = 0.3409 days)
Log BCF Arnot-Gobas method (upper trophic):	2.096 (BCF = 124.7)
Log BAF Arnot-Gobas method (upper trophic):	2.096 (BAF = 124.7)
log Kow used:	3.93 (expkow database)

"Based on the very low log Kow of 3.9, linalyl acetate may accumulate (BCF of 412). However, in view of the rapid hydrolysis of the substance it is unlikely that significant uptake may occur."

As taken from OECD-SIDS (2002). Linalyl acetate (115-95-7).

The Ecological Categorization Results from the Canadian Domestic Substances List state that 1,6-octadien-3-ol, 3,7-dimethyl-, acetate (CAS RN 115-95-7) is not bioaccumulative in the environment:

Empirical Log Kow	3.93

Log Kow predicted by KowWin	4.39
Log BAF T2MTL predicted by Gobas	2.79621616880891
Log BCF 5% T2LTL predicted by Gobas	2.63595056026556
Log BCF Max predicted by OASIS	3.69578962080815
Log BCF predicted by BCFWIN	2.326

Data accessed June 2017 on the OECD website

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13. Last audited

May 2024

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8.¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 29 flavouring substances in the Flavouring Group Evaluation 18, as laid down in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have a genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. This revision deals with new toxicity data on the supporting substance myrcene [FL-no: 01.008], providing an appropriate NOAEL for the evaluation of candidate substance [FL-no: 02.146]. The Panel concluded that all 29 substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.146, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. However, based on mTAMDI calculations, for all candidate substances except [FL-no: 02.146] more reliable intake data are required for a re-evaluation. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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KEY WORDS

flavourings, safety, aliphatic, alicyclic, saturated, unsaturated, tertiary alcohols, FGE.18

¹ On request from the European Commission, Question No EFSA-Q-2013-00852, adopted on 07 May 2015.

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³ Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby and EFSA staff: Annamaria Rossi, Maria Carfi and Maria Anastassiadou for the support provided to this scientific opinion.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8. EFSA Journal 2015;13(5):4118, 115 pp. doi:10.2903/j.efsa.2015.4118

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 29 flavouring substances in the Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 29 flavouring substances belong to chemical groups 6 and 8, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision 3 of FGE.18 (FGE.18Rev3) is due to the availability of new toxicity data for myrcene, [FL-no: 01.008] which was used for the safety evaluation of the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

FGE.18Rev3 deals with 29 saturated and unsaturated aliphatic acyclic and alicyclic tertiary alcohols, aromatic tertiary alcohols and their esters. Based on their structures, the candidate substances can be subdivided into 8 subgroups.

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four substances [FL-nos: 02.129, 02.147, 02.168 and 02.197] the stereoisomeric composition of the mixture has not been specified.

Seventeen of the 29 candidate substances are classified into structural class I, eleven into structural class II and one into structural class III according to the decision tree approach.

Twenty-one out of the 29 candidate substances have been reported to occur in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, 28 of the 29 flavouring substances in this group have intakes in Europe that vary from 0.0012 to 27 $\mu\text{g}/\text{capita}$ per day, which are below the thresholds of concern for structural classes I, II and III substance of 1800, 540 and 90 μg per person per day, respectively. The remaining substance, terpineol [FL-no: 02.230] belonging to structural class I, has an MSDI of 1200 $\mu\text{g}/\text{capita}$ per day, which is also below the threshold for this structural class.

2-Methylpropan-2-ol [FL-no: 02.052] provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence and the lack of structural alerts for genotoxicity for this substance and its metabolites do not raise concern for *in vivo* genotoxicity. For the other substances in this group the available data considered valid do not give rise to any safety concerns with respect to genotoxicity.

Twenty-eight of the candidate substances are anticipated to be metabolised to innocuous products. For the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no metabolism data are available and therefore it cannot be predicted to be metabolised to innocuous products.

In Revision 2 of FGE.18, for substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no appropriate NOAEL was available and additional data were requested. In the present revision of FGE.18 (Revision 3), new toxicity data on the supporting substance myrcene [FL-no: 01.008] became available, providing an appropriate NOAEL of 44 mg/kg bw per day which was used for the evaluation of the candidate substance [FL-no: 02.146]. Based on the MSDI intake of 12 µg/capita per day, the margin of safety calculated for [FL-no: 02.146] is 2.2×10^5 .

Therefore, none of the 29 candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] would give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances, on the basis of the default MSDI approach.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for 25 candidate substances. However, information on purity criteria and/or stereoisomeric composition has not been specified sufficiently for four substances [FL-no: 02.129, 02.147, 02.168 and 02.197]. Thus, the final evaluation of the materials of commerce cannot be performed for these substances, pending further information.

Thus, for 25 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach: [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.146, 02.149, 02.150, 02.171, 02.181, 02.184, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808].

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI approach, are 3900 µg per person per day, and for the two remaining substances 7000 and 14000 µg per person per day respectively. These seventeen mTAMDI are all above the threshold of concern of 1800 µg per person per day for a structural class I substance. For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg per person per day and for the remaining two substances, 110 and 1600 µg per person per day, respectively. For ten substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg per person per day. For the one substance assigned to structural class III the mTAMDI is 5700 µg per person per day, which is above the threshold of concern for structural class III substances of 90 µg per person per day.

In conclusion, for all candidate substances except [FL-no: 02.146] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008⁴ on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012⁵. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000.⁶

FGE.78Rev1

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2.⁷

The substances [FL-nos: 01.008, 01.018, 01.040 and 01.061] were among the 14 substances for which the Panel had “reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing information on stereoisomerism/composition of mixture)” and also among those for which “additional toxicity data was requested”.

FGE.25Rev2

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31.⁸

The substances with [FL-nos: 01.035, 01.064, 01.070 and 01.035] were among the 27 candidate substances for which “additional toxicity data” were required by EFSA. For [FL-no: 01.035] also “additional information on composition” was requested.

FGE.18Rev2

On 30 September 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8.

For the flavouring substance [FL-no: 02.146], the Panel considered that “additional data” are needed including “information on specifications/stereoisomerism/composition of mixture”.

⁴ Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p.34-50.

⁵ Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1-161.

⁶ Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8-16.

⁷ EFSA Journal 2011;9(6):2178

⁸ EFSA Journal 2011;9(6):2177

On 21 November 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted a statement on the re-evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] based on additional data on a supporting substance.⁹

The Panel concluded that “linalool [FL-no: 02.013] is not sufficiently structurally related to 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for a re-evaluation of [FL-no: 02.146]. Accordingly, a 90-day study on 3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] or on a sufficiently structurally related substance has to be provided in order to establish on appropriate NOAEL”.

New data and relationship with other substances

On 5 and 11 July 2013, the applicant submitted additional data on the following acyclic terpene hydrocarbons [FL-nos: 01.008, 01.018, 01.040, 01.061, 01.035, 01.064, 01.070 and 02.146] represented by myrcene [FL-no: 01.008].

As regards the related substances also evaluated in these opinions, namely [FL-nos: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026, 01.029 and 01.059], data was submitted and are currently being evaluated (EFSA-Q-2013-00185 to – 00193).

As regards substance with [FL-no: 01.014], data should be submitted by 31 December 2013.¹⁰

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment on this group of flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

SUPPORTING DOCUMENTS

Submission by the European Flavour Association

INTERPRETATION OF THE TERMS OF REFERENCE

The above background and terms of reference include also a previous mandate received from the European Commission on 6 February 2013.¹¹ The present scientific opinion FGE.18Rev3 covers the safety assessment of the following flavouring substance: 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

⁹ EFSA Journal 2012;10(12):2995

¹⁰ This substance is in the process of being deleted from the Union List (DG SANTE, 2015)

¹¹ SANCO.E3/SH/km D (2013) Ares(2013)15188

ASSESSMENT

1. History of the Evaluation of the Substances in the Present FGE

The first version of the Flavouring Group Evaluation 18, FGE.18, dealt with 24 aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters.

The first Revision of FGE.18, FGE.18Rev1, included the assessment of six additional candidate substances [FL-nos: 02.120, 02.206, 02.226, 02.230, 02.253 and 09171]. Information on toxicity and/or metabolism on three of the substances were included [FL-nos: 02.120, 02.206 and 02.230]. Since the publication of FGE.18 additional information on specifications on 12 substances became available [FL-nos: 02.052, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.191, 02.197, 09.614, 09.617 and 09.671].

The second Revision of FGE.18, FGE.18Rev2, included the assessment of two additional candidate substances [FL-nos: 02.129 and 02.146]. Information on toxicity and/or metabolism on the substance [FL-no: 02.129] was included. No information on toxicity and/or metabolism was available for [FL-no: 02.146]. A search in open literature did not provide any further data on toxicity or metabolism for these substances. Since the publication of FGE.18Rev1 additional information on toxicity on [FL-nos: 02.120, 02.140, 02.144 and 02.197] was included. Furthermore, additional information on specifications on six substances was included [FL-nos: 02.147, 02.168, 02.197, 02.226, 02.230 and 02.253] (EFFA, 2010). Based on the additional information made available by Industry, it was also considered appropriate to merge subgroups 7 and 8 into one (subgroup 7).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.18	3 March 2006	http://www.efsa.europa.eu/en/scdocs/scdoc/331.htm	24
FGE.18Rev1	29 January 2008	http://www.efsa.europa.eu/en/scdocs/scdoc/978.htm	30
FGE.18Rev2	30 September 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1847.htm	32
FGE.18Rev3	7 May 2015	http://www.efsa.europa.eu/en/efsajournal/pub/4118.htm	29

The present Revision of FGE.18, FGE.18Rev3, includes the safety assessment of (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for which a 90-day toxicity study on a supporting substance has been provided. No data on toxicity and/or metabolism are available for the substance itself and a search in the open literature did not provide any pertinent information either. In the EFSA statement of 2012 (EFSA, 2012) the Panel has proposed a suitable supporting substance for [FL-no: 02.146], namely myrcene [FL-no: 01.008]. The safety evaluation of myrcene has been finalised in FGE.78Rev2 based on a new 90-day toxicity study (Bauter, 2013) for this substance. These new data will accordingly be used in the safety evaluation of (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146]. Substances in subgroup 3 with [FL-nos: 02.185, 02.191 and 09.669] are no longer used as flavouring substances in the EU according to the Industry (DG SANCO, 2012) and therefore they will not be considered in the present FGE.

1.1. Description

The present Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000) (The Procedure – shown in schematic form in Appendix B of this FGE), deals with 29 saturated and unsaturated aliphatic acyclic and alicyclic tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000). The 29 flavouring substances under consideration (candidate substances), with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

The present FGE consists of seven aliphatic saturated tertiary alcohols and one ester of such [FL-nos: 02.041, 02.052, 02.147, 02.181, 02.184, 02.219, 02.253 and 09.356]; five are aliphatic unsaturated tertiary alcohols which possess isolated terminal double bonds and two are esters thereof [FL-nos: 02.123, 02.144, 02.150, 02.168, 02.226, 09.614 and 09.671]; one aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond [FL-no: 02.146]; one aliphatic unsaturated tertiary alcohol which does not possess terminal double bonds [FL-no: 02.140]; two monocyclic saturated tertiary alcohols and one ester thereof [FL-nos: 02.054, 02.171 and 09.617]; two monocyclic unsaturated tertiary alcohols [FL-nos: 02.129 and 02.230]; two mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond [FL-nos: 02.149 and 02.206]; one bicyclic unsaturated ester [FL-no: 09.808], one tricyclic saturated ester [FL-no: 09.171] and two bi- and tricyclic tertiary alcohols [FL-nos: 02.197 and 02.120] and one tertiary alcohol with an aromatic substituent [FL-no: 02.203].

A summary of the safety evaluation is summarised in Appendix A, Table 7.

The hydrolysis products of the candidate esters are listed in Appendix A, Table 8.

The 29 candidate substances are closely related structurally to 26 flavouring substances (supporting substances). Twenty-three of these were evaluated in the group of “Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances” and one, menthol, was evaluated in the group “Substances structurally related to menthol” at the 51st JECFA meeting (JECFA, 2000a). Two supporting substances were evaluated at the 63rd JECFA meeting (JECFA, 2005b), one in the group “Aliphatic and alicyclic hydrocarbons” and the other in the group “Aromatic hydrocarbons”. The names and structures for the 26 supporting substances are listed in Appendix A, Table 9, together with their evaluation status.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the FGE.18Rev3

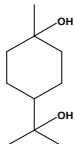
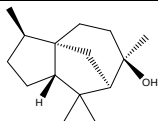
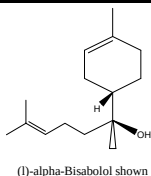
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.041	2-Methylbutan-2-ol		515 75-85-4	Liquid C ₅ H ₁₂ O 88.15	Slightly soluble Freely soluble	102 MS 96 %	1.402-1.408 0.805-0.813	
02.052	2-Methylpropan-2-ol		698 75-65-0	Liquid C ₄ H ₁₀ O 74.12	Slightly soluble Freely soluble	82 MS 95 %	1.384-1.390 0.780-0.790	
02.054	p-Menthane-1,8-diol		701 80-53-5	Solid C ₁₀ H ₂₀ O ₂ 172.27	Slightly soluble Freely soluble	260 116 MS 95 %	n.a. n.a.	
02.120 2030	(+)-Cedrol		4503 10190 77-53-2	Solid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Freely soluble	286 86 MS 95 %	n.a. n.a.	
02.123	2-Methylbut-3-en-2-ol		11794 115-18-4	Liquid C ₅ H ₁₀ O 86.13	Practically insoluble or insoluble Freely soluble	97 MS 98 %	1.413-1.419 0.818-0.827	
02.129 2031	(l)-alpha-Bisabolol		4666 10178 23089-26-1	Liquid C ₁₅ H ₂₆ O 222.37	Insoluble Soluble	^(g) MS >94 %	1.493-1.499 0.927-0.935	Secondary components (no amount given): (d)-alpha- Bisabolol (CAS No. 23178-88-3), (l)-epi-alpha-

Table 1: Specification Summary of the Substances in the FGE.18Rev3

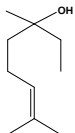
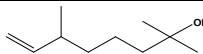
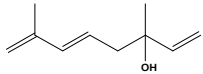
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
								Bisabolol (CAS No.78148-59-1), (d)-epi-alpha-Bisabolol (CAS No. 76738-75-5). Stereoisomeric composition to be specified.
02.140	1,2-Dihydrolinalool		2270-57-7	Liquid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	90 (16 hPa) MS 95 %	1.452-1.458 0.857-0.863	Racemate.
02.144	2,6-Dimethyloct-7-en-2-ol		18479-58-8	Liquid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	86 (15 hPa) MS 95 %	1.434-1.440 0.824-0.830	Racemate.
02.146	(E)-3,7-Dimethylocta-1,5,7-trien-3-ol ^(f)		10202 53834-70-1	Liquid C ₁₀ H ₁₆ O 152.24	Insoluble	62 (6 hPa) MS 93 %	1.489-1.495 0.878-0.886	Racemate; Secondary components 2-3 % linalool, 1-2 % linalool oxide and up to 1 % nerol oxide (Flavour Industry, 2012).

Table 1: Specification Summary of the Substances in the FGE.18Rev3

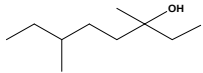
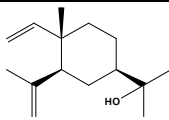
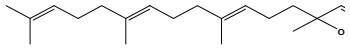
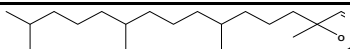
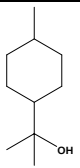
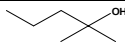
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.147	3,6-Dimethyloctan-3-ol ^(f)		151-19-9	Liquid C ₁₀ H ₂₂ O 158.28	Practically insoluble or insoluble Freely soluble	195 MS 95 %	1.434-1.440 0.831-0.837	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.149	(-)-alpha-Elemol		10205 639-99-6	Solid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Freely soluble	123 (5 hPa) 50 MS 95 %	n.a. n.a.	Register name (1R,3S,4S)- isomer.
02.150	(E,E)-Geranyl linalool		1113-21-9	Solid C ₂₀ H ₃₄ O 290.49	Practically insoluble or insoluble Freely soluble	153 (5 hPa) 51 MS 95 %	1.484-1.490 0.878-0.881	Racemate of (6E,10E)-isomer.
02.168	Isophytol ^(f)		10233 505-32-8	Liquid C ₂₀ H ₄₀ O 296.54	Practically insoluble or insoluble Freely soluble	138 (0.1 hPa) MS 95 %	1.457-1.462 0.847-0.853	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.171	p-Menthan-8-ol		498-81-7	Solid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	208 35 MS 95 %	1.460-1.466 0.909-0.915	
02.181	2-Methylpentan-2-ol		10274 590-36-3	Liquid C ₆ H ₁₄ O 102.18	Slightly soluble Freely soluble	122 MS 95 %	1.409-1.415 0.823-0.829	

Table 1: Specification Summary of the Substances in the FGE.18Rev3

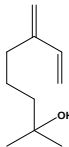
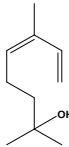
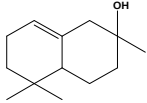
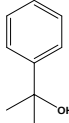
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.184	3-Methylpentan-3-ol		10277 77-74-7	Liquid C ₆ H ₁₄ O 102.18	Practically insoluble or insoluble Freely soluble	121 MS 95 %	1.415-1.421 0.824-0.830	
02.185	Myrcenol		543-39-5	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	91 (13 hPa) MS 95 %	1.470-1.476 0.873-0.879	Not longer supported by industry (DG SANCO, 2012)
02.191	Ocimenol		5986-38-9	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	94 (16 hPa) MS 95 %	1.480-1.486 0.871-0.877	Mixture of (E)- and (Z)-isomers (EFFA, 2010). Not longer supported by industry (DG SANCO, 2012)
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol ^(f)		10173 41199-19-3	Solid C ₁₃ H ₂₂ O 194.32	Practically insoluble or insoluble Freely soluble	82 (1 hPa) 68 MS 95 %	n.a. n.a.	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.203	2-Phenylpropan-2-ol		11704 617-94-7	Liquid C ₉ H ₁₂ O 136.19	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.529-1.535 0.971-0.977	

Table 1: Specification Summary of the Substances in the FGE.18Rev3

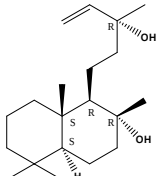
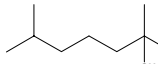
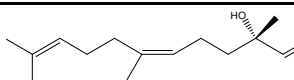
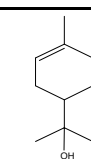
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.206 2029	(-)-Sclareol		4502 10311 515-03-7	Solid C ₂₀ H ₃₆ O ₂ 308.5	Practically insoluble or insoluble Freely soluble	182 (1.33 hPa) 106 MS 98 %	n.a. n.a.	
02.219	2,6-Dimethyl-2-heptanol		13254-34-7	Liquid C ₉ H ₂₀ O 144.26	Practically insoluble or insoluble Freely soluble	171 MS 98 %	1.421-1.427 0.816-0.822	
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol		67 142-50-7	Liquid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Soluble	291.92 20.98 MS 95 %	1.478-1.483 0.870-0.876	
02.230	Terpineol	 alpha-Terpineol shown	8000-41-7	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	210-24 to 235-6 MS 91-99 %	1.480-1.488 0.928-0.937	The specification covers alpha-, beta-, gamma- and delta-terpineol. Composition of mixture: 55-75 % alpha, 16-23 % gamma, 1-10 % cis-beta, 1-13 % trans-beta, 0-1 % delta (EFFA, 2007b).

Table 1: Specification Summary of the Substances in the FGE.18Rev3

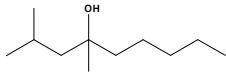
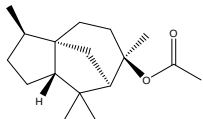
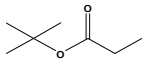
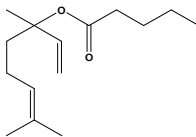
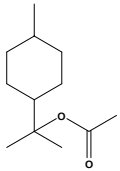
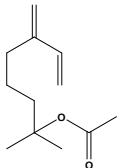
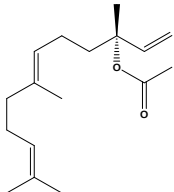
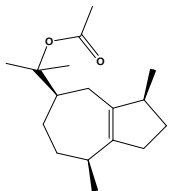
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.253 1850	2,4-Dimethyl-4- Nonanol		4407 74356-31-3	Liquid C ₁₁ H ₂₄ O 172.31	Practically insoluble or insoluble Freely soluble	212 MS 95 %	1.434-1.440 0.825-0.833	Racemate (EFFA, 2010).
09.171	Cedryl acetate		527 77-54-3	Solid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	158 (10.7 hPa) 156 MS 95 %	n.a. n.a.	
09.356	1,1-Dimethylethyl propionate		20487-40-5	Liquid C ₇ H ₁₄ O ₂ 130.19	Practically insoluble or insoluble Freely soluble	118 MS 95 %	1.390-1.396 0.862-0.868	
09.614	Linalyl valerate		10738 10471-96-2	Liquid C ₁₅ H ₂₆ O ₂ 238.37	Practically insoluble or insoluble Freely soluble	238 MS 95 %	1.450-1.456 0.897-0.903	Racemate
09.617	p-Menthan-8-yl acetate		58985-18-5	Liquid C ₁₂ H ₂₂ O ₂ 198.29	Practically insoluble or insoluble Freely soluble	115 (13 hPa) MS 95 %	1.449-1.455 0.930-0.940	
09.669	Myrcenyl acetate		10857 1118-39-4	Liquid C ₁₂ H ₂₀ O ₂ 196.29	Practically insoluble or insoluble Freely soluble	112 (13 hPa) MS 95 %	1.456-1.462 0.915-0.921	Not longer supported by industry (DG SANCO, 2012)

Table 1: Specification Summary of the Substances in the FGE.18Rev3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
09.671	(3S,6Z)-Nerolidyl acetate		10862 56001-43-5	Liquid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	107 (0.4 hPa) MS 95 %	1.467-1.473 0.901-0.907	
09.808	Guaiyl acetate		10659 134-28-1	Solid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	269 96 MS 95 %	n.a. n.a.	

(a): Solubility in water, if not otherwise stated.

(b): Solubility in 95 % ethanol, if not otherwise stated.

(c): At 1013.25 hPa, if not otherwise stated.

(d): At 20°C, if not otherwise stated.

(e): At 25°C, if not otherwise stated.

(f): Stereoisomeric composition not specified.

(g): Boiling point missing.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavours may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.)

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four of these flavouring substances [FL-nos: 02.129, 02.147, 02.168 and 02.197] the stereoisomeric composition has not been sufficiently specified (see Table 1).

1.3. Natural Occurrence in Food

Twenty-one out of 29 candidate substances have been reported to occur widely in fruits, liquorice, milk powder, cabbage, mushroom, various herbs, coffee, tea, chicken, wine and rum. Quantitative data on the natural occurrence in food have been reported for nine of these 29 substances:

Table 2: Candidate Substances Reported to Occur in Food (TNO, 2000)

FL-no:	Name:	Quantitative data reported
02.041	2-Methylbutan-2-ol	Up to 0.1 mg/kg in cherimoya, up to 0.1 mg/kg in loquat, up to 0.01 mg/kg in passion fruit, 0.00002 mg/kg in sapodilla fruit, 0.0007 mg/kg in chicken, 0.2 mg/kg in rum
02.052	2-Methylpropan-2-ol	0.25 mg/kg in grape, up to 0.1 mg/kg in mango, 0.0021 mg/kg in guava fruit
02.054	p-Menthane-1,8-diol	Up to 0.37 mg/kg in cranberry
02.120	Cedrol	1900 mg/kg in calamus (European), up to 100 mg/kg in cinnamon
02.123	2-Methylbut-3-en-2-ol	3.3 mg/kg in mango, up to 2.5 mg/kg in coffee, 1 mg/kg in tea, 0.6 mg/kg in black currants, 0.1 mg/kg in cranberry, up to 0.1 mg/kg in passion fruit, 0.05 mg/kg in bilberry, up to 0.05 mg/kg in cherimoya, 0.002 mg/kg in papaya, trace amounts in cabbage, trace amounts in cardamom, 0.003 mg/kg in milk powder
02.149	Elemol	0.37 mg/kg in grapefruit juice
02.171	p-Menthan-8-ol	0.01 mg/kg in grapefruit juice
02.181	2-Methylpentan-2-ol	0.01 mg/kg in plumcot
02.184	3-Methylpentan-3-ol	0.034 mg/kg in plumcot

According to TNO eight of the substances have not been reported to occur naturally in any food items:

Table 3: Candidate Substances Not Reported to Occur in Food (TNO, 2000)

FL-no:	Name:
02.140	1,2-Dihydrolinalool
02.147	3,6-Dimethyloctan-3-ol
02.150	Geranyl linalool
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol
02.219	2,6-Dimethyl-2-heptanol
02.253	2,4-Dimethyl-4-nonanol
09.671	Nerolidyl acetate
09.808	Guaiyl acetate

2. Specifications

Purity criteria for the 29 substances have been provided by the Flavouring Industry (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007b; EFFA, 2010; Flavour Industry, 2009a; Flavour Industry, 2009b) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for [FL-no: 02.129] are insufficient. Furthermore, the stereoisomeric composition / composition of mixture need to be specified for [FL-nos: 02.129,02.147, 02.168 and 02.197]. The specifications are adequate for 25 candidate substances (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population¹² (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation the total annual volume of production of the 29 candidate substances from use as flavouring substances in Europe has been reported to be approximately 10700 kg (EFFA, 2004b; EFFA, 2005b; EFFA, 2006a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2009b). For the supporting substances the total annual volume of production is approximately 58000 kg (JECFA, 2000a).

On the basis of the annual volume of production reported for the 29 candidate substances, MSDI values for each of these flavourings have been estimated (Appendix A, Table 7).

Ninety-four percent of the total annual volume of production for the candidate substances is accounted for by terpineol [FL-no: 02.230] with 10200 kg. The estimated MSDI of terpineol from use as a flavouring substance is 1200 µg/*capita* per day. The daily *per capita* intakes for each of the remaining substances are less than 30 µg (Appendix A, Table 7).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the 29 candidate substances, information on food categories and normal and maximum use levels^{13,14,15} were submitted for the 29 candidate substances by the Flavour Industry (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012). The 29 candidate substances are used in flavoured food products divided into

¹² EU figure 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

¹³ "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

¹⁴ The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

¹⁵ The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), as shown in Table 4. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 4: Use of 29 Candidate Substances in Various Food Categories

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except [FL-no: 02.197]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-nos: 02.146, 02.197, 02.226]
03.0	Edible ices, including sherbet and sorbet	All except [FL-nos: 02.197, 09.671]
04.1	Processed fruits	All except [FL-nos: 02.146, 02.197, 02.226, 02.230]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	[FL-no: 09.171]
05.0	Confectionery	All except [FL-no: 02.197]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-nos: 02.146, 02.197, 02.230]
07.0	Bakery wares	All except [FL-nos: 02.146, 02.197]
08.0	Meat and meat products, including poultry and game	All except [FL-nos: 02.129, 02.146, 02.197, 02.226]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-nos: 02.129, 02.146, 02.197, 02.226, 02.230, 02.253]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-nos: 02.146, 02.197]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-nos: 02.146, 02.129, 02.197, 02.226, 02.230]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 02.197]
15.0	Ready-to-eat savouries	All except [FL-nos: 02.146, 02.129, 02.197]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-nos: 02.146, 02.197, 02.226, 02.230]

According to the Flavour Industry the normal use levels for the 29 candidate substances are in the range of 0.2 - 200 mg/kg food, and the maximum use levels are in the range of 2 - 500 mg/kg (EFFA, 2002; EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012) (see Appendix C).

The mTAMDI values for the seventeen candidate substances from structural class I (See Section 6) range from 3900 to 14000 µg/person per day. For ten candidate substances from structural class II the

mTAMDI range from 1600 to 3900 µg/person per day whereas for [FL-no: 02.146] from structural class II is 110 µg/person per day. For the one substance from structural class III the mTAMDI value is 5700 µg/person per day (see Section 6).

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Appendix C.

4. Absorption, Distribution, Metabolism and Elimination

Six of the candidate substances in this group are esters [FL-nos: 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808]. Hydrolysis data are not available for any of these esters. However, *in vitro* hydrolysis data for the supporting substance linalyl acetate, indicate that these seven esters can be anticipated to be hydrolysed. The carboxylic acids resulting from the hydrolysis of these seven candidate substances are acetic acid, propanoic acid and valeric acid, which will all be incorporated in normal metabolic processes such as beta-oxidation and the citric acid cycle. The alcohols resulting from the hydrolysis of these esters are tertiary alcohols and their metabolisms are considered further below, together with the candidate tertiary alcohols in this Flavouring Group Evaluation.

A consideration of the chemical structures of the candidate substances, their anticipated pathways of metabolism and the extent to which data on one substance may support the metabolism of another substance has indicated that it is appropriate to divide the candidate substances in FGE.18Rev2 into eight subgroups of more closely related structures. This subdivision is shown in Table 5.

Table 5: Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
1	02.041	2-Methylbutan-2-ol	Aliphatic saturated tertiary alcohols and one ester thereof
	02.052	2-Methylpropan-2-ol	
	02.147	3,6-Dimethyloctan-3-ol	
	02.181	2-Methylpentan-2-ol	
	02.184	3-Methylpentan-3-ol	
	02.219	2,6-Dimethyl-2-heptanol	
	02.253	2,4-Dimethyl-4-nonanol	
	09.356	1,1-Dimethylethyl propionate	
2	02.123	2-Methylbut-3-en-2-ol	Aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two esters thereof
	02.144	2,6-Dimethyloct-7-en-2-ol	
	02.150	Geranyl linalool	
	02.168	Isophytol	
	02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	
	09.614	Linalyl valerate	
	09.671	Nerolidyl acetate	
3	02.146	3,7- Dimethylocta-1,5,7-trien-3-ol	Aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond
4	02.140	1,2-Dihydrolinalool	Aliphatic unsaturated tertiary alcohol (without terminal double bond)
5	02.054	p-Menthane-1,8-diol	Monocyclic saturated and unsaturated tertiary alcohols and one ester thereof
	02.129	Bisabola-1,12-dien-8-ol	
	02.171	p-Menthan-8-ol	

Table 5: Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
6	02.230	Terpineol	Monocyclic and bicyclic unsaturated tertiary alcohols with isolated terminal double bonds
	09.617	p-Menthan-8-yl acetate	
	02.149	Elemol	
	02.206	Sclareol	
7	02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	Bi- and tricyclic tertiary alcohols and esters
	02.120	Cedrol	
	09.171	Cedryl acetate	
	09.808	Guaiyl acetate	
8	02.203	2-Phenylpropan-2-ol	Tertiary alcohol with an aromatic substituent

Subgroup 1: Metabolism studies of the three candidate substances 2-methylbutan-2-ol [FL-no: 02.041], 2-methylpropan-2-ol [FL-no: 02.052] and 2-methylpentan-2-ol [FL-no: 02.181] show that these are conjugated with glucuronic acid before excretion in the urine. When rats were given 2-methylpropan-2-ol by gavage, acetone was excreted in small amounts, and when given 2-methylbutan-2-ol by gavage, diols were excreted. This indicates that an additional metabolic pathway of the three candidate substances is oxidation of methyl groups. From these metabolism studies it is anticipated that the candidate substances 2-methylbutan-2-ol [FL-no: 02.041], 2-methylpropan-2-ol [FL-no: 02.052], 3,6-dimethyloctan-3-ol [FL-no: 02.147], 2-methylpentan-2-ol [FL-no: 02.181], 3-methylpentan-3-ol [FL-no: 02.184], 2,6-dimethyl-2-heptanol [FL-no: 02.219], 2,4-dimethyl-4-nonanol [FL-no: 02.253] and the hydrolysis product 2-methylpropan-2-ol from the candidate substance 1,1-dimethylethyl propionate [FL-no: 09.356] are conjugated with glucuronic acid and excreted in the urine, or that they can undergo oxidation to yield the corresponding diols, which are also expected to be excreted as their respective glucuronic acid conjugates.

Subgroup 2: Linalool is a supporting substance to the candidate substances 2-methylbut-3-en-2-ol, linalyl valerate, nerolidyl acetate, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol and geranyl linalool [FL-nos: 02.123, 09.614, 09.671, 02.168, 02.226 and 02.150], which all have an isolated terminal double bond in close proximity to the tertiary alcohol group. As these substances or their respective alcohol moieties have a free hydroxyl group, they may be directly conjugated. Seventy-two hours after intragastrical application of 500 mg/kg bw ¹⁴C-labelled linalool to 12 weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetrahydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut (Rahman, 1974). In addition, the metabolism of linalool indicates that these candidate substances may also be metabolised by omega-oxidation of methyl groups and excreted in the urine as the oxidation product as such or after conjugation with glucuronic acid. No oxidation of the terminal double bond in linalool was observed, indicating no formation of epoxide intermediates.

For 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] the structure differs from the supporting substance linalool and the other candidate substances in this group in that the isolated terminal double bond is located distant from the tertiary alcohol group. However, any risk from epoxide formation of this compound is considered to be low since at low dose such epoxides formed are anticipated to be efficiently metabolised by conjugation with glutathione or by epoxide-hydrolase mediated hydrolysis,

and in line with the discussion in FGE.07Rev2, the candidate substance 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] can be evaluated via the A-side of the Procedure scheme. Moreover, the tertiary alcohol group can be directly conjugated with glucuronic acid.

Subgroup 3: The Panel stated that for the evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146], the monoterpene myrcene [FL-no: 01.008] could be used as a supporting substance (EFSA, 2012). This substance also has alcohol moiety that can be directly conjugated. In addition, further oxidation of methyl groups may occur. As shown for myrcene, oxidation of conjugated terminal double bonds in the candidate substance may occur, resulting in epoxide intermediates. However the available genotoxicity data for myrcene do not indicate a genotoxic potential for this substance even in the presence of metabolic activation. Nevertheless, it cannot be anticipated that these candidate substances will be metabolised to innocuous products.

Subgroup 4: 1,2-Dihydrolinalool [FL-no: 02.140] has been shown to be directly conjugated with glucuronic acid like the supporting substance linalool, and excreted. After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep dihydrolinalool and tetrahydrolinalool are formed as metabolites (Rahman, 1974). *In vivo* metabolism studies in rats on ¹⁴C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucuronides (Rahman, 1974). Additionally, it might be oxidised at the methyl groups, introducing new hydroxyl groups that also can be conjugated and excreted.

Subgroup 5: From the metabolism studies of alpha-terpineol and menthol it is anticipated that the candidate substances terpineol [FL-no: 02.230], p-menthane-1,8-diol [FL-no: 02.054], bisabola-1,12-dien-8-ol [FL-no: 02.129] and p-menthan-8-ol [FL-no: 02.171] (also an hydrolysis product of candidate substance: p-menthan-8-yl acetate [FL-no: 09.617]) may undergo allylic oxidation of the exocyclic methyl group. This could be further oxidised to a carboxylic acid group. Alternative or subsequent metabolism may occur by conjugation with glucuronic acid, followed by excretion in the urine.

Subgroup 6: A metabolism study on elemol [FL-no: 02.149] indicates that the substance is absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of elemol [FL-no: 02.149] and sclareol [FL-no: 02.206], which have the same structural features as elemol, would not be anticipated.

Subgroup 7: Two metabolism studies on cedrol indicate that the candidate substances cedrol [FL-no: 02.120], cedryl acetate [FL-no: 09.171] guaiyl acetate [FL-no: 09.808] and 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197] will be further hydroxylated and excreted in urine as such or as conjugates.

Subgroup 8: In metabolism studies, the supporting substance 1-isopropyl-4-methylbenzene [FL-no: 01.002] (synonym: p-cymene) was oxidised at the isopropyl side chain yielding 2-(p-tolyl)-2-propanol, which is not further oxidised, but excreted unchanged or as a glucuronic acid conjugate. It is anticipated that the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203] will follow the same pathway and be excreted unchanged or in conjugation with glucuronic acid.

In summary, 28 of the candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] are anticipated to be metabolised to innocuous products.

For the candidate substance [FL-no: 02.146], which contains a conjugated terminal double bond, data from a supporting substance, myrcene [FL-no: 01.008] indicate that [FL-no: 02.146] may be oxidised,

giving rise to epoxide intermediates. Thus, it cannot be anticipated that this candidate substance will be metabolised to innocuous products. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies and the Procedure can be applied for the safety evaluation of this candidate substance.

A more detailed discussion of the metabolism of the candidate substances in this evaluation is provided in Appendix D.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the 29 candidate substances the Procedure as outlined in Appendix B was applied, based on the MSDI approach. The stepwise evaluations of the 29 substances are summarised in Appendix A, Table 7.

Step 1

Seventeen of the 29 candidate substances are classified into structural class I, eleven candidate substances are classified into structural class II and one candidate substance is classified into structural class III according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

Twenty-eight of the candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] are anticipated to be metabolised to innocuous products and proceed via the A-side of the Procedure scheme.

One candidate substance [FL-no: 02.146] contains a conjugated terminal double bond and data from the supporting substance myrcene [FL-no: 01.008] indicate that this terminal double bond may be oxidised, giving rise to epoxide intermediates. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies and therefore the Panel decided to evaluate [FL-no: 02.146] according to the Procedure. Since it cannot be anticipated that [FL-no: 02.146] is metabolised to innocuous products, the evaluation of this candidate substance will proceed via the B-side of the Procedure scheme.

Step A3

Seventeen of the candidate substances [FL-nos: 02.054, 02.120, 02.140, 02.144, 02.149, 02.168, 02.171, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.614, 09.617, 09.671 and 09.808], which are anticipated to be metabolised to innocuous products have been assigned to structural class I. These substances have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1200 µg. These intakes are below the threshold of concern of 1800 µg/person per day for structural class I.

Ten of the candidate substances [FL-nos: 02.041, 02.052, 02.123, 02.147, 02.150, 02.181, 02.184, 02.197, 02.203 and 09.356], which are predicted to be metabolised to innocuous products have been assigned to structural class II. These substances have European daily *per capita* intakes (MSDI) of 0.0012 to 12 µg. These intakes are below the threshold of concern of 540 µg/person per day for structural class II.

One of the candidate substances [FL-no: 02.129] which is predicted to be metabolised to innocuous products has been assigned to structural class III. This substance has European daily *per capita* intake (MSDI) of 27 µg. This intake is below the threshold of concern of 90 µg/person per day for structural class III.

Based on results of the safety evaluation sequence these 28 candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] proceeding via the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances at estimated levels of intake.

Step B3

The candidate substance [FL-no: 02.146] which could not be predicted to be metabolised to innocuous products,] was assigned to structural class II according to the Cramer classification. The estimated European daily *per capita* intake (MSDI) is of 12 µg for [FL-no: 02.146] is below the threshold of concern of 540 µg per person per day and accordingly, [FL-no: 02.146] proceeds to step B4 of the Procedure.

Step B4

In its statement of 2012 (EFSA CEF Panel, 2012) the Panel identified myrcene [FL-no: 01.008] as supporting substance for [FL-no: 02.146] in the present FGE. The safety evaluation of myrcene has been finalised in FGE.78Rev2 based on a new 90-day toxicity study (Bauter, 2013) which provided a NOAEL of 44 mg/kg bw per day. When the MSDI of [FL-no: 02.146] of 12 µg/*capita* per day is compared to this NOAEL a margin of safety of 2.2×10^5 for [FL-no: 02.146] can be calculated.

6. Comparison of the Intake Estimations Based on the MSDI and the mTAMDI Approach

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI, are 3900 µg/person per day, and for the two remaining substances 7000 and 14000 µg/person per day. These seventeen mTAMDI are all above the threshold of concern of 1800 µg/person per day for a structural class I substance.

For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg/person per day and for the remaining two substances, 110 and 1600 µg/person per day, respectively. For 10 substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg/person per day. For the candidate substance [FL-no: 02.146] the estimated intake is below the threshold of concern for structural class II substances of 540 µg/person per day for a structural class II substance.

For substance [FL-no: 02.129] assigned to structural class III the mTAMDI is 5700 µg/person per day, which is above the threshold of concern for structural class III substances of 90 µg/person per day.

Thus, for 28 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, see Table 6.

Table 6: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI µg/capita per day	mTAMDI µg/person per day	Structural class	Threshold of concern µg/person per day
02.054	p-Menthane-1,8-diol	11	3900	Class I	1800
02.120	(+)-Cedrol	13	3900	Class I	1800
02.140	1,2-Dihydrolinalool	0.044	3900	Class I	1800
02.144	2,6-Dimethyloct-7-en-2-ol	0.0012	3900	Class I	1800
02.149	(-)-alpha-Elemol	1.6	3900	Class I	1800
02.168	Isophytol	0.037	3900	Class I	1800
02.171	p-Menthan-8-ol	0.012	3900	Class I	1800
02.206	(-)-Sclareol	0.67	3900	Class I	1800
02.219	2,6-Dimethyl-2-heptanol	0.012	3900	Class I	1800
02.226	[S-(cis)]-3,7,11-Trimethyl- 1,6,10-dodecatrien-3-ol	0.049	7000	Class I	1800
02.230	Terpineol	1200	14000	Class I	1800
02.253	2,4-Dimethyl-4-Nonanol	0.24	3900	Class I	1800
09.171	Cedryl acetate	0.99	3900	Class I	1800
09.614	Linalyl valerate	0.43	3900	Class I	1800
09.617	p-Menthan-8-yl acetate	0.012	3900	Class I	1800
09.671	(3S,6Z)-Nerolidyl acetate	0.061	3900	Class I	1800
09.808	Guaiyl acetate	0.0012	3900	Class I	1800
02.041	2-Methylbutan-2-ol	2.7	3900	Class II	540
02.052	2-Methylpropan-2-ol	0.012	3900	Class II	540
02.123	2-Methylbut-3-en-2-ol	0.0012	3900	Class II	540
02.147	3,6-Dimethyloctan-3-ol	0.0012	3900	Class II	540
02.150	(E,E)-Geranyl linalool	0.026	3900	Class II	540
02.181	2-Methylpentan-2-ol	0.12	3900	Class II	540
02.184	3-Methylpentan-3-ol	0.0012	3900	Class II	540
02.197	1,2,3,4,4a,5,6,7- Octahydro-2,5,5- trimethylnaphthalen-2-ol	0.026	1600	Class II	540
02.203	2-Phenylpropan-2-ol	0.0012	3900	Class II	540
09.356	1,1-Dimethylethyl propionate	0.0012	3900	Class II	540
02.146	(E)-3,7-Dimethylocta- 1,5,7-trien-3-ol	12	110	Class II	540
02.129	(l)-alpha-Bisabolol	27	5700	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same

pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2004b, 2005b, 2006a, 2007b; Flavour Industry, 2009a,b) the estimated combined daily *per capita* intake as flavourings of the 17 candidate substances assigned to structural class I is approximately 1250 µg, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 µg per person per day.

For the 11 candidate substances assigned to structural class II the combined intake is approximately 15 µg, which does not exceed the threshold of concern for a compound belonging to structural class II of 540 µg/person per day.

The 29 candidate substances are structurally related to 26 supporting substances of which 23 were evaluated by JECFA at its 51st meeting (JECFA, 2000a) and three were evaluated by the JECFA at its 63rd meeting (JECFA, 2005b). The total combined intake (in Europe) of the candidate and the supporting substances all assigned to structural class I is approximately 25500 µg/*capita* per day, which exceeds the threshold of concern for the corresponding structural class of 1800/person per day.

For one of the supporting substances from structural class I, menthol [FL-no: 02.015], The JECFA established an acceptable daily intake (ADI) of 4 mg/kg bw per day at their 51st meeting (JECFA, 2000a). The combined intake of the candidate and supporting substances from structural class I of 25500 µg/*capita* per day ~ 0.425 mg/kg bw per day is approximately 10 times below this ADI.

In Europe, the total estimated combined intake of the candidate and the supporting substances assigned to structural class II is 16 µg/*capita* per day, which does not exceed the threshold of concern for the corresponding structural class (540 µg/person per day).

The intake of the one candidate substance assigned to structural class III is 27 µg/*capita* per day. No supporting substances are assigned to structural class III and thus calculation of a combined intake for this substance is not applicable.

8. Toxicity

8.1. Acute Toxicity

Data are available for 16 of the 32 candidate substances and for 19 of the 26 supporting substances. The oral LD₅₀ values in rats, mice or rabbits ranged from 230 to 50000 mg/kg body weight (bw). The magnitudes of the LD₅₀ values indicate that the oral acute toxicity is low for the candidate and supporting substances.

The acute toxicity data are summarised in Appendix E, Table 16.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data are available for six of the candidate substances, 2-methylpropan-2-ol [FL-no: 02.052], cedrol [FL-no: 02.120], 2-methylbut-3-en-2-ol [FL-no: 02.123], bisabol-1,12-dien-8-ol [FL-no: 02.129], 2,6-Dimethyloct-7-en-2-ol [FL-no: 02.144], and sclareol [FL-no: 02.206] (see Appendix E, Table 17).

A number of studies have been conducted on 2-methyl-propan-2-ol [FL-no: 02.052], as described below:

In a 10-week study in male rats, exposure to drinking water containing 0.5 % 2-methylpropan-2-ol [FL-no: 02.052] (equivalent to 500 mg/kg bw per day) resulted in histopathological changes in the liver and kidney. No other tissues were examined or analyses performed (Acharya et al., 1997).

Ninety-day studies were performed in Fisher F344/N rats and B6C3F₁ mice, in conjunction with 2 year chronic toxicity/carcinogenicity studies, which are described below. The main findings after 90 days were, in rats, hyperplasia and inflammation of the urinary bladder in males receiving 2 % and males and females receiving 4 % 2-methylpropan-2-ol in the drinking water (equivalent to 2000 and 4000 mg/kg bw per day, respectively), increased incidence of nephropathy in females receiving 0.5 %, 1 % and 4 % (equivalent to 500, 1000 and 4000 mg/kg bw per day) and increased severity of nephropathy in males at all treatment doses (intakes of 250 mg/kg bw per day and above). In mice, transitional epithelial hyperplasia and inflammation were observed in the urinary bladder in 2 % and 4 % group males (equivalent to 5000 and 10,000 mg/kg bw per day, respectively) and 4 % group females (equivalent to 10,000 mg/kg bw per day) (Lindamood et al., 1992; NTP, 1995). These observations were consistent with earlier 90-day studies using the same species and strains and the same dose levels, except that there was no reference in the short report to renal effects in rats (Brown and Wheeler, 1979).

In the 2-year study on 2-methylpropan-2-ol [FL-no: 02.052] in F344/N rats, the animals (60/sex/group) were dosed via drinking water containing, for males 0, 0.125, 0.25 or 0.5 % (equivalent to 0, 90, 200 or 420 mg/kg bw per day) and for females 0, 0.25, 0.5 or 1 % (equivalent to 0, 180, 330 or 650 mg/kg bw per day), 2-methylpropan-2-ol. Survival was significantly reduced in the 0.5 % male group and 1 % female group. Severity of nephropathy and incidence and severity of transitional cell nephropathy were reported to be increased in all treated groups. Foci of mineralisation were observed in the renal papillas in all treated groups, and the incidence of renal mineralisation was significantly increased in the 0.5 % male group. The incidences of focal renal tubular hyperplasia and adenoma were observed to be increased in a dose-related manner in all treated male groups but did not reach statistical significance. Combined incidences of renal tubular adenomas and carcinomas were significantly increased in the 0.25 % male group, although not in the 0.5 % dose group. Renal tubular hyperplasia was observed in one female in the 1 % dose group (NTP, 1995). A review subcommittee for the US National Toxicology Program (NTP) concluded that the study showed 'some evidence of carcinogenic activity' in the males and 'no evidence of carcinogenic activity' in the females (NTP, 1995).

Further examination of renal samples from the 90-day study in F344/N male rats revealed a significant increase in the quantity of hyaline droplets and number of intracytoplasmic deposits of abnormal shape (crystalline rhomboid structures) in treated groups compared to controls, indicating that the nephropathy observed in male rats was at least in part due to alpha-2u-globulin (Takahashi et al., 1993). The NTP review subcommittee noted that the increased severity of nephropathy also seen in females indicated that the mechanism for renal toxicity is not limited to increased accumulation of alpha-2 μ -globulin (NTP, 1995); however, tumours were observed in males only and appear to be related to a dose-related increase in renal tubule hyperplasia, which occurred in males only.

The B6C3F₁ mice (60/sex/group) received drinking water containing 0, 0.5, 1 or 2 % 2-methylpropan-2-ol [FL-no: 02.052] (equivalent to 0, 540, 1040, 2070 mg/kg bw per day in males and 0, 510, 1020 or 2100 mg/kg bw per day in females) for 2 years. Survival of the 2 % male group was significantly reduced compared to controls. Incidence of follicular cell hyperplasia of the thyroid were significantly increased in all treated male groups and in the 1 % and 2 % female groups. Incidence of follicular cell adenoma was significantly increased in the 2 % female group. The combined incidences of follicular cell adenomas and carcinomas was observed to be increased in the 1 % male group, although this did not reach statistical significance. However, the incidence of adenoma exceeded the highest incidence seen in historic NTP drinking water controls. Chronic inflammation of the urinary bladder was significantly increased in the 2 % male and female groups, and transitional cell hyperplasia in the urinary bladder was significantly increased in the 2 % female group. No NOAEL could be derived from this study for males; the NOAEL in females was 0.5 % in the diet, equivalent to 510 mg/kg bw

per day (NTP, 1995). An NTP review subcommittee concluded that the study demonstrated ‘equivocal evidence of carcinogenic activity’ in male mice and ‘some evidence of carcinogenic activity’ in females (NTP, 1995). The Panel concluded that these tumours appear to be secondary to follicular cell hyperplasia, which was observed in all treated male groups and mid- and high-dose female groups.

The Panel concluded, taking into account the clear lack of a genotoxic potential *in vivo*, that the carcinogenic effects observed in male rats and in mice appear likely to be due to threshold-based mechanisms. Therefore, the overall results do not preclude evaluating 2-methylpropan-2-ol and structurally related candidate substances through the Procedure.

In a 90-day study, 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] was administered by gavage to ten male and ten female Sprague-Dawley rats at dose levels of 10, 50, 500 and 1000 mg/kg bw per day. A control group of ten males and ten females was dosed with vehicle (corn oil) alone (Dunster et al. 2006). In female rats, treatment related effects such as decrease in body weight, platelet counts, thromboplastin time, haemoglobin, hematocrit, erythrocyte counts, increase in serum aspartate aminotransferase, alanine aminotransferase, urinary creatinine were observed doses of 500 and/or 1000 mg/kg bw per day. No such changes were observed in females at the lower doses tested and therefore, 50 mg/kg bw per day was considered to constitute a No Observed Adverse Effect Level (NOAEL) for females. In the kidney of male rats, a greater incidence and/or severity of groups of basophilic tubules and/or globular accumulations of eosinophilic material were observed at all doses tested. Dunster et al., 2007 considered these findings to be consistent with the presence of hydrocarbon nephropathy, which results from the excessive accumulation of alpha2-urinaryglobulin. The latter was indicated by positive staining with Mallory' s Heidenhain stain in renal proximal tubular epithelial cells, frequently associated with tubular degenerative changes. alpha2-Urinaryglobulin is found only in the proximal tubular epithelium of adult male rats. Dunster et al. 2007, concluded that this effect is not indicative of a hazard to human health and that 10 mg/kg per day should be regarded as the NOAEL for males (Dunster et al., 2007).

A NOAEL of 150 mg/kg bw per day in rats is available from a 28-day oral toxicity study conducted on 2-methylbut-3-en-2-ol [FL-no: 02.123] (BASF, 1994).

A NOAEL of 850 mg/kg bw per day in rats is available from a 28-day oral toxicity study conducted on bisabola-1,12-dien-8-ol [FL-no: 02.129] (Habersang et al., 1979).

A 32-day oral toxicity study on both sclareol [FL-no: 02.206] and cedrol [FL-no: 02.120] was conducted in Charles River Laboratories CD(SD) rats. Two groups of 10 rats received dose levels of approximately 8.8 mg/kg bw per day of sclareol and two groups of 10 rats received dose levels of approximately 8.4 mg/kg bw per day of cedrol. The animals were dosed seven days per week via gavage. No adverse effects were observed (IOFI, 2006).

Data are available for six supporting substances [FL-nos: 01.008, 02.013, 02.015, 09.013, 09.423 and 09.830] and two other related substances (linalyl cinnamate and geranyl acetate) administered as a mixture together with citronellyl acetate (See Annex E, Table 17).

Linalool [FL-no: 02.013] was reported to result in no significant adverse effects compared to control when administered to rats at 50 mg/kg bw per day as a 50 % mixture with citronellol via the diet for 84 days. No further details are available. Similarly linalyl acetate [FL-no: 09.013], linalyl isobutyrate [FL-no: 09.423] and geranyl acetate [FL-no: 09.011] administered as a mixture at doses of dosed 24, 27 and 48 mg/kg bw per day respectively resulted in no adverse effects (Oser, 1967).

No adverse effects were reported in rats (10/sex/group) given diets containing 0, 1000, 2500 or 10,000 ppm linalyl isobutyrate [FL-no: 09.423] (equivalent to 0, 50, 125 or 500 mg/kg bw per day) (Hagan et al., 1967) for 18 weeks. Few study details are available. Similarly no adverse effects were reported in a similar study on terpinyl acetate [FL-no: 09.830] given diet containing 0, 1000, 2500 or 10,000 ppm (equivalent to 0, 50, 125 or 500 mg/kg bw per day) for 20 weeks (Hagan et al., 1967).

Since the publication of FGE.18Rev2 (EFSA CEF Panel, 2011) new data on myrcene have become available (Bauter, 2013). The results are described in more detail in FGE.78Rev2 (EFSA, 2015). In this FGE a NOAEL of 44 mg/kg bw per day was derived which can be used for the evaluation of [FL-no: 02.146] in the current FGE.

The repeated dose toxicity data are summarised in Appendix E, Table 17.

8.3. Developmental / Reproductive Toxicity Studies

Data are available for two candidate substances: 2-methylpropan-2-ol [FL-no: 02.052] and 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144].

In a study on 2-methylpropan-2-ol [FL-no: 02.052], doses of 0.5, 0.75 or 1 % liquid diet (equivalent to approximately 3400, 4900 and 6400 mg/kg bw per day) administered to mice on days 6-20 of gestation were associated with reduced performance of offspring in four neurobehavioural tests: righting reflex, cliff avoidance and open field (conducted on days 2, 4, 6, 8 and 10 post-parturition) and roto-rod (conducted on days 14, 16, 18, 20 and 22). The authors indicated that there was some evidence of recovery in the first three tests but not the latter during the periods of study (Daniel and Evans, 1982). In a second study, administration of 1557 mg/kg bw per day to pregnant CBA/J and C57BL/6J mice by oral gavage during days 6-18 of gestation resulted in a significant increase in the incidence of foetal resorptions and a significant decrease in the number of live births per litter. No foetal malformations were observed (Faulkner et al., 1989). The Panel noted that these experimental studies were conducted using very high doses and were not of concern when compared with the low exposure arising from use as flavouring substances.

In a study on 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], doses of 0, 250, 500 or 1000 mg/kg bw per day were administered to Charles River Laboratory CD(SD) rats via corn oil gavage on gestational days 7-17.

Observations for viability, adverse clinical signs, abortion, and premature delivery were conducted before and approximately one hour following treatment and once thereafter. Body weight gains in the high-dose group were reduced by 5 % when compared to controls; weight losses were observed after the first two doses. Although these observations were not significant, they were considered to be evidence of a threshold level for maternal toxicity. Both maternal absolute and relative feed consumption values were significantly reduced in the 1000 mg/kg bw per day group compared to vehicle control. Reduced feed consumption was most prominent on gestational days 7-10, which correlated with the weight losses and reduced weight gains that occurred during the initial days of the dosing period.

Body weights for combined male and female foetuses were reduced approximately 3% in the 1000 mg/kg bw per day group compared to vehicle controls (the reduction was statistically significant for females). No other litter parameters were affected by dosages of 2,6-dimethyloct-7-en-2-ol as high as 1000 mg/kg bw per day. Upon inspection, there were no foetal gross external alterations or foetal soft tissue or skeletal malformations observed in the experiment. There were no observable soft tissue variations, and skeletal variations were limited to two reversible minor changes. First, there was evidence of a threshold (but statistically significant) increase in supernumerary ribs, along with associated significant increases and decreases in the respective numbers of thoracic and lumbar vertebrae. Second, there was evidence of a small but statistically significant retardation in ossification of the metatarsal bones in the hind paws, evident as a reduction in the mean number of ossified metatarsal bones.

The results indicate that 1000 mg 2,6-dimethyloct-7-en-2-ol/kg bw per day produced threshold levels of maternal and developmental toxicity. As such, the maternal and developmental no-observable-adverse-effect levels (NOAELs) for 2,6-dimethyloct-7-en-2-ol are considered to be 500 mg/kg per day (Politano et al., 2008).

Data are available for three supporting substances [FL-nos: 02.013, 02.015 and 01.008] (See Annex E, Table 18).

A NOAEL of 365 mg/kg bw per day was reported for linalool [FL-no: 02.013]. A higher dose of 729 mg/kg bw per day resulted in decreased live litter size and increased pup mortality; maternal toxicity was observed at all doses tested (Hoberman and Christian, 1989). NOAELs of 185 to 425 mg/kg bw per day (highest doses tested) were reported for menthol [FL-no: 02.015] in teratological studies in rats, mice, hamsters and rabbits (Food and Drug Research Laboratories, Inc., 1973). Three studies have been performed in rats on myrcene [FL-no: 01.008]; two developmental toxicity studies (one in which myrcene was administered to dams on days 6-15 of gestation and one in which dosing was from day 15 of gestation until weaning) and a single generation study. NOAELs were 500 mg/kg bw per day, 250 mg/kg bw per day and 300 mg/kg bw per day, respectively (Delgado et al., 1993a; Delgado et al., 1993b; Paumgartten et al., 1998).

The developmental/reproductive toxicity data are summarised in Appendix E, Table 18.

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for nine candidate [FL-nos: 02.052, 02.041, 02.120, 02.123, 02.129, 02.140, 02.144, 02.168 and 02.197] and for eight supporting substances [FL-nos: 01.002, 01.008, 02.013, 02.014, 02.015, 02.097, 09.013 and 09.830]. Data from *in vivo* tests are available for two candidate [FL-nos: 02.052 and 02.123] and for three supporting substances [FL-nos: 01.008, 02.013 and 02.015].

2-Methylpropan-2-ol [FL-no: 02.052] was negative in reversion tests in *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100, without and with metabolic activation by rat and hamster liver S9 (Zeiger et al., 1987). A borderline (less than two-fold) increase in revertants in strain TA1535 was observed in two other studies (Haworth et al., 1981a; Haworth et al., 1981b), which were not available for evaluation. A marginal increase in sister chromatid exchange (SCE) was reported from two studies with Chinese hamster ovary (CHO) cells, which could not be evaluated because the papers were submitted incompletely (Putman, 1985; Thilagar et al., 1981). A borderline increase in mutant frequency was observed in mouse-lymphoma TK +/- cells in a single test in the absence of metabolic activation, whereas negative results were obtained in repeated experiments with S9 (McGregor et al., 1988). Again, similar results are quoted in the summary of an unpublished study not available for evaluation (Kirby et al., 1981). Finally, a slight increase of petite (mitochondrial) mutations was reported in yeast after treatment with 2-methylpropan-2-ol (Jiménez et al., 1988), but this effect is not considered relevant for genotoxicity assessment.

1,2-Dihydrolinalool [FL-no: 02.140] was negative in *S. typhimurium* TA97, TA100, TA102, TA1535, without and with metabolic activation (Gocke, 1999). In one test, 1,2-dihydrolinalool was negative without S9 but increased the number of revertants in TA98 with metabolic activation. This positive finding could not be reproduced in subsequent tests with TA98 (Gocke, 1999).

The remaining candidate substances, for which genotoxicity data have become available in this Revision 2 of FGE.18; Bisabola-1,12-dien-8-ol [FL-no: 02.129], cedrol [FL-no: 02.120], 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197] were all negative in reversion tests in the following test objects, without and with metabolic activation by rat liver S9: [FL-no: 02.129]: *S. typhimurium* TA97a, TA98, TA100, TA102, TA1535, TA1537 up to 5000 µg/plate; [FL-no: 02.120]: *S. typhimurium* TA97a, TA98, TA102, TA1535 up to 5000 µg/plate; [FL-no: 02.144]: *S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 up to 5000 µg/plate; and [FL-no: 02.197]: *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA up to 250 µg/plate without metabolic activation and up to 500 µg/plate with metabolic activation. The studies were considered valid.

A negative result was obtained in a sister chromatid exchange (SCE) study on bisabola-1,12-dien-8-ol [FL-no: 02.129] with Chinese hamster ovary (CHO) cells. The data were presented in a review, but considered valid.

In vivo, 2-methylpropan-2-ol gave clearly negative results in a rat bone marrow micronucleus test, after intraperitoneal (i.p.) administration of a range of doses (six doses from 39 to 1250 mg/kg bw), which reached complete lethality at the highest dose (NTP, 1997). Negative results were also obtained in the mouse peripheral blood micronucleus assay, after 13 weeks of oral exposure to 3000 to 40000 ppm in drinking water. There was no deviation in the PCE/NCE ratio in treated animals, but signs of general toxicity were observed at the two highest doses, indicating significant systemic exposure (NTP, 1995). In another study, 2-methylpropan-2-ol was negative in the mouse bone marrow micronucleus assay when given by ip injections at doses up to 1250 mg/kg bw (three daily administrations) (NTP, 1996). The alleged positive result obtained with 2-methylpropan-2-ol in a rat bone marrow chromosomal aberration test after oral administration of 1/5 of the LD₅₀ (Barilyak and Kozachuk, 1988) is considered inconclusive, because the result is not adequately supported by experimental data.

Terpineol [FL-no: 02.230] (the mixture of alpha-terpineol, beta-terpineol, delta-terpineol and gamma-terpineol) was tested negative in a rec assay in *Bacillus subtilis* (Oda et al., 1978).

Alpha-terpineol [FL-no: 02.014] was reported to give weakly positive results (as a dose-dependent increase in mutation frequency both with and without S9 activation with a maximum increase of 2.2-fold compared with the control) in an Ames-type mutagenicity assay in one (TA102) of four *S. typhimurium* strains tested (TA97a, TA98, TA100, TA102). alpha-Terpineol was incorporated into agar plates up to 2500 µg/plate, either with or without S9 metabolic activation (Gomes-Carneiro et al., 1998).

In other studies, alpha-terpineol gave consistently negative results in Ames assays in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, either with or without S9 metabolic activation (Florin et al., 1980; Lorillard, 1983; Heck et al., 1989).

In an *in vivo/in vitro* study designed to investigate the mutagenicity of the metabolites of beta-terpineol [FL-no: 02.097], Sprague-Dawley rats were administered a single dose of 0.5 ml (452 mg) of beta-terpineol by gavage and the urine was collected for 24-hours. The urine (500 microl) was hydrolysed with beta-glucuronidase. Hydrolysed and un-hydrolysed urine samples, ether extracts of the urine, and aqueous fractions of the urine-ether extracts were then separately incubated with *S. typhimurium* strains TA98 and TA100 without S9 activation. Neither beta-terpineol, nor any of the urinary solutions isolated from the urine of rats given 452 mg doses of beta-terpineol, showed any evidence of mutagenicity in either TA98 or TA100 without metabolic activation (Rockwell and Raw, 1979).

In gene mutation tests in mouse lymphoma cells, alpha-terpinol was non-mutagenic when applied of doses up to 250 nl/ml (with S9) and 300 nl/ml (without S9) (Lorillard, 1982); negative results were also obtained in another study in which alpha-terpineol was tested up to 0.5 µl/ml (without S9) and 0.75 µl/ml (with S9) (Kirby et al., 1984). Based on the negative results obtained in gene mutation tests in mammalian cells, and in view of the sensibility of the TK+/- system to mutagens specifically active toward the *S. typhimurium* strain TA102, the Panel concluded that alpha-terpineol does not raise concern for genotoxicity.

Overall, 2-methylpropan-2-ol provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence does not raise concern for *in vivo* genotoxicity.

2-Methylbut-3-en-2-ol [FL-no: 02.123] was reported to be negative in two bacterial gene mutation tests and in an *in vivo* micronucleus test; however, the unpublished study reports are not available for re-evaluation.

The available data considered valid do not give rise to safety concerns with respect to genotoxicity.

The genotoxicity data are summaries in Appendix E, Tables 19 and 20.

CONCLUSIONS

The present revision 3 of the Flavouring Group Evaluation 18 (FGE.18Rev3) is due to the availability of new toxicity data for myrcene, [FL-no: 01.008] which was used for the safety evaluation of the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

In this revision seven of the candidate substances are aliphatic saturated tertiary alcohols and one is an ester of such, five are aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two are esters of such, one is an aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond, one is an aliphatic unsaturated tertiary alcohol without terminal double bond, two are monocyclic saturated tertiary alcohols and one is an ester of such, two are monocyclic unsaturated tertiary alcohols, two are mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond, two are bicyclic unsaturated esters, two are bi- and tricyclic tertiary alcohols and one is a tertiary alcohol with an aromatic substituent.

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four of these substances [FL-nos: 02.146, 02.147, 02.168 and 02.197] the stereoisomeric composition has not been specified sufficiently.

Seventeen of the 29 candidate substances are classified into structural class I, eleven candidate substances are classified into structural class II and one is classified into structural class III according to the decision tree approach.

Twenty-one out of the 29 candidate substances have been reported to occur in a wide range of food items.

According to the default MSDI approach, 28 of the 29 flavouring substances in this group have intakes in Europe that vary from 0.0012 to 27 $\mu\text{g/capita}$ per day, which are below the thresholds of concern for structural classes I, II and III substance of 1800, 540 and 90 $\mu\text{g/person}$ per day, respectively. The remaining substance, terpineol [FL-no: 02.230] belonging to structural class I, has an MSDI of 1200 $\mu\text{g/capita}$ per day, which is also below the threshold for this structural class.

On the basis of the reported annual production volumes in Europe, the combined intakes of seventeen candidate substances belonging to class I and of eleven candidate substances belonging to class II would result in combined intakes of approximately 1250 and 15 $\mu\text{g/capita}$ per day, respectively. These values are lower than the thresholds of concern for structural class I and class II substances of 1800 and 540 $\mu\text{g/person}$ per day, respectively.

For flavouring substances belonging to structural class I, the total combined intake of the candidate and supporting substances is approximately 25500 $\mu\text{g/capita}$ per day. This total combined intake exceeds the threshold for structural class I of 1800 $\mu\text{g/person}$ per day. For one of the supporting substances from structural class I, menthol [FL-no: 02.015], the JECFA established an acceptable daily intake (ADI) of 4 mg/kg bw per day at their 51st meeting. The combined intake of the candidate and supporting substances from structural class I of 25500 $\mu\text{g/capita}$ per day $\sim$ 0.425 mg/kg bw per day is approximately 10 times below this ADI. For flavouring substances belonging to structural class II, the total combined intake of the candidate and supporting substances is approximately 16 $\mu\text{g/capita}$ per day, which is below the threshold of concern for structural class II (540 $\mu\text{g/person}$ per day).

2-Methylpropan-2-ol [FL-no: 02.052] provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence and the lack of structural alerts for genotoxicity for this substance and its metabolites do not raise concern for *in vivo* genotoxicity. For

the other substances in this group the available data do not give rise to safety concerns with respect to genotoxicity.

Twenty-eight of the candidate substances are anticipated to be metabolised to innocuous products. For the candidate substance (E)-3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no metabolism data are available and therefore it cannot be predicted to be metabolised to innocuous products.

In Revision 2 of FGE.18, for substance (E)-3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no appropriate NOAEL was available and additional data were requested. In the present revision of FGE.18 (Revision 3), new toxicity data on the supporting substance myrcene [FL-no: 01.008] became available, providing an appropriate NOAEL of 44 mg/kg bw per day which was used for the evaluation of the candidate substance [FL-no: 02.146]. Based on the MSDI intake of 12 µg/capita per day, the margin of safety calculated for [FL-no: 02.146] is 2.2×10^5 .

Therefore, none of the 29 candidate substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] would give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances, on the basis of the default MSDI approach.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for 25 candidate substances. However, information on purity criteria and/or stereoisomeric composition has not been specified sufficiently for four substances [FL-no: 02.129, 02.147, 02.168 and 02.197]. Thus, the final evaluation of the materials of commerce cannot be performed for these substances, pending further information.

Thus, for 25 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach: [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.146, 02.149, 02.150, 02.171, 02.181, 02.184, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808].

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI approach, are 3900 µg/person per day, and for the two remaining substances 7000 and 14000 µg/person per day respectively. These seventeen mTAMDI are all above the threshold of concern of 1800 µg/person per day for a structural class I substance. For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg/person per day and for the remaining two substances, 110 and 1600 µg/person per day, respectively. For ten substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg/person per day. For the one substance assigned to structural class III the mTAMDI is 5700 µg/person per day, which is above the threshold of concern for structural class III substances of 90 µg/person per day.

In conclusion, for all candidate substances except [FL-no: 02.146] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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Appendix A. Summary of Safety Evaluation

Table 7: Summary of Safety Evaluation Applying the Procedure

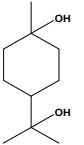
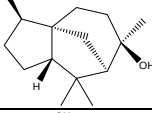
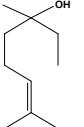
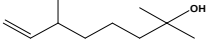
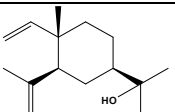
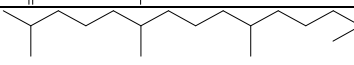
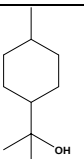
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.054	p-Menthane-1,8-diol		11	Class I A3: Intake below threshold	d	f	
02.120 2030	(+)-Cedrol		13	Class I A3: Intake below threshold	d	f	
02.140	1,2-Dihydrolinalool		0.044	Class I A3: Intake below threshold	d	f	
02.144	2,6-Dimethyloct-7-en-2-ol		0.0012	Class I A3: Intake below threshold	d	f	
02.149	(-)-alpha-Elemol		1.6	Class I A3: Intake below threshold	d	f	
02.168	Isophytol		0.037	Class I A3: Intake below threshold	d	g	
02.171	p-Menthan-8-ol		0.012	Class I A3: Intake below threshold	d	f	

Table 7: Summary of Safety Evaluation Applying the Procedure

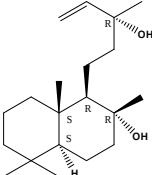
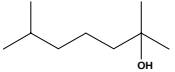
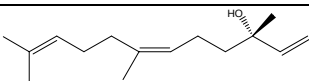
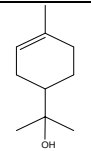
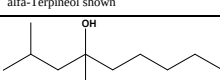
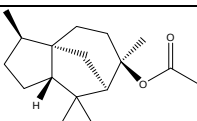
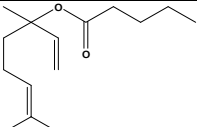
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.206 2029	(-)-Sclareol		0.67	Class I A3: Intake below threshold	d	f	
02.219	2,6-Dimethyl-2-heptanol		0.012	Class I A3: Intake below threshold	d	f	
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol		0.049	Class I A3: Intake below threshold	d	f	
02.230	Terpineol	 alpha-Terpineol shown	1200	Class I A3: Intake below threshold	d	f	
02.253 1850	2,4-Dimethyl-4-Nonanol		0.24	Class I A3: Intake below threshold	d	f	
09.171	Cedryl acetate		0.99	Class I A3: Intake below threshold	d	f	
09.614	Linalyl valerate		0.43	Class I A3: Intake below threshold	d	f	

Table 7: Summary of Safety Evaluation Applying the Procedure

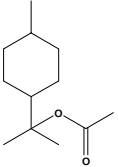
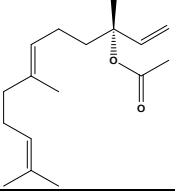
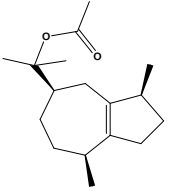
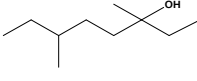
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
09.617	p-Menthan-8-yl acetate		0.012	Class I A3: Intake below threshold	d	f	
09.671	(3S,6Z)-Nerolidyl acetate		0.061	Class I A3: Intake below threshold	d	f	
09.808	Guaiyl acetate		0.0012	Class I A3: Intake below threshold	d	f	
02.041	2-Methylbutan-2-ol		2.7	Class II A3: Intake below threshold	d	f	
02.052	2-Methylpropan-2-ol		0.012	Class II A3: Intake below threshold	d	f	
02.123	2-Methylbut-3-en-2- ol		0.0012	Class II A3: Intake below threshold	d	f	
02.147	3,6-Dimethyloctan-3- ol		0.0012	Class II A3: Intake below threshold	d	g	

Table 7: Summary of Safety Evaluation Applying the Procedure

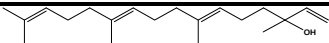
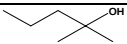
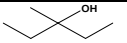
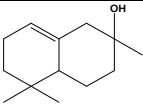
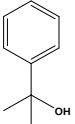
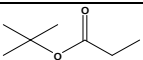
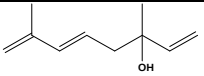
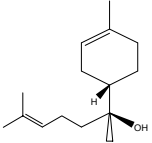
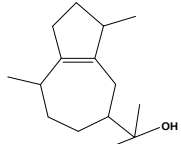
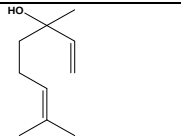
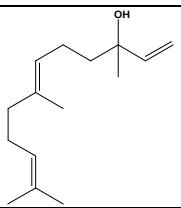
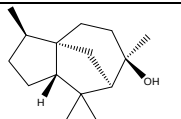
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.150	(E,E)-Geranyl linalool		0.026	Class II A3: Intake below threshold	d	f	
02.181	2-Methylpentan-2-ol		0.12	Class II A3: Intake below threshold	d	f	
02.184	3-Methylpentan-3-ol		0.0012	Class II A3: Intake below threshold	d	f	
02.197	1,2,3,4,4a,5,6,7- Octahydro-2,5,5- trimethylnaphthalen- 2-ol		0.026	Class II A3: Intake below threshold	d	g	
02.203	2-Phenylpropan-2-ol		0.0012	Class II A3: Intake below threshold	d	f	
09.356	1,1-Dimethylethyl propionate		0.0012	Class II A3: Intake below threshold	d	f	
02.146	(E)-3,7- Dimethylocta-1,5,7- trien-3-ol		12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		No safety concern at the estimated level of intake based on the MSDI approach.

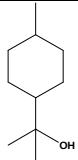
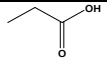
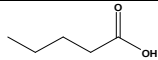
Table 7: Summary of Safety Evaluation Applying the Procedure

FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.129 2031	(l)-alpha-Bisabolol	 (l)-alpha-Bisabolol shown	27	Class III A3: Intake below threshold	d	g	
(a):	EU MSDI: Amount added to food as flavour in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.						
(b):	Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.						
(c):	Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.						
(d):	No safety concern based on intake calculated by the MSDI approach of the named compound.						
(e):	Data must be available on the substance or closely related substances to perform a safety evaluation.						
(f):	No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).						
(g):	Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.						
(h):	No conclusion can be drawn due to lack of information on the purity of the material of commerce.						

EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 8: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status ^a JECFA status ^b CoE status ^c EFSA status	Structural class ^d Procedure path (JECFA) ^e	Comments
	Guaiol				Not evaluated as flavour
02.013	Linalool 356		No safety concern (JECFA, 2000a) Category A (CoE, 1992)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
02.018	Nerolidol 1646		Category B (CoE, 1992)	Class I A3: Intake below threshold	
02.052	2-Methylpropan-2-ol		Category B (CoE, 1992) FGE.18	Class II A3: Intake below threshold	FGE.18: No safety concern based on the intake calculation by the MSDI approach
02.120	(+)-Cedrol 2030		FGE.18	Class I A3: Intake below threshold	

02.171	p-Menthan-8-ol		FGE.18	Class I A3: Intake below threshold	FGE.18: No safety concern based on the intake calculation by the MSDI approach
08.002	Acetic acid 81		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.003	Propionic acid 84		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake below threshold	

- (a): Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4: Not acceptable due to evidence of toxicity.
- (b): No safety concern at estimated levels of intake.
- (c): Category A: Flavouring substance which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.
- (d): Threshold of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.
- (e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

SUPPORTING SUBSTANCES SUMMARY

Table 9: Supporting Substances Summary

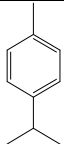
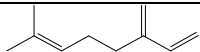
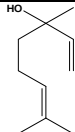
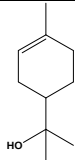
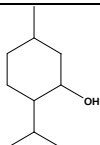
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
01.002	1-Isopropyl-4-methylbenzene		2356 620 99-87-6	1325 JECFA specification (JECFA, 2005a).	926	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	
01.008	Myrcene		2762 2197 123-35-3	1327 JECFA specification (JECFA, 2005a)	290	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	EFSA conclusion: B4: Adequate NOAEL exists (EFSA, 2015).
02.013	Linalool		2635 61 78-70-6	356 JECFA specification (JECFA, 1998)	2200	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)
02.014	alpha-Terpineol		3045 62 98-55-5	366 JECFA specification (JECFA, 1998)	2600	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998)	16000	No safety concern (JECFA, 2000a) Category A	ADI: 0-4 (JECFA, 2000a).

Table 9: Supporting Substances Summary

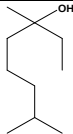
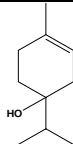
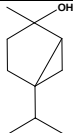
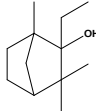
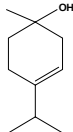
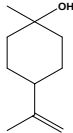
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
						(CoE, 1992)	
02.028	3,7-Dimethyloctan-3-ol		3060 77 78-69-3	357 JECFA specification (JECFA, 1998)	47	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.072	4-Terpinenol		2248 2229 562-74-3	439 JECFA specification (JECFA, 2000b)	150	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.085	Sabinene hydrate		3239 10309 546-79-2	441 JECFA specification (JECFA, 2000b)	0.91	No safety concern (JECFA, 2000a)	
02.095	2-Ethylfenchol		3491 10208 18368-91-7	440 JECFA specification (JECFA, 2001)	0.74	No safety concern (JECFA, 2000a)	
02.096	1-Terpinenol		3563 10252 586-82-3	373 JECFA specification (JECFA, 2000b)	35	No safety concern (JECFA, 2000a)	
02.097	beta-Terpineol		3564 10254 138-87-4	374 JECFA specification (JECFA, 2001)	1.3	No safety concern (JECFA, 2000a)	

Table 9: Supporting Substances Summary

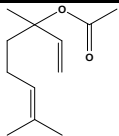
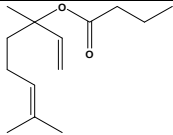
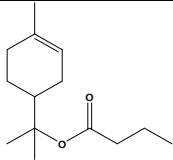
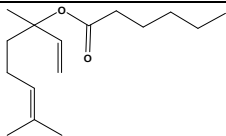
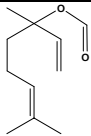
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.013	Linalyl acetate		2636 203 115-95-7	359 JECFA specification (JECFA, 1998)	1700	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)
09.050	Linalyl butyrate		2639 276 78-36-4	361 JECFA specification (JECFA, 1998)	8.4	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.052	Terpinyl butyrate		3049 278 2153-28-8	370 JECFA specification (JECFA, 2001)	5.1	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.068	Linalyl hexanoate		2643 318 7779-23-9	364 JECFA specification (JECFA, 1998)	0.85	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.080	Linalyl formate		2642 347 115-99-1	358 JECFA specification (JECFA, 2003)	6.9	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)

Table 9: Supporting Substances Summary

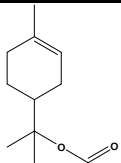
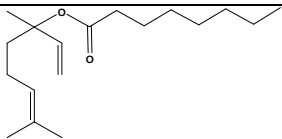
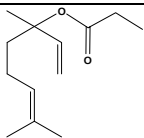
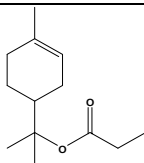
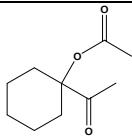
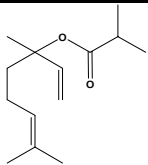
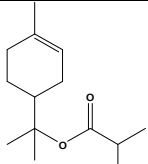
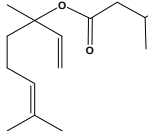
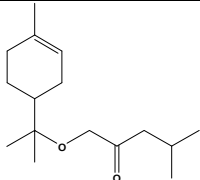
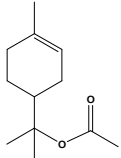
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.081	alpha-Terpinyl formate		3052 348 2153-26-6	367 JECFA specification (JECFA, 2001)	0.12	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.116	Linalyl octanoate		2644 397 10024-64-3	365 JECFA specification (JECFA, 2000b)	0.12	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.130	Linalyl propionate		2645 411 144-39-8	360 JECFA specification (JECFA, 2003)	13	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.142	Terpinyl propionate		3053 423 80-27-3	369 JECFA specification (JECFA, 1998)	0.024	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.293	1-Acetoxy-1-acetylcyclohexane		3701 52789-73-8	442 JECFA specification (JECFA, 2001)	ND	Additional data required (JECFA, 2000a)	

Table 9: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.423	Linalyl isobutyrate		2640 298 78-35-3	362 JECFA specification (JECFA, 1998)	30	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.425	Terpinyl 2-methylpropionate		3050 300 7774-65-4	371 JECFA specification (JECFA, 2000b)	0.61	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.454	Linalyl isovalerate		2646 449 1118-27-0	363 JECFA specification (JECFA, 2000b)	4.6	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.461	Terpinyl isovalerate		3054 456 1142-85-4	372 JECFA specification (JECFA, 2001)	0.12	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.830	Terpineol acetate		3047 205 8007-35-0	368 JECFA specification (JECFA, 1998)	220	No safety concern (JECFA, 2000a)	

(a): EU MSDI: Amount added to food as flavouring substance in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.

(b): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

(c): No safety concern at estimated levels of intake.

(d): Category A: Flavouring substance which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

Appendix B. Procedure for the Safety Evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure A.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 µg/person per day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products¹⁶ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous¹⁷ (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

¹⁶ "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

¹⁷ "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

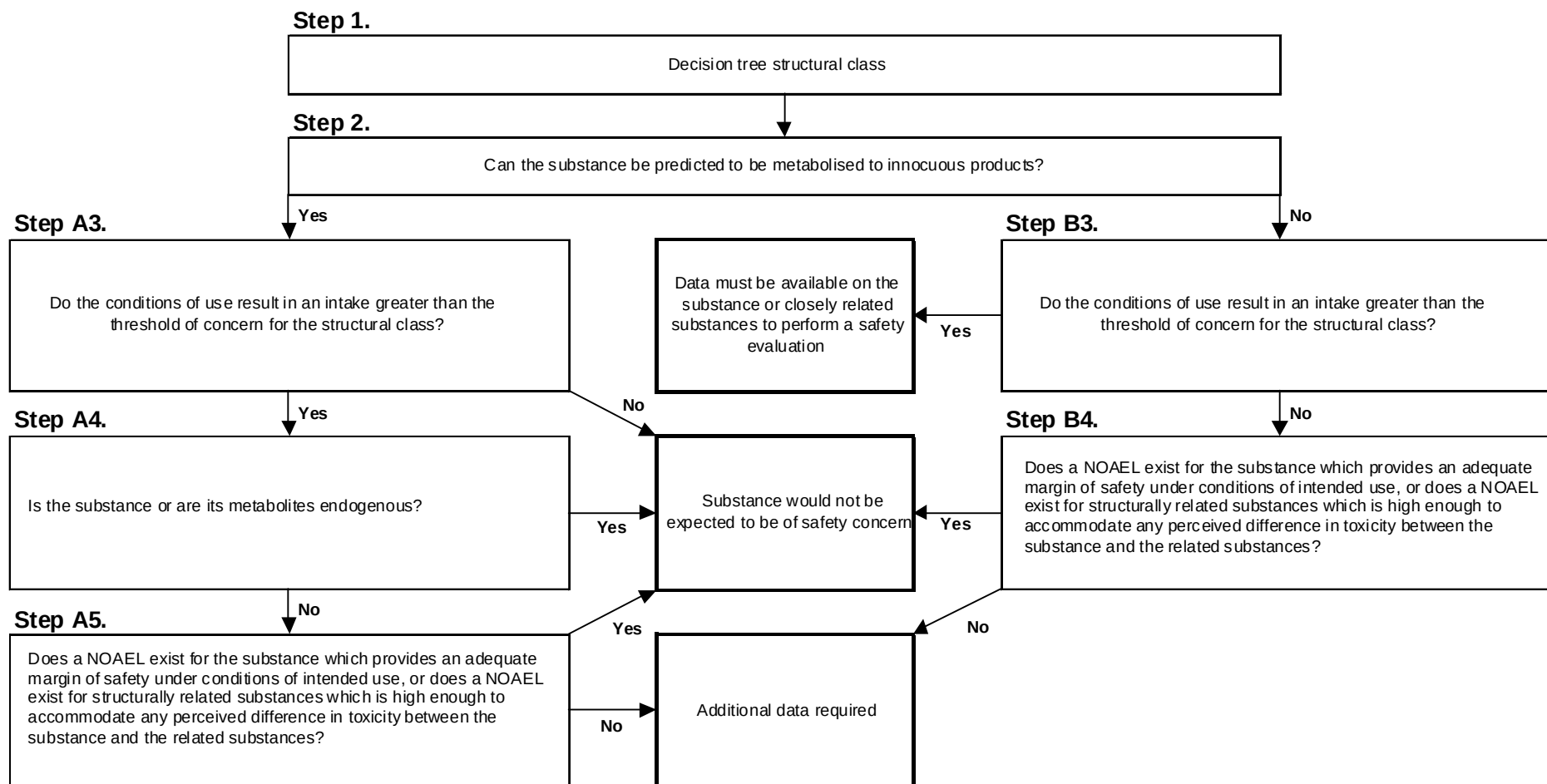


Figure A.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

Appendix C. Use Levels / mTAMDI

C.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table 10) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004c).

Table 10: Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic (“soft”) beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The “normal and maximum use levels” are provided by Industry for the 29 candidate substances in the present flavouring group (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012) (Table 11).

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFSA, 2005a; EFSA, 2006b; EFSA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.041	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.052	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.054	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.120	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.123	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.129	10	10	8	10	-	20	10	4	-	-	-	-	20	-	10	10	-	10
	50	50	50	30	-	100	30	20	-	-	-	-	100	-	30	30	-	500
02.140	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.144	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.146	0,2	-	0,2	-	-	0,5	-	-	-	-	-	-	-	-	0,2	0,2	-	-
	2	-	2	-	-	5	-	-	-	-	-	-	-	-	2	2	-	-
02.147	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.149	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.150	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.168	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFFA, 2005a; EFFA, 2006b; EFFA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)									Maximum use levels (mg/kg)								
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.171	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.181	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.184	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.185	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.191	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-
02.203	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.206	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.219	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.226	2	-	2	-	-	200	2	2	-	-	-	-	1	-	2	30	2	-
	8	-	80	-	-	500	7	6	-	-	-	-	5	-	5	100	10	-
02.230	20	10	50	-	-	50	-	50	5	-	-	-	50	-	10	60	50	-
	150	50	300	-	-	300	-	300	10	-	-	-	300	-	300	300	300	-
02.253	7	5	10	7	-	10	5	10	2	-	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	-	-	-	25	50	25	50	100	25
09.171	7	5	10	7	7	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	35	50	25	50	10	10	-	-	25	50	25	50	100	2

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFFA, 2005a; EFFA, 2006b; EFFA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
09.356	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.614	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.617	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.669	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.671	7	5	-	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	-	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.808	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

C.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table 12. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table 12: Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g per day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table 13):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table 13: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (See Table 14) are presented for the 29 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012). The mTAMDI values are only given for highest reported normal use levels (See Table 14).

Table 14: Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per day)
02.054	p-Menthane-1,8-diol	3900	Class I	1800
02.120	(+)-Cedrol	3900	Class I	1800
02.140	1,2-Dihydrolinalool	3900	Class I	1800
02.144	2,6-Dimethyloct-7-en-2-ol	3900	Class I	1800
02.149	(-)-alpha-Elemol	3900	Class I	1800
02.168	Isophytol	3900	Class I	1800
02.171	p-Menthan-8-ol	3900	Class I	1800
02.206	(-)-Sclareol	3900	Class I	1800
02.219	2,6-Dimethyl-2-heptanol	3900	Class I	1800
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	7000	Class I	1800
02.230	Terpineol	14000	Class I	1800
02.253	2,4-Dimethyl-4-Nonanol	3900	Class I	1800
09.171	Cedryl acetate	3900	Class I	1800
09.614	Linalyl valerate	3900	Class I	1800
09.617	p-Menthan-8-yl acetate	3900	Class I	1800
09.671	(3S,6Z)-Nerolidyl acetate	3900	Class I	1800
09.808	Guaiyl acetate	3900	Class I	1800
02.041	2-Methylbutan-2-ol	3900	Class II	540
02.052	2-Methylpropan-2-ol	3900	Class II	540
02.123	2-Methylbut-3-en-2-ol	3900	Class II	540
02.147	3,6-Dimethyloctan-3-ol	3900	Class II	540
02.150	(E,E)-Geranyl linalool	3900	Class II	540
02.181	2-Methylpentan-2-ol	3900	Class II	540
02.184	3-Methylpentan-3-ol	3900	Class II	540
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	1600	Class II	540
02.203	2-Phenylpropan-2-ol	3900	Class II	540
09.356	1,1-Dimethylethyl propionate	3900	Class II	540
02.146	(E)-3,7-Dimethylocta-1,5,7-trien-3-ol	110	Class II	540
02.129	(l)-alpha-Bisabolol	5700	Class III	90

Appendix D. Metabolism

D.1 Introduction

A consideration of the chemical structures of the candidate substances in this Flavouring Group Evaluation, their anticipated pathways of metabolism and the extent to which data on one substance may support the metabolism of another substance has indicated that it is appropriate to divide the candidate substances in this evaluation into eight subgroups of more closely related structures (See Table 15).

Table 15: Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
1	02.041	2-Methylbutan-2-ol	Aliphatic saturated tertiary alcohols and one ester thereof
	02.052	2-Methylpropan-2-ol	
	02.147	3,6-Dimethyloctan-3-ol	
	02.181	2-Methylpentan-2-ol	
	02.184	3-Methylpentan-3-ol	
	02.219	2,6-Dimethyl-2-heptanol	
	02.253	2,4-Dimethyl-4-nonanol	
2	09.356	1,1-Dimethylethyl propionate	Aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two esters thereof
	02.123	2-Methylbut-3-en-2-ol	
	02.144	2,6-Dimethyloct-7-en-2-ol	
	02.150	Geranyl linalool	
	02.168	Isophytol	
	02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	
	09.614	Linalyl valerate	
3	09.671	Nerolidyl acetate	Aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond
	02.146	3,7- Dimethylocta-1,5,7-trien-3-ol	
4	02.140	1,2-Dihydrolinalool	Aliphatic unsaturated tertiary alcohol (without terminal double bond)
5	02.054	p-Menthane-1,8-diol	Monocyclic saturated and unsaturated tertiary alcohols and one ester thereof
	02.129	Bisabola-1,12-dien-8-ol	
	02.171	p-Menthan-8-ol	
	02.230	Terpineol	
	09.617	p-Menthan-8-yl acetate	
6	02.149	Elemol	Monocyclic and bicyclic unsaturated tertiary alcohols with isolated terminal double bonds
	02.206	Sclareol	
7	09.808	Guaiyl acetate	Bi- and tricyclic tertiary alcohols and esters
	02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	
	02.120	Cedrol	
	09.171	Cedryl acetate	
8	02.203	2-Phenylpropan-2-ol	Tertiary alcohol with an aromatic substituent

D.2 Absorption, Distribution and Elimination

Specific information on absorption, distribution and excretion is available for the candidate substances, 2-methylpropan-2-ol (tertiary butanol) [FL-no: 02.052], elemol [FL-no: 02.149], sclareol [FL-no: 02.206] and terpineol [FL-no: 02.230].

2-Methylpropan-2-ol [FL-no: 02.052] was administered by gastric intubation to female Wistar rats at a single dose of 1853 mg/kg bw and blood concentrations were monitored for 20 hours. 2-Methylpropan-2-ol was eliminated slowly from the blood of rats in this study, with concentrations in blood at 2, 5 and 20 hours measured as 13.2, 12.6 and 11.4 mM, respectively (Beaugé et al., 1981). The high bolus dose of 2-methylpropan-2-ol may have exceeded the capacity of the metabolic pathways for detoxication and contributed to the relatively slow clearance time from the blood.

The pharmacokinetic profile for 2-methylpropan-2-ol was characterised in male (four/dose) and female (three/dose) F-344 rats following intravenous administration of 37.5, 75, 150 and 300 mg 2-methylpropan-2-ol/kg bw. Blood samples were collected in heparinised syringes at 5, 10, 20, 30, 40 and 60 min, and 4, 8, 12, 16 and 24 hours from the cannula implanted in the right jugular vein of each animal. The data fit a two-compartment model with the distribution half-life ($T_{1/2\alpha}$) about 3 min and the elimination half-life ($T_{1/2\beta}$) about 3.8 hours in male and female rats for doses less than 300 mg/kg bw. Consistent with another study (Beaugé et al., 1981), the elimination of 2-methylpropan-2-ol appears to get saturated at higher doses, as evidenced by a disproportional increase in area under the concentration – time curve and decreased rate of clearance in this study. After administration of a 300 mg/kg bw dose of 2-methylpropan-2-ol, the means of $T_{1/2\beta}$ were increased to 5.0 and 4.3 hours in male and female rats, respectively. The steady-state volume of distribution for 2-methylpropan-2-ol was approximately 4.5-fold greater than total body water, suggesting significant tissue distribution (Poet et al., 1997).

Potential pharmacokinetic outcomes in mammalian plasma were analysed by intravenous administration of sclareol [FL-no: 02.206] at 100 mg/kg bw to two male Wistar rats. Plasma samples were collected at 5, 15, 30, 60, 180, 360, 720 and 1440 min after administration, and sclareol concentrations were quantified by gas-liquid chromatography using an internal standard. At 5 min post-injection, plasma levels of sclareol were 84.9 µg/ml. The sclareol plasma concentration dropped to 42.9 µg/ml after 180 min, and was not detectable at 720 min. This study indicated a rapid biphasic disappearance of sclareol from plasma following intravenous treatment. The authors suggest that sclareol may be distributed in fatty tissue due to its high lipophilicity. Sclareol was administered to two male Wistar rats by intravenous administration at 100 mg/kg bw, and to male Wistar rats by oral gavage at 1 g/kg bw in 3:1 propylene glycol-ethanol. Urine and faecal samples were collected from all rats at periodic intervals over 144 or 72 hours, respectively. Bile samples were collected only from rats given intravenous injections at periodic intervals over 30 hours. No sclareol was detected in urine or urine treated with β-glucuronidase at any time. No sclareol was detected in faecal samples from intravenoustreated rats, but 9 % of the initial sclareol dose was found in faecal samples from rats given oral administration. The bile samples from rats given intravenous administration showed very low levels (0.02 %) of sclareol over a 3 hour period. Very low levels (0.04 %) of oxidised metabolites were found in bile after 3 hours, including 3-α-hydroxysclareol (0.24 %), 3-β-hydroxysclareol (0.075 %), 18-hydroxysclareol (0.056 %), and 3-ketosclareol (0.03 %). Sclareol or its oxidised metabolites were not observed in bile samples collected at any other time during the 30 hours study. The authors hypothesized that the low sensitivity of the assay technique may have prevented detection of low levels of sclareol and its metabolites over the course of the experiment. While only a very small percentage of intravenously administered sclareol (<0.05 %) could be accounted for in these experiments, the authors suggested that other mammalian metabolites were formed that were not detected in the assays used (Kouzi et al., 1993).

Incubation of sclareol with 37 different microorganisms found that sclareol is oxidised to more polar metabolites, including 3-ketosclareol, 2α-hydroxysclareol, 3β-hydroxysclareol, 18-hydroxysclareol, 2α,18-dihydroxysclareol, and three glucoside conjugates (Kouzi et al., 1993).

Intravenous administration of sclareol to rats, however, found very low levels of the hydroxylated and glycosidic metabolites or the parent compound in the urine.

A metabolism study on the structurally related elemol [FL-no: 02.149] indicates that substances of this type are absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of this candidate substance would not be anticipated.

alpha-Terpineol, the major component of terpineol [FL-no: 02.230], undergoes allylic oxidation of the exocyclic methyl group, which can then be further oxidised to a carboxylic acid group (Madyastha and Srivatsan, 1988). In a minor pathway, the double bond of alpha-terpineol is epoxidized and then hydrolysed to yield the triol metabolite 1,2,8-trihydroxy-p-menthane, which also has been reported in humans following inadvertent oral ingestion of a pine oil disinfectant containing alpha-terpineol (Horning et al., 1976). It is expected that after single dose exposures, alpha-terpineol would undergo metabolism via glucuronic acid conjugation and excretion in the urine (Chadha and Madyastha, 1984; Hill et al., 1975; Wright, 1945).

(-)-Elemol (2 g) was orally given to rabbits (2-3 kg) and the urine was collected for the three following days (72 hours). In total 80 % of the administered dosage was recovered from the urine (Asakawa et al., 1986).

In addition, there is information on absorption and excretion for the four supporting substances linalool, myrcene, menthol and 1-isopropyl-4-methylbenzene (synonym: *p*-cymene).

[1,2-¹⁴C]-Linalool was orally administered to rats at a single dose of 500 mg/kg bw. The majority (55 %) of the radioactivity was excreted in the urine as the glucuronic acid conjugate, whereas 23 % was excreted as CO₂ in expired air, and 15 % was excreted in the faeces within 72 hours of administration. Only 3 % of the radioactivity was detected in tissues after 72 hours, with 0.5 % in the liver, 0.6 % in the gut, 0.8 % in the skin and 1.2 % in the skeletal muscle (Parke et al., 1974).

When given to male Japanese White rabbits by gavage at a dose of 670 mg/kg bw per day for two days, approximately 25 % of the total administered amount (19 g to six rabbits) of myrcene could be recovered from the urine excreted over a period of three days following administration (Ishida et al., 1981).

In humans 79 % of a 1000 mg oral dose (Quick, 1928) or 78 % of a 10 to 20 mg oral dose (Atzl et al., 1972) of menthol administered to volunteers was eliminated as the glucuronic acid conjugate. For eight days 750 mg l-menthol was administered orally to two human volunteers followed by oral or intravenous administration of 200 mg [6-¹³C]-glucuronolactone or [6-¹³C]-sodium glucuronate. For two days after administration of the isotopic compound, menthyl glucuronide was excreted in an average daily yield ranging from approximately 27 % to 84 % of the l-menthol administered (Eisenberg et al., 1955). Four males were given an oral dose of 180 mg peppermint oil. Between 37 and 116 mg menthol glucuronide were excreted in the urine after 14 hours (Kaffenberger and Doyle, 1990).

Non-cannulated and bile duct-cannulated male Fischer 344 rats were administered a single oral dose of 500 mg [3-³H]-1-menthol/kg bw. Urine and faeces were collected over the next 24 and 48 hours from non-cannulated rats. In the bile duct-cannulated rats, bile samples were collected at 2-hours intervals for the first 6 hours and then from 6 to 24 hours. The 0-24 hours urine was collected in the same rats. In the bile duct-cannulated rats, total recovery of the dose of the radiolabeled substance in the urine or bile was 74.2 % after 24 hours. The amount of radioactivity found in the urine after 24 hours from the non-cannulated and the bile duct-cannulated rats differed from 19 to 7.3 %, but indicates that most of the compound is excreted in the bile during the first 24 hours (Yamaguchi et al., 1994).

Of an oral dose of 100 mg/kg bw of p-cymene given to male Wistar rats or Dunkin Hartley guinea pigs, 80 % or 71 %, respectively, was excreted in the urine within the following 48 hours in the form of extractable metabolites. It was speculated that the rest of the dose was either excreted via the faeces or as unextractable metabolites in the urine (Walde et al., 1983).

In conclusion, the candidate substance 2-methylpropan-2-ol is anticipated to be absorbed and to undergo rapid excretion when administered at lower doses. However, when administered at higher doses the metabolic capacity may be exceeded. The candidate substances elemol and sclareol are absorbed but excreted rather slowly. The candidate substance terpineol is anticipated to be absorbed and excreted. The supporting substances, menthol and p-cymene, are readily absorbed and excreted rapidly with approximately 70 % recovery within 24 hours and 70-80 % recovery within 48 hours, respectively. Linalool is absorbed but rather slowly excreted. The slowest absorption and excretion was observed for myrcene where only 25 % of administered dose was recovered in urine after 72 h.

D.3 Biotransformation

Ester hydrolysis

Aliphatic esters are hydrolysed to the component alcohols and carboxylic acid by carboxyl-esterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980). In mammals these enzymes occur within the body in most tissues including the gut lumen and intestinal wall, but predominate in the hepatocytes (Heymann, 1980). The wide range of tissue distribution and the multiplicity of the esterases generally give rapid hydrolyse of esters *in vivo*.

No hydrolysis studies on the candidate esters [FL-no: 09.171, 09.356, 09.614, 09.669, 09.671, 09.808 and 09.617] are available. Two hydrolysis studies on a supporting substance, linalyl acetate [FL-no: 09.013], were found. In an *in vitro* hydrolysis study, linalyl acetate was easily hydrolysed in water and simulated gastric and pancreatic fluids. The mean half-lives for linalyl acetate hydrolysis were 5.5 and 52.5 min in gastric and pancreatic fluids, respectively (Hall, 1979). In neutral gastric juice, linalyl acetate is slowly ($t_{1/2} = 121$ min) hydrolysed to a mixture of linalool and the ring-closed isomer alpha-terpineol. In acidic artificial gastric juice, linalyl acetate is rapidly hydrolysed ($t_{1/2} < 5$ min) to yield linalool (Buck and Renwick, 1998). Linalyl acetate was slowly hydrolysed ($t_{1/2} = 153 - 198$ min) in intestinal fluid with or without pancreatin. Linalyl acetate also hydrolyses in homogenates of rat intestinal mucosa, blood and liver, but at rates much slower than in acidic gastric juice (rate constant for hydrolysis, $k = 0.01$ to 0.0055 min^{-1} vs. $> 5 \text{ min}^{-1}$ in gastric juice). Based on these observations it can be concluded that linalyl acetate hydrolyses in gastric juice to yield linalool and acetic acid (Buck and Renwick, 1998). Further, it has been demonstrated that linalyl acetate can be hydrolysed to linalool in *in vitro* studies after incubation with rat caecal flora (Rahman, 1974).

Subgroup 1 - Aliphatic saturated tertiary alcohols and ester

For three of the eight candidate substances in this subgroup, 2-methylpropan-2-ol [FL-no: 02.052], 2-methylbutan-2-ol [FL-no: 02.041] and 2-methylpentan-2-ol [FL-no: 02.181], there are one or more metabolism studies.

2-Methylpropan-2-ol (tert-Butanol [FL-no: 02.052])

[2-¹³C]-2-Methylpropan-2-ol was orally administered in a gel capsule at a dose of 5 mg/kg bw to one human volunteer (44 year old, 80 kg male). Urine was collected in 12-hours intervals for 48 hours and analysed by ¹³C-NMR. All of the urine samples of this human volunteer showed the presence of 2-methylpropan-2-ol, 2-methylpropan-2-ol glucuronide, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol. In contrast to the rat urine samples in which 2-methylpropan-2-ol sulphate was observed as a major metabolite, the sulphate was only present in trace amounts in the human volunteer urine samples. The low recovery of 2-methylpropan-2-ol sulphate in the urine of the human volunteer is likely based on a low affinity of human sulphotransferase for 2-methylpropan-2-ol as compared to rats. On the other hand, 2-hydroxyisobutyrate was the major metabolite excreted by the human volunteer. The likely pathway for the formation of the 2-hydroxyisobutyrate and 2-methyl-1,2-

propanediol metabolites involves methyl group oxidation of 2-methylpropan-2-ol by CYP-450 to yield 2-methyl-1,2-propanediol. Further oxidation of 2-methyl-1,2-propanediol results in the formation of 2-hydroxyisobutyrate, and acetone is likely formed by further oxidation of the intermediate diol and/or 2-hydroxyisobutyrate (Bernauer et al., 1998).

Three male rats per experiment were administered a single 250 mg/kg bw dose of [12C]- or [13C]- 2-methylpropan-2-ol dissolved in corn oil by oral gavage. All animals were maintained in individual metabolism cages for 72 hours, after which they were sacrificed by cervical dislocation. Urine was collected at 24 and 48 hours and analysed by 13C-NMR. In the 24-hour urine samples, it was determined that 2-methylpropan-2-ol sulphate, 2-methyl-1,2-propanediol, and 2-hydroxyisobutanoate (i.e. alpha-hydroxyisobutanoate) are the major metabolites of 2-methylpropan-2-ol in rats; minor metabolites were identified as free 2-methylpropan-2-ol, 2-methylpropan-2-ol glucuronide, and [13C]-acetone. Identical metabolites were present at lower concentrations in the urine collected between 24 and 48 hours after dosing. Extensive biotransformation of tert-butyl alcohol in rats by conjugation and oxidation was confirmed by the results of these experiments (Bernauer et al., 1998).

2-Methylpropan-2-ol was administered by gastric intubation to chinchilla rabbits at a 297 mg/kg bw. 2-Methylpropan-2-ol was conjugated to a large extent with glucuronic acid, and conjugates were readily isolated from urine. Of the administered dose, 24.4 % was excreted as glucuronic acid conjugate in the urine within 24 hours after administration. The investigators suggested that the volatile alcohol may also be eliminated to some extent via the lungs. No aldehydes or ketones were detected in the expired air of a rabbit administered 6 ml of 2-methylpropan-2-ol (Kamil et al., 1953).

2-Methylbutan-2-ol (tert-amyl-alcohol [FL-no: 02.041])

2-Methylbutan-2-ol was administered by gastric intubation to chinchilla rabbits at a single dose of 441 mg/kg bw. 2-Methylbutan-2-ol was conjugated to a large extent with glucuronic acid and 58 % of the dose was excreted as such conjugates via the urine within 24 hours post dosing (Kamil et al., 1953).

Three male rats were administered a single 250 mg/kg bw dose of [2-13C]-2-methylbutan-2-ol dissolved in corn oil by oral gavage. All animals were maintained in individual metabolism cages for 48 hours. Urine was collected at 24 and 48 hours and analysed by ¹³C-NMR. In the 24-hour urine samples, it was determined that 2-methylbutan-2-ol glucuronide, 2-methyl-2,3-butanediol and 2-methyl-2,3-butanediol glucuronide are the major metabolites of 2-methylbutan-2-ol; minor metabolites were identified as free 2-methylbutan-2-ol, 2-hydroxy-2-methylbutanoic acid and 3-hydroxy-3-methylbutanoic acid. The low concentration of 2-methylbutan-2-ol recovered in the urine suggests extensive metabolism of the alcohol. In the 48-hour urine samples, only 2-methyl-2,3-butanediol and 2-methyl-2,3-butanediol glucuronide were detected, suggesting the rapid excretion of 2-methylbutan-2-ol glucuronide following oral exposure. Glucuronidation appears to be the major pathway of metabolism, resulting in urinary excretion of the 2-methylbutan-2-ol glucuronide. In addition, it appears that 2-methylbutan-2-ol is oxidised to 2-methyl-2,3-butanediol, which is further conjugated to 2-methyl-2,3-butanediol glucuronide and excreted in the urine. A minor metabolic pathway seems to be oxidation of the carbon atom at the C4 position resulting in 2-methyl-2,4-butanediol as an intermediate, which is further oxidised to 3-hydroxy-3-methylbutanoic acid. Finally, oxidation of the methyl side chain is another minor transformation pathway that results in the formation of 2-methyl-1,2-butanediol as an intermediate, which is further oxidised to produce 2-hydroxy-2-methylbutanoic acid (Amberg et al., 1999) (See Figure D.1.)

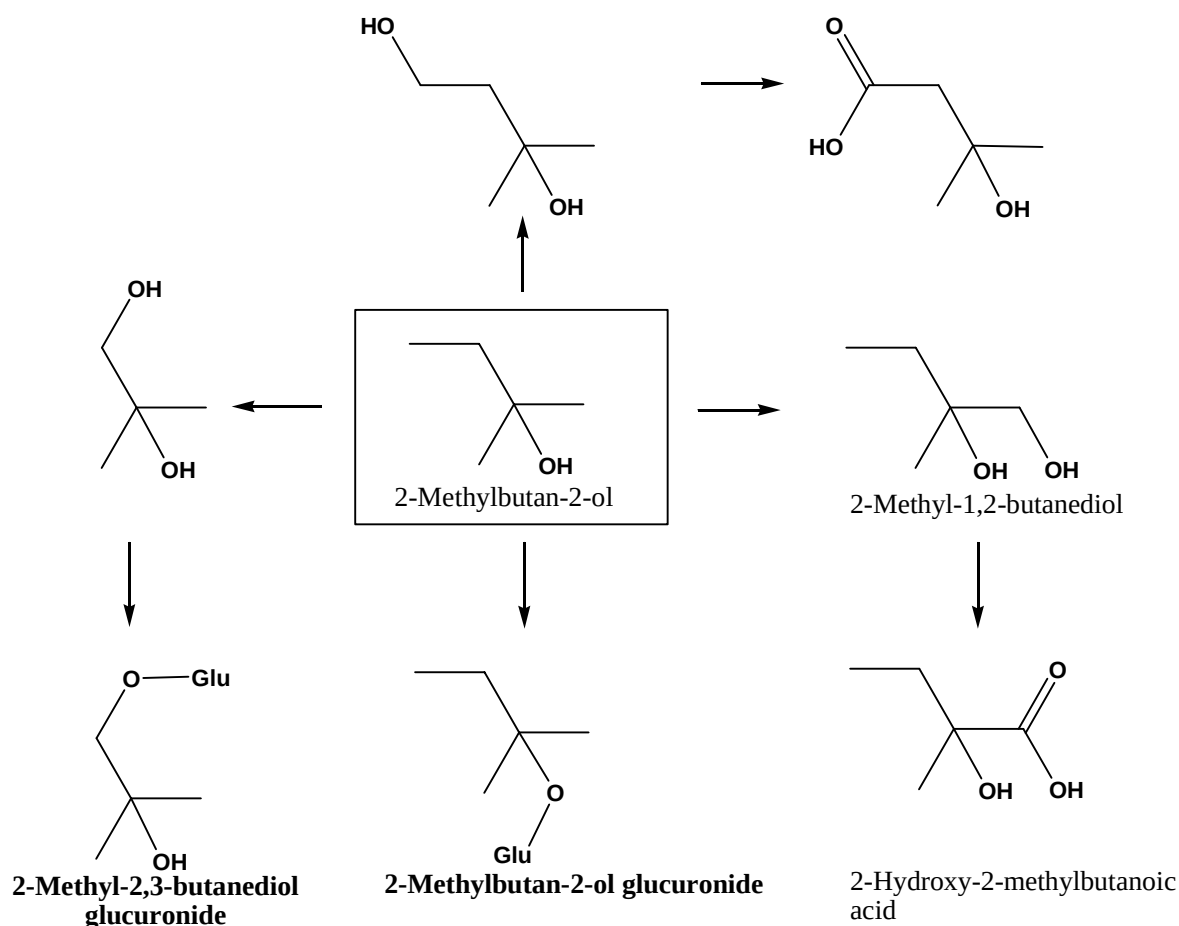


Figure D.1. Metabolism of 2-methylbutan-2-ol (tert-amyl-alcohol) in rats (Amberg et al., 1999). Main metabolism products are in bold.

2-Methylpentan-2-ol (tert-hexylalcohol [FL-no: 02.181])

2-Methylpentan-2-ol was administered by gastric intubation to chinchilla rabbits at a single dose of 851 mg/kg bw. 2-Methylpentan-2-ol was conjugated to a large extent with glucuronic acid, and 57 % of the dose was excreted within 24 hours post dosing as such conjugates via the urine (Kamil et al., 1953).

Subgroup 2 - Aliphatic unsaturated tertiary alcohols and esters with isolated terminal double bonds

There are no metabolism studies on the candidate substances 2-methylbut-3-en-2-ol, 2,6-dimethyloct-7-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate, [FL-no: 02.123, 02.144, 02.150, 02.168, 02.226, 09.614 and 09.671]. However, there is a metabolism study for linalool, supporting substance to 2-methylbut-3-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate [FL-no: 02.123, 02.150, 02.168, 02.226, 09.614 and 09.671,], which have the isolated double bond in close proximity to the tertiary alcohol group.

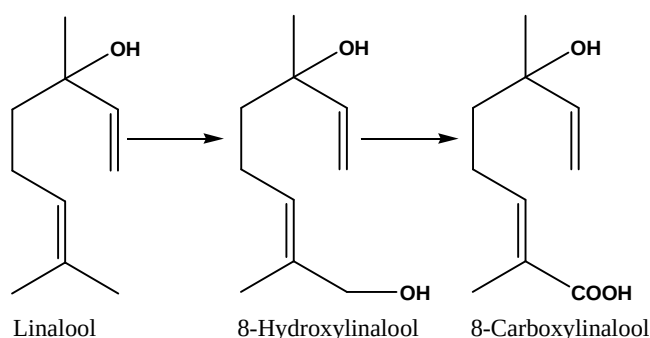


Figure D.2 Metabolism of linalool (Chadha & Madyastha 1984).

Seventy-two hours after intragastrical administration of 500 mg/kg bw ^{14}C -labelled linalool/kg bw to 12-weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetra hydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut (Rahman, 1974).

In another study, male rats were given a daily 800 mg/kg bw oral dose of linalool for 20 days. Urinary metabolites formed by CYP-450-mediated allylic oxidation of linalool included 8-hydroxylinalool and 8-carboxylinalool (Chadha and Madyastha, 1984) (Figure D.2). No oxidation of the terminal double bond was observed, indicating no formation of epoxide intermediates.

No metabolism studies on supporting substances to 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], which has the isolated double bond far from the tertiary alcohol group, are available.

Subgroup 3: Aliphatic unsaturated tertiary alcohols with a conjugated terminal double bond

No metabolism studies are found for the candidate substance, 3,7-dimethylocta-1,5,7-trien-3-ol, [FL-no: 02.146]. For the supporting substance, myrcene, two studies on metabolism are found.

In the urine of rabbits orally administered myrcene (single dosage of 670 mg/kg bw) via gavage, more than 80 % of the metabolites were neutral metabolites, the rest were acidic metabolites. The main metabolites identified in urine were myrcene-3,10-glycol, myrcene-1,2-glycol and uroterpenol (40.7, 20.8 and 11.8 %, respectively, of the neutral metabolites after 72 hours). Additionally, the glycols underwent further oxidation to yield 2-hydroxymyrcene-1-carboxylic acid and 3-hydroxymyrcene-10-carboxylic acid (no quantitative data were given for these acidic metabolites).

The authors suggested that uroterpenol (or limonene-8,9-diol) may have been formed from limonene, which is derived from cyclization of myrcene in the acidic conditions of the rabbit stomach (Ishida et al., 1981).

When rats were administered 800 mg/kg bw myrcene per day via gavage for 20 days, the principal metabolites isolated from the urine were 10-hydroxylinalool (or myrcene-3,10-glycol) and, to a lesser extent, 7-methyl-3-methylene-oct-6-ene-1,2-diol (or myrcene-1,2-glycol). Other minor metabolites included the hydroxy acids of both the 3,10- and 1,2-glycols (10-carboxylinalool (or 3-hydroxymyrcene-10-carboxylic acid) and 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid (or 2-hydroxymyrcene-1-carboxylic acid), respectively) and a cyclic diol, 1-hydroxymethyl-4-isopropenylcyclohexanol (or p-menth-8-ene-1,7-diol), formed by intramolecular cyclization of an open chain metabolite (Madyastha and Srivatsan, 1987).

It was demonstrated that the biotransformation of myrcene was cytochrome P450 (CYP)-mediated and that it could be enhanced by pretreatment of animals with phenobarbital (Madyastha & Srivatsan,

1987). These studies indicate the oxidation of the terminal double bonds of myrcene via intermediate epoxidation of the 1,2- and 3,10- double bonds (Figure D.3).

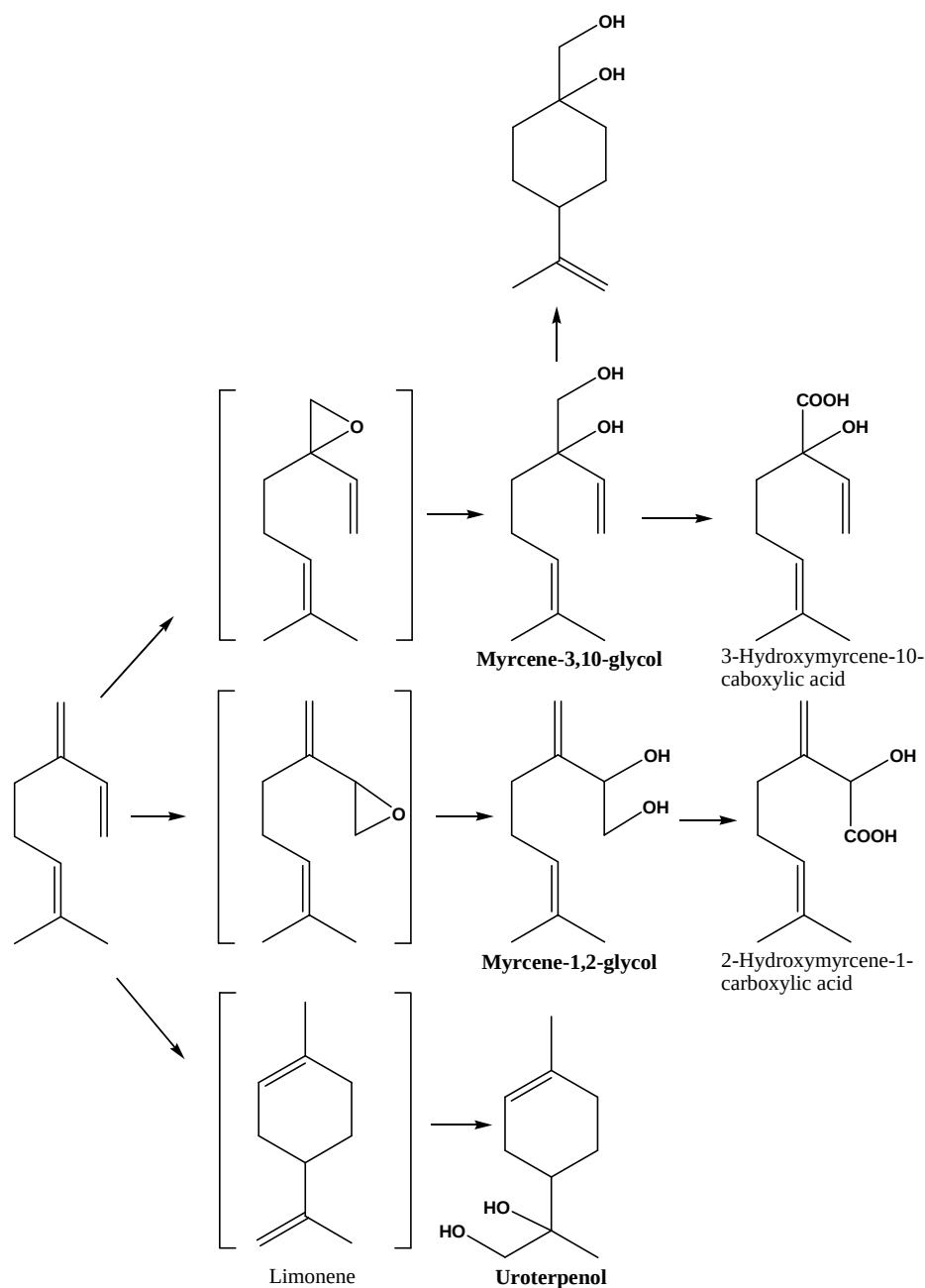


Figure D.3 Metabolism of myrcene (Ishida et al., 1981 and Madyastha & Srivatsan 1987). Intermediate products are in brackets and main metabolism products are in bold.

Subgroup 4: Aliphatic unsaturated tertiary alcohols

After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep, dihydrolinalool and tetrahydrolinalool are formed as metabolites (Rahman, 1974). *In vivo* metabolism studies in rats on ¹⁴C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucuronides (Rahman, 1974).

Subgroup 5: Monocyclic saturated tertiary alcohols and esters

There are no metabolism studies on the candidate alicyclic substances p-menthane-1,8-diol, bisabolol, 1,12-dien-8-ol, p-menthan-8-ol, terpineol and p-menthan-8-yl acetate [FL-no: 02.054, 02.129, 02.171, 02.230 and 09.617]. p-Menthan-8-yl acetate is anticipated to be hydrolysed to give p-menthan-8-ol. There are metabolism studies on two substances, alpha-terpinol and menthol, which are supporting substances to p-menthane-1,8-diol, p-menthan-8-ol, and p-menthan-8-yl acetate (Figure D.4).

In a repeated dose study, male albino rats were orally administered the alicyclic tertiary alcohol alpha-terpineol at a daily dose of 600 mg/kg bw for 20 days. Oxidation of the allylic methyl group was observed to yield the corresponding carboxylic acid, which was hydrogenated, to a small extent, to yield the corresponding saturated carboxylic acid (Madyastha and Srivatsan, 1988).

In rats, the vast majority of orally administered menthol is eliminated in either the urine or faeces as the glucuronic acid or various oxidation products (Madyastha and Srivatsan, 1988; Yamaguchi et al., 1994).

Non-cannulated and bile duct-cannulated male Fischer 344 rats were given a single oral dose of 500 mg/kg bw [3-³H]-1-menthol. Urine and faeces were collected over the next 24 and 48 hours from non-cannulated rats. In the bile duct-cannulated rats, bile samples were collected at 2-hours intervals for the first 6 hours and then from 6 to 24 hours. The 0-24 hours urine was collected in the same rats (Yamaguchi et al., 1994). The major metabolites found were menthol glucuronide in the bile and a variety of oxidation products in the urine. Menthol glucuronide formed in the liver passes into the bile with subsequent elimination or entry into enterohepatic circulation. Oxidation products of menthol are primarily p-menthane-3,8-diol, 3,8-dihydroxy-p-menthane-7-carboxylic acid and 3-hydroxy-p-menthane-7-carboxylic acid (Figure III.4). Minor metabolites are p-menthane-3,7-diol, p-menthane-3,7,8-triol, p-menthane-3,9-diol and 3-hydroxy-p-menthane-9-carboxylic acid. The monohydroxylated menthols are excreted in the urine in part as glucuronide (Yamaguchi et al., 1994).

The study by Madyastha & Srivatsan (Madyastha and Srivatsan, 1988) supports the finding of p-menthane-3,8-diol and 3,8-dihydroxy-p-menthane-7-carboxylic acid as the main metabolites of menthol in urine after giving rats an oral dosage of 800 mg/kg bw. They also found p-menthane-3,9-diol as a minor metabolite.

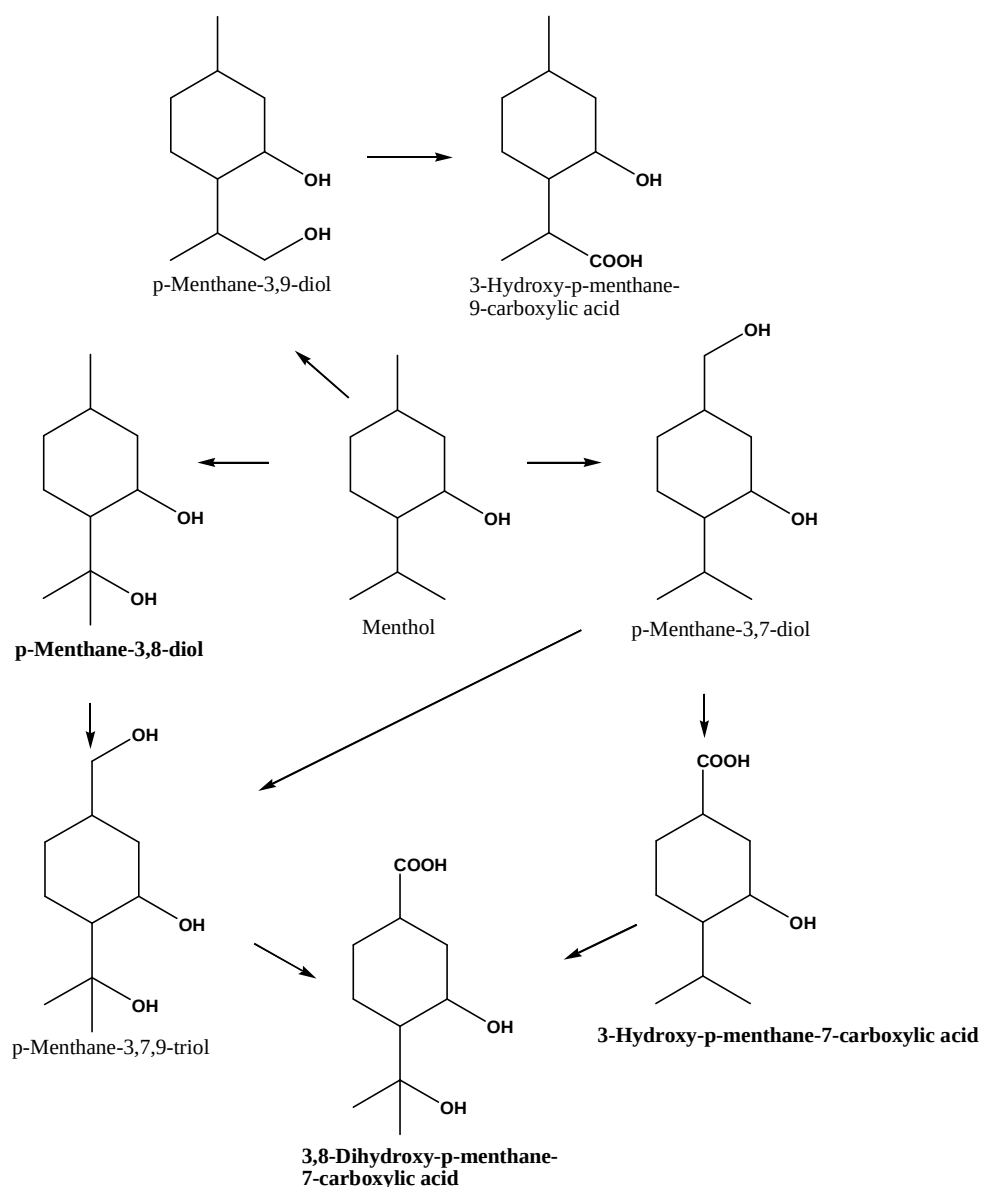


Figure D.4. Metabolism of menthol in rats (Yamaguchi et al., 1994). Main metabolism products are in bold.

Subgroup 6: Monocyclic unsaturated tertiary alcohols with isolated terminal double bonds

There is one metabolism study on one of the two candidate substances in this group, elemol [FL-no: 02.149]. (-)-15-Hydroxyelemol was the only metabolite (10 % of the natural metabolites) detected after 72 hours in the urine of rabbits (2-3 kg) given 2g (-)-elemol. Most of the (-)-elemol (70 %) was recovered as elemol conjugated with glucuronic acid or sulphate. No oxidation of the double bond of the vinyl group was observed, indicating that no epoxide intermediates was formed.

Subgroup 7: Bi- and tricyclic tertiary alcohols and esters

For the candidate substances guaiyl acetate [FL-no: 09.808], 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphtalen-2-ol [FL-no: 02.197] and cedryl acetate [FL-no: 09.171] there are no metabolism studies available. Two metabolism studies were found for the candidate substance cedrol [FL-no: 02.120]. In the study by Bang and Ourisson (1975) rabbits were given natural cedrol (1 g) suspended in a 1 % methyl-cellulose mucilage (20 ml) administered by gavage followed by 25 ml water. The urine was collected at 24, 48 and 96 hours after administration. The urine was acidified to pH 4.5 and hydrolysed at 37° C for 24 hours with 6 ml snail (D-glucuronide)-glucuronidase. Five percent of the

dose was excreted as conjugation product of unchanged cedrol. Thirtyfive percent of the dose was recovered as a mixture of stereochemically different 3-hydroxycedrol metabolites. Twelve percent of the dose was recovered as a C7-C8 dehydrated C3-hydroxylation product of cedrol (both 3-R and 3-S) (Bang and Ourisson, 1975).

Trifilieff et al. (1975) administered 2 grams of cedrol to a phenobarbital-pretreated fasted dog. The metabolites described below (see also Figure III.5) were found in the first 24 hours urine. Urine was acidified with hydrochloric acid and treated with glucuronidase-sulphatase to hydrolyse conjugates. Three metabolite fraction, isolated by chromatography on silica columns and acetylation, were obtained in the following amounts: 130 mg of diol (1), 100 mg of a mixed fraction of diols (2 plus 3), and 40 mg of an acid (4). Diol (1) was identified by spectral data and correlation with α -epi-isobiotol (= 3-hydroxycedrol) and the corresponding ketone. In contrast to the rabbit the dog produced only the 3S isomer. Diols (2 plus 3) were identified as 15-hydroxycedrol and 2-hydroxycedrol, respectively, by separation after dehydration and further derivation and identification of the reaction products by comparison of the spectral data with literature data. The acid (4) was identified as the C14 carboxylic acid derivation product of 2-hydroxycedrol. In total only 270 mg (13 % of the dose) was recovered, but elimination of cedrol (-conjugates) was not studied / reported (Trifilieff et al., 1975). For structures of cedrol and the four metabolites in the dog see scheme below (figure III.5):

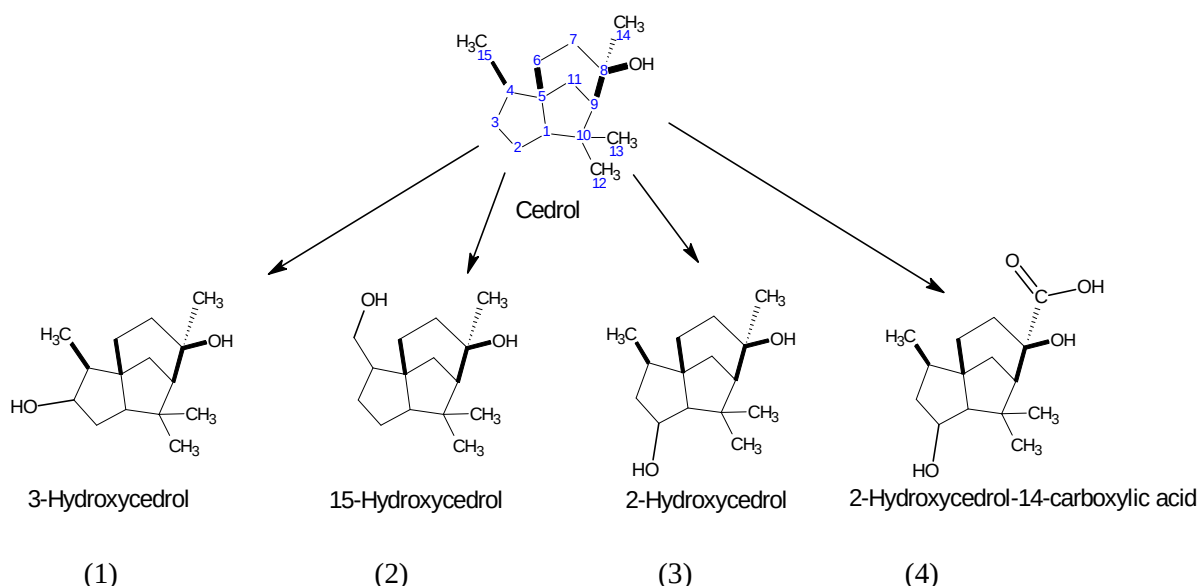


Figure D.5 Metabolism of cedrol in a dog treated with phenobarbital. The carboxylic acid is presumably formed from 2-hydroxycedrol, but this was not further studied (after Trifilieff et al, 1975).

Subgroup 8: Aromatic tertiary alcohols

No metabolism studies were found for the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203]. However, several metabolism studies were found for the supporting substance p-cymene.

The main metabolites in the urine of rabbits given a single oral dose of 670 mg p-cymene /kg bw were p-cymen-9-ol and p-cymen-8-ol (50 % and 28 %, respectively, of the neutral metabolites). Acidic metabolites identified were α -p-tolylpropionic acid, α -tolyl- α -hydroxypropionic acid, p-isopropylbenzoic acid and p-1-hydroxyisopropylbenzoic acid. Ring hydroxylation did not occur (Ishida et al., 1981).

Following an oral dose of 100 mg/kg bw of p-cymene to male rats, the principal urinary metabolites were p-isopropylbenzoic acid (19 % of the administered dose) and 2-p-carboxyphenylpropionic acid (16 %). Other less important urinary metabolites included 2-p-tolylpropan-1-ol (8 %), 2-p-tolylpropan-

2-ol (9 %), 2-p-carboxyphenylpropan-2-ol (9 %), 2-p-(hydroxymethyl)phenylpropionic acid (4 %), 2-p-carboxyphenylpropan-1-ol (11 %), p-isopropylbenzoylglycine (2 %), p-isopropylbenzyl alcohol (1 %), and 2-p-tolylpropionic acid (1 %) (Walde et al., 1983). When the same dose was given to male guinea pigs, similar urinary metabolites were identified, however in different quantities. The primary urinary metabolite in guinea pigs was p-isopropylbenzoylglycine (31 %), indicating that conjugation with glycine was more prevalent in guinea pigs than in rats. Another major metabolite in guinea pigs was 2-p-tolylpropan-2-ol (14 %). In addition, whereas ring hydroxylation of p-cymene was not reported in rats (Bakke and Scheline, 1970; Walde et al., 1983) and rabbits (Ishida et al., 1981), trace amounts of the ring hydroxylation metabolites hydroxyl-p-cymene and hydroxycarvacrol (dehydroxyl-p-cymene) were detected in guinea pig urine. Ring hydroxylation in guinea pigs only occurred in ortho position to the methyl group (Walde et al., 1983).

Boyle et al. (1999) studied the metabolite pattern of p-cymene in rats following oral doses equivalent to 50 and 200 mg/kg bw. The major metabolites in 0-48 hours urine after administration of the 50 mg/kg bw dose were 2-p-tolylpropan-2-ol (39 % of recovered dose) and 2-p-carboxyphenylpropan-2-ol (19 %). The former metabolite is the product of allylic hydroxylation of the isopropyl substituent, while the latter metabolite is the product of allylic hydroxylation of both the isopropyl substituent and the methyl substituent. Minor metabolites in rat urine were 2-p-carboxyphenylpropan-1-ol (10 %), 2-p-carboxyphenylpropionic acid (14 %), and p-isopropylbenzoic acid (17 %). A large percentage of the urinary metabolites at this dose was conjugated (66 % conjugated vs 34 % free) both to glucuronic acid and glycine. The same metabolites were observed after the high dose, but conjugation was considerably reduced (18 % conjugated vs 82 % free), suggesting saturation of the conjugation pathway (Boyle et al., 1999).

Oxidation of terminal double bonds

Ten candidate substances [FL-no: 02.123, 02.168, 02.144, 02.146, 02.150, 02.206, 02.226, 09.614, 09.671 and 02.149] possess terminal double bonds. These double bonds may be oxidised to the corresponding epoxide. Epoxides are highly reactive molecules, due to the large strain associated with the three membered ring structures, and they react easily with nucleophilic sites of cellular macromolecules. For this reason, several aliphatic alkene-derived epoxides have been demonstrated to be carcinogenic (Melnick, 2002).

Two of the main metabolites of one of the supporting substances, myrcene, were found to be produced through epoxidation of the double bonds followed by hydrolysis to diols by epoxide hydroxylase (see above). Theoretically these reactions may also occur in the candidate substances with conjugated terminal double bonds. However, linalool or elemol were not found to give oxidation of terminal double bonds, indicating that terminal double bonds as such will not give rise to intermediate epoxides.

D.4 Summary and Conclusion

Six candidate flavouring substances in this group are esters [FL-no: 09.171, 09.356, 09.614, 09.671, 09.808 and 09.617]. Hydrolysis data are not available for any of these esters. However, for the supporting substance, linalyl acetate, *in vitro* hydrolysis data indicate that these esters may be hydrolysed, which would also be expected based on general knowledge about ester hydrolysis. The carboxylic acids resulting from the hydrolysis of these six candidate flavouring substances are acetic acid, propanoic acid and valeric acid, which will be incorporated in normal physiological processes such as beta-oxidation and citric acid cycle. The alcohols resulting from the hydrolysis of these esters are tertiary alcohols as are all the remaining candidate substances in this flavouring group. The tertiary alcohols in this FGE are subdivided into eight subgroups according to their chemical structures.

Subgroup 1: Metabolism studies of the three candidate substances, 2-methylpropan-2-ol [FL-no: 02.052], 2-methylbutan-2-ol [FL-no: 02.041] and 2-methylpentan-2-ol [FL-no: 02.181] show that these are conjugated with glucuronic acid before excretion in the urine. When rats were treated with 2-methylpropan-2-ol, acetone was excreted in small amounts, and when administered 2-methylbutan-2-

ol, diols were excreted. This indicates that an additional metabolism pathway of the three candidate substances is oxidation of the methyl group. From these metabolism studies it is anticipated that the candidate substances 2-methylpropan-2-ol, 2-methylbutan-2-ol, 3-methylpenta-3-ol, 2-methylpentan-2-ol, 2,6-dimethyl-2-heptanol, 2,4-dimethyl-4-nonanol, 3,6-dimethyloctan-3-ol and 1,1-dimethylethyl propionate [FL-no: 02.052, 02.041, 02.184, 02.181, 02.219, 02.253, 02.147 and 09.356] are conjugated with glucuronic acid and excreted in the urine, or that they can undergo oxidation to yield the corresponding diols. They are also expected to be excreted as their respective glucuronic acid conjugates.

Subgroup 2: Linalool is a supporting substance to the candidate substances 2-methylbut-3-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate [FL-no: 02.123, 02.150, 02.168, 02.226, 09.614 and 09.671,], which have the isolated double bond in close proximity to the tertiary alcohol group. As these substances or their respective alcohol moieties have a free hydroxyl group, they may be directly conjugated. Seventy-two hours after intragastrical administration of 500 mg/kg bw ¹⁴C-labelled linalool to 12-weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetra hydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut. In addition, the metabolism of linalool indicates that these candidate substances may also be metabolised by omega-oxidation of methyl groups, excreted in the urine as such or in conjugation with glucuronic acid. No oxidation of the terminal double bond in linalool was observed, indicating no formation of epoxide intermediates.

For 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] the structure differs from the supporting substance linalool and the other candidate substances in this group in that the isolated terminal double bond is located distant from the tertiary group. However, any risk from epoxide formation of this compound is considered to be low since the substance can be directly conjugated with glucuronic acid or the alcohol group can be expected to be readily attacked by oxidation processes, ultimately yielding the corresponding carboxylic acid. Biochemical attack of this carboxylic acid via beta-oxidation or conjugation with glucuronic acid is expected to be more efficient and rapid than microsomal oxidation. Any epoxides formed may be metabolised by conjugation with glutathione or by epoxide-hydrolase mediated hydrolysis.

Subgroup 3: Myrcene is a supporting substance to the candidate substance 3,7-dimethylocta-1,5,7-trien-3-ol, [FL-no: 02.146]. This substance also has an alcohol moiety that can be directly conjugated. In addition, further oxidation of methyl groups may occur. As shown for myrcene, oxidation of conjugated terminal double bonds in the candidate substances may occur, giving rise to epoxide intermediates. It should be noted that the available genotoxicity data for myrcene do not indicate a genotoxic potential for this substance even in the presence of metabolic activation. However, it cannot be anticipated that these candidate substances will be metabolised to innocuous products.

Subgroup 4: 1,2-Dihydrolinalool [FL-no: 02.140] can be expected to be directly conjugated to glucuronic acid like the supporting substance linalool, and excreted. After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep, dihydrolinalool and tetrahydrolinalool are formed as metabolites. *In vivo* metabolism studies in rats on ¹⁴C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucuronides. Additionally, it might be oxidised at the methyl groups, introducing new hydroxyl groups that also can be conjugated and excreted.

Subgroup 5: From the metabolism studies of alpha-terpineol and menthol it is anticipated that the candidate substances terpineol [FL-no: 02.230], p-menthane-1,8-diol [FL-no: 02.054], bisabola-1,12-dien-8-ol [FL-no: 02.129] and p-menthan-8-ol [FL-no: 02.171] (also an hydrolysis product of candidate substance [FL-no: 09.617]) may undergo allylic oxidation of the exocyclic methyl group. This could be further oxidised to a carboxylic acid group. Alternative or subsequent metabolism may occur by conjugation to glucuronic acid, followed by excretion in the urine.

Subgroup 6: A metabolism study on elemol indicate that the substance is absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of elemol [FL-no: 02.149] and sclareol [FL-no: 02.206], which have the same structural features as elemol, would not be anticipated.

Subgroup 7: Two metabolism studies on cedrol [FL-no: 02.120] indicate that the candidate substances 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197], cedrol [FL-no: 02.120] and cedryl acetate [FL-no: 09.171] and guaiyl acetate [FL-no: 09.808] will be further hydroxylated and excreted in urine as such or as conjugates.

Subgroup 8: In metabolism studies, the supporting substance 1-isopropyl-4-methylbenzene [FL-no: 01.002] (synonym: p-cymene) was oxidised at the isopropyl side chain yielding 2-(p-tolyl)-2-propanol, which is not further oxidised, but excreted unchanged or as a glucuronic acid conjugate. It is anticipated that the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203] will follow the same pathway and be excreted unchanged or in conjugation with glucuronic acid.

In summary, 28 of the candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.808, 09.171, 09.356, 09.614, 09.617 and 09.671] are anticipated to be metabolised to innocuous products.

For the remaining candidate substance [FL-no: 02.146] (subgroup 3), which contains a conjugated terminal double bond, data from the supporting substance myrcene [FL-no: 01.008] indicate that this terminal double bond may be oxidised, giving rise to epoxide intermediates. Thus, it cannot be anticipated that this candidate substance will be metabolised to innocuous products. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies.

Appendix E. Toxicity

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Rat	NR	Oral	3500	(Schaffarzick and Brown, 1952)	
	Rat	M, F	Gavage	3046	(Johnson, 1981a)	
	Rat	M, F	Gavage	2733	(Johnson, 1981b)	
	Rat	NR	Oral	> 3800	(Eastman Kodak Co., 1994)	
	Rabbit	M, F	Gavage	3560	(Munch, 1972)	
2-Methylbutan-2-ol [02.041]	Rat	NR	Oral	1000	(Schaffarzick and Brown, 1952)	
	Rat	NR	Oral	1000 – 2000	(Dow Chemical Company, 1982)	
	Rabbit	M, F	Gavage	2027	(Munch, 1972)	
3-Methylpentan-3-ol [02.184]	Rat	NR	Oral	710	(Brown et al., 1955)	
2,6-Dimethyl-2-heptanol [02.219]	Rat	NR	Oral	6800	(BASF, 1979)	
	Rat	NR	Oral	> 5000	(Moreno, 1976a)	
3,6-Dimethyloctan-3-ol [02.147]	Rat	NR	Oral	> 5000	(Moreno, 1973a)	
2-Methylbut-3-en-2-ol [02.123]	Rat	NR	Oral	1800	(BASF, 1972)	
(Linalool [02.013])	Rat	M, F	Gavage	2790	(Jenner et al., 1964)	
	Mouse	M, F	Oral	2200	(Rhône-Poulenc, Inc., 1992)	
	Mouse	M, F	Oral	3918	(Hoffman-LaRoche, Inc., 1967)	
	Mouse	M, F	Oral	3000 ¹ 1700 ²	(Rhône-Poulenc, Inc., 1992)	
(3,7-Dimethyloctan-3-ol [02.028])	Rat	NR	Oral	> 5000	(Moreno, 1976b)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat	NR	Oral	3600	(Moreno, 1973b)	
Myrcenol [02.185]	Rat	NR	Oral	5300	(Moreno, 1972a)	
Ocimenol [02.191]	Rat	NR	Oral	1700	(Wohl, 1974)	
Geranyl linalool [02.150]	Rat	NR	Oral	> 5000	(Moreno, 1982a)	
	Mouse	M, F	Oral	14,632	(Hoffman-LaRoche Inc., 1967)	
(Linalyl formate [09.080])	Rat	NR	Oral	> 5000	(Russell, 1973)	
	Mouse	M	Oral	5389	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl acetate [09.013])	Rat	M, F	Gavage	14,550	(Jenner et al., 1964)	
	Rat	NR	Oral	10,000	(Zeller, 1969)	
	Mouse	NR	Gavage	13,360	(Jenner et al., 1964)	
	Mouse	M	Oral	13,539	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl propionate [09.130])	Rat	NR	Oral	> 5000	(Moreno, 1973c)	
	Mouse	M	Oral	13,874	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl butyrate [09.050])	Rat	NR	Oral	> 5000	(Levenstein, 1975)	
	Mouse	M	Oral	> 8907	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl isobutyrate [09.423])	Rat	M, F	Gavage	> 36,300	(Jenner et al., 1964)	
	Mouse	NR	Gavage	15,100	(Jenner et al., 1964)	
	Mouse	M	Oral	> 17,698	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl isovalerate [09.454])	Rat	NR	Oral	> 5000	(Moreno, 1975)	
	Mouse	M, F	Oral	25,165	(Hoffman-LaRoche, Inc., 1967)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Linalyl hexanoate [09.068])	Mouse	M, F	Oral	37,869	(Hoffman-LaRoche Inc., 1967)	
Myrcenyl acetate [09.669]	Rat	NR	Oral	6300	(Moreno, 1972b)	
(Linalyl octanoate [09.116])	Mouse	M, F	Oral	48,849	(Hoffman-LaRoche Inc., 1967)	
Nerolidyl acetate [09.671]	Rat	NR	Oral	> 5000	(Moreno, 1976c)	
(alpha-Terpineol [02.014])	Rat	NR	Oral	4300 ³	(Moreno, 1971)	
	Mouse	M	Gavage	2830	(Yamahara et al., 1985)	
(alpha-Terpinyol formate [09.081])	Rat	NR	Oral	> 5000	(Moreno, 1976d)	
(Terpinyl acetate [09.830])	Rat	M, F	Gavage	5075	(Jenner et al., 1964)	
(Terpinyl propionate [09.142])	Rat	NR	Oral	> 5000	(Moreno, 1973d)	
(Terpinyl 2-methylpropionate [09.425])	Rat	NR	Oral	> 5000	(Moreno, 1982b)	
(4-Terpinenol [02.072])	Rat	NR	Oral	1300	(Moreno, 1977)	
Guaiyl acetate [09.808]	Rat	NR	Oral	> 5000	(Moreno, 1973e)	
2-Phenylpropan-2-ol [02.203]	Rat	NR	Oral	1037 ⁴	(Patty, 1982)	
(beta-Terpineol) [02.097])	Rat	NR	Oral	4300 ³	(Moreno, 1971)	
<i>p</i> -Menthan-8-ol [02.171]	Rat	NR	Oral	> 5000	(Moreno, 1973f)	
(1-Acetoxy-1-acetylcyclohexane [09.293])	Rat	M, F	Gavage	2150	(Piccirillo and Hartman, 1980)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Menthol [02.015])	Mouse	M	Gavage	2652	(Food and Drug Research Laboratories, Inc., 1975)	
	Mouse	M	Gavage	4384	(Food and Drug Research Laboratories, Inc., 1975)	
	Mouse	NR	Gavage	3100	(Wokes, 1932)	
	Rat	M, F	Gavage	3180	(Jenner et al., 1964)	
	Rat	M	Gavage	940	(Food and Drug Research Laboratories, Inc., 1975)	
Bisabola-1,12-dien-8-ol [02.129]	Rat	NR	Oral	> 5000	(CIR, 1999)	
	Mice	NR	Oral	0.633	(CIR, 1999)	
	Rat	M, F	Oral	M: 14,9 ml/kg bw F: 15.6 ml/kg bw	(Habersang et al., 1979)	
	Mice	M, D	Oral	15.1 ml/kg bw	(Habersang et al., 1979)	

¹Value represents calculated LD₅₀ when substance was administered in peanut oil.

²Value represents calculated LD₅₀ when substance was administered as an emulsion in an aqueous Arabic gum solution at 10 %.

³Reported for a mixture of alpha- and beta-terpineol.

⁴LD₅₀ value reported as 1.07 ml; conversion based on a density of 0.97 g/ml.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Rat; M 5-6	Drinking water	0 or 0.5 %, equivalent to 0 or 500 mg/kg bw per day	10 weeks	None	(Acharya et al., 1997)	Histological examination of liver and kidney only.
	Rat; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	90 days	None	(Lindamood et al., 1992); (NTP, 1995)	Fully described NTP study. No NOAEL for males or females.
	Rat; M 10	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	90 days	None	(Takahashi et al., 1993)	Good quality investigative study using extra renal samples from the above 90-day study; indicates nephropathy in male F344 rats is due to alpha-2 μ -globulin. No NOAELs.
	Rat; M, F 120 ^(d)	Drinking water	Males: 0, 0.125, 0.25 or 0.5 %, equivalent to 0, 90, 200 or 420 mg/kg bw per day Females: 0, 0.25, 0.5 or 1 %, equivalent to 0, 180, 330 or 650 mg/kg bw per day	2 years	None	(Cirvello et al., 1995); (NTP, 1995)	Fully described NTP study. No NOAELs. NTP conclusions on carcinogenicity: some evidence of carcinogenic activity in males, no evidence of carcinogenicity in females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
	Rat; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	90 days	Male: 0.5 %, equivalent to 500 mg/kg bw per day Female: 1 %, equivalent to 1000 mg/kg bw per day	(Brown and Wheeler, 1979)	The report is incomplete as it is lacking tables.
	Mouse; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 625, 1250, 2500, 5000 or 10,000 mg/kg bw per day	90 days		(Brown and Wheeler, 1979)	The report is incomplete as it is lacking tables.
	Mouse; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 625, 1250, 2500, 5000 or 10,000 mg/kg bw per day	90 days	Male: 1 %, equivalent to 2500 mg/kg bw per day Female: 2 %, equivalent to 5000 mg/kg bw per day	(Lindamood et al., 1992); (NTP, 1995)	Fully described NTP study.
	Mouse; M, F 120	Drinking water	0, 0.5, 1 or 2 %, equivalent to 0, 540, 1040 or 2070 mg/kg bw per day in males and 0, 510, 1020 or 2100 mg/kg bw per day in females	2 years	M: None F: 0.5 %, equivalent to 510 mg/kg bw per day	(Cirvello et al., 1995); (NTP, 1995)	Fully described NTP study. NTP conclusions on carcinogenicity: equivocal evidence of carcinogenic activity in males, some evidence of carcinogenic activity in females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
2-Methylbut-3-en-2-ol [02.123]	Rat; M, F 10	Gavage	0, 30, 150 or 750 mg/kg bw per day 5 days/week	4 weeks	150	(BASF, 1994)	Full study details provided. Study considered valid. Dosing only 5 days/week but a clear NOAEL.
(Linalool [02.013])	Rat; M, F NR	Diet ^(b)	0 or 50 mg/kg bw per day ^(a)	84 days	50 ^(a)	(Oser, 1967)	Unpublished report. Administered as 50 % mixture of linalool and citronellol. Slight retardation of growth in males at 50 mg/kg bw per day, not related to food intake.
	Mouse; F 30	Gavage	0 or 365 mg/kg bw per day	5 days	375 ^{(a),(c)}	(Gaworski et al., 1994)	Immunotoxicity study only.
	Rat; F 100	Gavage		21 days	500	(Lewis, 2006)	
(Linalyl acetate [09.013])	Rat; M, F NR	Diet ⁵	0 or 24 mg/kg bw per day ^(a)	84 days	24 ^(a)	(Oser, 1967)	Unpublished report. Administered as mixture of linalyl acetate (24 mg/kg bw per day), linalyl isobutyrate (27 mg/kg bw per day) and geranyl acetate (48 mg/kg bw per day). Slight growth retardation of females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
(Linalyl isobutyrate [09.423])	Rat; M, F NR	Diet ⁶	0 or 27 mg/kg bw per day ^(a)	84 days	27 ^(a)	(Oser, 1967)	
	Rat; NR 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw per day	18 weeks	500 (10,000 ppm)	(Hagan et al., 1967)	Very limited details provided.
(Linalyl cinnamate [09.736] ⁷)	Rat; M, F 10	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw per day	17 weeks	500 ^(a) (10,000 ppm)	(Hagan et al., 1967)	Very limited details provided.
(Geranyl acetate [09.011] /Citronellyl acetate[09.012] ⁸)	Mouse; M, F 50	Gavage		103 weeks	- ⁹	(NTP, 1987)	
	Rat; M, F 50	Gavage		103 weeks	1000 (710; 290) ⁸	(NTP, 1987)	
(Myrcene [01.008])	Rat; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	13 weeks	None	(NTP, 2004)	Draft results only available. Evaluated by the JECFA. No NOAEL as most sensitive adverse effect (nephropathy) present at lowest dose tested (JECFA, 2006).
	Mouse; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day, 5 days/week	13 weeks	Male: None Female: 250 mg/kg bw per day	(NTP, 2004)	Draft results only available. Evaluated by the JECFA. Liver hypertrophy in males at all doses (JECFA, 2006).

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
	Rats; M, F 10	Diet	0, 8.0, 40 and 44 mg/kg bw per day (males) 0, 9.6, 48 and 53 mg/kg bw per day (females)	90 days	Male: 44 mg/kg bw per day Female: 53 mg/kg bw per day	(Bauter, 2013)	Compliant with OECD Guideline 408.
(Menthol [02.015])	Mouse; M, F 50	Diet	0, 2000 or 4000 ppm, equivalent to 0, 300 or 600 mg/kg bw per day	103 weeks	600 ^(a)	(National Cancer Institute, 1979)	Good quality.
	Mouse; F 30	I.P. injection	0, 500 or 2000 mg/kg, 3 times/week	24 weeks	None	(Stoner et al., 1973)	Good quality.
	Rat; M, F 20	Gavage	0, 200, 400 or 800 mg/kg bw day	28 days	None	(Thorup et al., 1983)	Relative good quality.
	Rat; M, F 80	Diet	0, 100 or 200 mg/kg bw	5.5 weeks	200 ^(a)	(Herken, 1961)	Limited information.
	Rat; M, F 50	Diet	0, 3750 or 7500 ppm, equivalent to 0, 188 or 375 mg/kg bw per day	103 weeks	375 ^(a)	(National Cancer Institute, 1979)	Good quality.
(Terpinyl acetate [09.830])	Rat; M, F 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw per day	20 weeks	500 ^(a)	(Hagan et al., 1967)	Very limited details provided.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
Sclareol [02.206]	Rat; M, F 20	Gavage	8.8 mg/kg bw per day	28 days	8.8	(IOFI, 2006)	
Cedrol [02.120]	Rat; M, F 20	Gavage	8.4 mg/kg bw per day	28 days	8.4	(IOFI, 2006)	
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat; M, F 40	Gavage	10, 50, 500, 1000 mg/kg bw per day	90 days	50 (F) 10 (M)	(Dunster et al., 2007)	
Bisabola-1,12-dien-8-ol [02.129]	Dog, NR 20	Gavage	1 ml/kg bw	14 days	850	(Habersang et al., 1979)	
	Rat, M,F 20	Gavage	2, 3 ml/kg bw	28 days	850	(Habersang et al., 1979)	

NR Not Reported

(a): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(b): Administered with citronellol as part of a 50/50 mixture.

(c): Immunotoxicity study.

(d): Three treatment groups per sex. Each dose group included 60 male and female animals.

⁵ Administered with linalyl isobutyrate and geranyl acetate as a part of a mixture.

⁶ Administered with linalyl acetate and geranyl acetate as a part of a mixture.

⁷ Structurally related ester of linalool not evaluated as part of the supporting chemicals group.

⁸ Structurally related terpenoid esters administered as a mixture: geranyl acetate, 71 %; citronellyl acetate, 29 %.

⁹ A NOAEL could not be established due to a high incidence of gavage errors and low survival associated with pneumonia.

Table 18: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Duration	Species/Species ex No/group	Route	Dose levels	NOAEL (mg/kg per day)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Developmental Toxicity Gestation Days 6 - 20	Mouse; F 15	Liquid Diet	0, 0.5, 0.75 or 1 % (equivalent to approximately 0, 3 400, 4 900 and 6 400 mg/kg bw per day)	Maternal: 0.5 % Foetal: None	(Daniel and Evans, 1982)	Study considered valid. Dose related reduction of offspring performance in neurobehavioural tests (righting reflex, negative geotaxis, open field behaviour, cliff avoidance, roto-rod performance).
	Developmental Toxicity: Gestation Days 6– 18	Mouse; F 9 - 12	Gavage ^(a)	0 or 10.5 mmol/kg bw per day, equivalent to 1 557 mg/kg bw per day	Maternal: None Foetal: None	(Faulkner et al., 1989)	Study considered valid. Increased foetal resorptions and decreased births per litter in treated group.
(Linalool [02.013])	Reproductive & Developmental Toxicity: 33 - 39 days ^(c)	Rat; F 10	Gavage	0, 250, 500 or 1 000 mg/kg bw per day coriander oil, equivalent to 0, 182.3, 364.5 or 729 mg/kg bw per day linalool	Maternal: None Foetal: 364.5	(Hoberman and Christian, 1989)	Test substance was coriander oil. Linalool content 72.9 %.
(Menthol [02.015])	Teratology Gestation days 6 - 15	Mouse; F 22	Gavage	0, 1.85, 8.59, 39.9, 185	185 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	
	Teratology Gestation days 6 - 15	Rat; F 22 - 23	Gavage	0, 2.18, 10.15, 47.05, 218	218 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	

Table 18: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Duration	Species/Species ex No/group	Route	Dose levels	NOAEL (mg/kg per day)	Reference	Comments
	Teratology Gestation days 6 - 15	Hamster; F 20 - 22	Gavage	0, 4.05, 21.15, 98.2, 405	405 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	
	Teratology Gestation days 6 - 18	Rabbit; F 9 - 11	Gavage	0, 4.25, 19.75, 91.7, 425	425 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	
(Myrcene [01.008])	Teratology Gestation days 6 - 15	Rat; F 16 - 29	Gavage	0, 250, 500 and 1 200 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal: 500 mg/kg bw per day	(Delgado et al., 1993a)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Gestation day 15 to postnatal day 21	Rat; F 12 - 18	Gavage	0, 250, 500, 1 000 and 1 500 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal/neonatal: 250 mg/kg bw per day	(Delgado et al., 1993b)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Prior to mating until postnatal day 21 ^(d)	Rat; M, F 60	Gavage	0, 100, 300, 500 mg/kg bw per day	Maternal/paternal: 500 mg/kg bw per day Foetal: 300 mg/kg bw per day	(Paumgartten et al., 1998)	Study considered valid. Evaluated by the JECFA.
2,6-Dimethyloct-7-en-2-ol [02.144]	Developmental Toxicity: Gestation Days 7 - 17	Rat, M, F 25	Gavage	0, 250, 500 and 1 000 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal: 500 mg/kg bw per day	(Politano et al., 2008)	

(a): Test substance was administered to two strains of mice at a dose level of 778 mg/kg (10.5 mmoles/kg) twice daily.

(b): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(c): Animals were dosed for seven days prior to mating, during mating (maximum of seven days), during gestation, delivery and four days post parturition.

(d): Males were dosed for 91 days prior to mating and during mating; females were dosed from 21 days prior to mating to 21 days after birth

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	0.1, 0.5, 2.5, 5, 10 µl/plate (7800 µg/plate)	Questionable ^(a)	(Haworth et al., 1981a)	Unpublished GLP study. According to the conclusion of the report, the test substance “did cause a weak but significant increase in TA1535 revertants per plate in both the presence and absence of rat liver microsomes.” However, this result cannot be re-evaluated because the corresponding page with results on TA1535 in Table format is lacking.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	probably 1 to 10 µl/plate (7800 µg/plate) (corresponding pages of the report are lacking)	Questionable ^(a)	(Haworth et al., 1981b)	Unpublished GLP study. According to the conclusion of the report, the test substance (purity 99.9 %) “did not cause a significant increase in the number of revertants per plate in any of the tester strains with or without metabolic activation. It should be noted, however, that there was a slight increase in TA1535 revertants per plate observed in the presence and absence of rat liver microsomes.” However, this result cannot be re-evaluated because 15 pages with all results in Table format are lacking.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	10000 µg/plate	Negative ^(a)	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD guideline 471. There was a slight increase in TA1535 revertants per plate observed in the presence and absence of rat and hamster liver microsomes. This effect is dose-related only with hamster liver S9. Overall, the effects were less than twice compared to control. The study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Yeast mitochondrial mutation assay	Several <i>Saccharomyces</i> strains	4 %	Positive	(Jiménez et al., 1988)	This non-GLP study was not in accordance with OECD guideline 480 (1986), and the study protocol does not belong to standard protocols used in routine testing. However, the result is considered valid since main details of method and results are reported. Endpoint not relevant for genotoxicity.
	Forward mutation assay	Mouse lymphoma L5178Y TK +/-	0, 1000, 2000, 3000, 4000, 5000 µg/ml	Negative ^(a)	(McGregor et al., 1988)	Non-GLP study in accordance with OECD guideline 476 (1984). Study is considered valid.
	Forward mutation assay	Mouse lymphoma L5178Y TK +/-	1.3 to 100 µl/ml (78000 µg/ml)	Negative	(Kirby et al., 1981)	Unpublished GLP study. According to the report's summary, test substance of high (99.9 %) purity did not induce any detectable increases in the mutant frequencies in the presence and absence of S9-mix. When cultures were tested in the presence of S9-mix with less pure test substance none of the cultures exhibited increases in mutant frequency. Without S9-mix this test substance did appear to induce an increase in the mutant frequency of cultures treated with the higher doses, but a dose-related response was not evident. In addition, in only one of two experiments was a greater than two-fold increase in mutant frequency observed. However, this result cannot be re-evaluated because 47 pages with all results in Table format are lacking in the report submitted.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration	Chinese hamster ovary cells	160 - 5000 µg /ml	Negative	(NTP, 1984)	Limited validity. This non-GLP study was in accordance with OECD guideline 473 (1983) except that only a single harvest time was used, however, the study protocol does not fully meet the criteria of the revised guideline from 1997. According to the version from 1997, a single sampling time should be equivalent to about 1.5 normal cell cycle lengths, duplicate cultures should be used at each concentration and 200 metaphases should be scored per concentration.
	Sister chromatid exchange	Chinese hamster ovary cells	6 concentrations ranging from 0.625 to 20 µl/ml (15 600 µg/ml)	Negative	(Putman, 1985)	Unpublished GLP study. According to the report's summary, test substance of high (99.9 %) purity caused a significant increase in sister chromatid exchanges at the high dose only in the assay without S9 and at the two highest doses in the assay with S9, however, the test article did not meet the criteria for a positive response (at least two-fold increase or a significant positive dose response over at least three doses). However, this result cannot be re-evaluated because pages with all results in Table format are lacking in the report submitted.
	Sister chromatid exchange	Chinese hamster ovary cells	20 µl/ml (15 600 µg/ml)	Negative	(Thilagar et al., 1981)	Unpublished GLP study is considered valid. A marginal increase in SCE frequency was observed in the tests with and without S9, while only the highest concentration without S9 resulted in a significant increase. Thus, the test article did not meet the criteria for a positive response (at least two-fold increase or a significant positive dose response over at least three doses). (All relevant tables

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
2-Methylbutan-2-ol [02.041]	Mutagenicity assays	<i>S. typhimurium</i> TA1535; TA1537; TA1538; <i>S. cerevisiae</i>	NR	Negative	(Dow Chemical Company, 1982)	Very short abstract only.
	Sister chromatid exchange	Chinese hamster ovary cells	160, 500, 1600, 5 000 µg/ml	Positive ^(c) Negative ^(b)	(NTP, 1997)	
	Sister chromatid exchange	Chinese hamster ovary cells	2 000, 3 000, 4 000, 5 000 µg/ml	Negative ^(a)	(NTP, 1997)	
2-Methylbut-3-en-2-ol [02.123]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	20 – 5 000 µg/plate	Negative ^(a)	(BASF, 1989)	Summary in IUCLID data set only. According to this summary, the assay was not in compliance with GLP but in accordance with OECD guideline 471. The unpublished study report is not available for re-evaluation.
	Liquid suspension assay	<i>S. typhimurium</i> TA98; TA100	20 - 5 000 µg/plate	Negative ^(a)	(BASF, 1991)	Summary in IUCLID data set only. According to this summary, the assay was not in compliance with GLP but in accordance with OECD guideline 471. The unpublished study report is not available for re-evaluation.
Isophytol [02.168]	Ames test	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535	100, 333, 1 000, 3 333, 10 000 µg/plate	Equivocal ^(a)	(NTP, 1994)	This non-GLP study is considered valid. It is in accordance with OECD guideline 471 (1983). The study is published in the Web and the report contains sufficient details.
	Ames test	<i>S. typhimurium</i> TA97, TA98, TA100, and TA1535	100 - 10000 µg/plate	Negative	(NTP, 2000)	This non-GLP study is considered valid. It is in accordance with OECD guideline 471 (1983). The study is published in the Web and the report contains sufficient details.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Linalool [02.013])	Ames test (modified)	<i>S. typhimurium</i> TA100	3 µl/2 ml (2 610 µg/2ml) incubation volume 1 mg/plate	Negative ^(a)	(Eder et al., 1980)	
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537	(1 000 µg/plate)	Negative ^(a)	(Ishidate et al., 1984)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	100 µl (87 000 µg)	Negative ^(a)	(Rockwell and Raw, 1979)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 nl/plate (8700 µg/plate)	Negative ^(a)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster fibroblasts	250 µg/ml	Negative ^(a)	(Ishidate et al., 1984)	
	Unscheduled DNA synthesis	Rat hepatocytes	50 nl/ml (43.6 µg/ml)	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	1 mg/plate (1 000 µg/plate)	Negative	(Yoo, 1986)	In Japanese (only summary and tables in English). Thus, the validity cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17(rec+); M45(rec-)	17 µg	Negative	(Oda et al., 1979)	
	Rec assay	<i>B. subtilis</i> H17(rec+); M45(rec-)	10 µl/disk (8700 µg/disk)	Positive	(Yoo, 1986)	In Japanese (only summary and tables in English). Thus, the validity cannot be evaluated.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell mutation	Mouse lymphoma L5178Y TK+/-	3.9 - 300 nl/ml	Negative ^(b) Positive ^(c)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	1 000 µM (15 4250 µg)	Negative ^{(a),(d)}	(Sasaki et al., 1989)	
(Linalyl acetate [09.013])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	25 000 nl/plate (22 575 µg/plate)	Negative ^(a)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Unscheduled DNA synthesis	Fischer or SD rat hepatocytes	300 nl/ml (271 µg/ml)	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17 (rec+); M45 (rec-)	18 µg	Negative	(Oda et al., 1979)	
	Chromosome aberration	Peripheral human lymphocytes	180 µg/ml	Negative ^(a)	(Bertens and van de Waart, 2000)	
(alpha-Terpineol [02.014])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 µg/plate	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA97a; TA98; TA100; TA102	2 500 µg/plate 2 500 µg/plate	Negative Weakly positive ^(e)	(Gomes-Carneiro et al., 1998)	The study is considered valid. A slight but dose-related response was noted with TA102 with and without the use of metabolic activation.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	1 000 µg/plate	Negative ^(a)	(National Cancer Institute, 1983)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 µg/plate	Negative ^(a)	(Lorillard, 1983)	
	Spot test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmol/plate (463 µg/plate)	Negative ^(a)	(Florin et al., 1980)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	0.5 µl/ml (467 µg/ml) 0.75 µl/ml (700 µg/ml)	Negative ^(c) Negative ^(b)	(Kirby et al., 1984)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	300 nl/ml (280 µg/ml) 250 nl/ml (233 µg/ml)	Negative ^(a)	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	15.6 - 250 nl/ml 15.6 - 300 nl/ml	Negative ^(b) Negative ^(c)	(Lorillard, 1982)	
	Rec assay	<i>S. cerevisiae</i>	NR	Negative	(Oda et al., 1979)	
(Terpinyl acetate [09.830])	Rec assay	<i>B. subtilis</i> H17; M45	19 µg	Negative	(Oda et al., 1979)	
(beta-Terpineol) [02.097])	Ames	<i>S. typhimurium</i> TA98; TA100	0.05 µl (100 µl)	Negative ^(a)	(Rockwell and Raw, 1979)	
	Rec assay	<i>S. cerevisiae</i>	NR	Negative ^(a)	(Oda et al., 1979)	Article does not specify alpha- or beta-terpineol.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(1-Isopropyl-4-methylbenzene [01.002])	<i>In vivo/in vitro</i> Ames test	<i>S. typhimurium</i> TA98 and TA100	0.5 ml (equivalent to 1 706 mg/kg bw) administered to Sprague-Dawley rats, urine collected and tested <i>in vitro</i>	Negative ^(e)	(Rockwell and Raw, 1979)	
(Myrcene [01.008])	Ames test	<i>S. typhimurium</i> TA100; TA1535; TA97; TA98	0, 33, 100, 333, 1 000, 3 333 and 10 000 µg/plate	Negative ^(a)	(NTP, 1999)	
	Chromosome aberration	Human lymphocytes	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	Sister chromatid exchange	Human lymphocytes	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	HPRT assay	V79 Chinese hamster cells	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	Sister chromatid exchange	V79 Chinese hamster cells	0, 100, 250 and 500 µg/ml	Negative ^(a)	(Röscheisen et al., 1991)	
	Sister chromatid exchange	HTC cells	0, 100, 250 and 500 µg/ml	Negative	(Röscheisen et al., 1991)	
(Menthol [02.015])	Ames test	<i>S. typhimurium</i> , TA92, TA100, TA94, TA98, TA1535, TA1537	0 – 5 000 µg/plate	Negative ^(a)	(Ishidate et al., 1984)	d,l-Menthol was used. The study is considered valid.
	Ames test (preincubation method)	<i>S. typhimurium</i> , TA1535, TA97, TA100, TA98	3 - 666 µg/plate	Negative ^(a)	(Zeiger et al., 1988)	d,l-Menthol was used. The study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> , TA2637, TA100, TA98	0 and 5 - 500 µg/plate	Negative ^(a)	(Nohmi et al., 1985)	d,l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> , TA2637, TA100, TA98	0 and 20 - 500 µg/plate	Negative ^(a)	(Nohmi et al., 1985)	l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> , TA1537, TA1535, TA100, TA98	0, 6.4, 32, 160 and 800 µg/plate	Negative ^(a)	(Andersen and Jensen, 1984)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.
	Ames test	<i>E. coli</i> WP2 <i>uvrA</i> (Trp)	100 - 800 µg/plate	Negative	(Yoo, 1986)	l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.
	Ames test	<i>S. typhimurium</i> TA97A; TA98; TA100; TA102	0, 5 - 800 µg/plate	Negative ^(a)	(Gomes-Carneiro et al., 1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.
	Rec assay	<i>B. subtilis</i> H17, M45	Up to 10 000 µg/disk	Positive	(Yoo, 1986)	l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method (Rec-assay) has poor predictive value.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay	<i>B. subtilis</i> H17, M45	20 µg/disk	Negative	(Oda et al., 1979)	l-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (Rec-assay) has poor predictive value.
	Alkaline elution assay	Rat hepatocytes	0 and 0.1 - 1.3 mM (203.2 µg/ml ^(d))	Negative	(Storer et al., 1996)	The experiment employed d-Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.
	Sister chromatid exchange	Chinese hamster ovary cells	5 – 50; 0 and 2 - 25 µg/ml ^(c) 0 and 16 - 167 µg/ml ²	Negative ^(a)	(Ivett et al., 1989)	d,l-Menthol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1 and 10 mM (1 563 µg/ml ^(d))	Negative ^(a)	(Murthy et al., 1991)	The study is considered valid.
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1 and 10 µg/ml	Negative	(Food and Drug Research Laboratories, Inc., 1975)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 - 200 µg/ml	Negative ^(c)	(Ishidate et al., 1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.
	Chromosome aberration	Chinese hamster ovary cells	0 and 50 - 250 µg/ml	Negative ^(a)	(Ivett et al., 1989)	d,l-Menthol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosome aberration	Human lymphocytes	0, 0.1, 1 and 10 mM (1 563 µg/ml ^(d))	Negative ^(a)	(Murthy et al., 1991)	The study is considered valid.
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0 and 12.5 - 200 µg/ml	Negative ^(a)	(Myhr and Caspary, 1991)	d,l-Menthol was used. The maximum concentration was selected by a preliminary test The study is considered valid.
Bisabola-1,12-dien-8-ol [02.129]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA102, TA1535, TA1537	0 and 0.5 – 1 500 µg/plate	Negative ^(a)	(King and Harnasch, 2002)	
	Ames test	<i>S. typhimurium</i> , TA97a, TA98, TA100, TA1535	0 and 1 - 400 µg/plate in ethanol	Negative ^(a)	(Gomes-Carneiro et al., 2005)	Publication in peer-reviewed journal. No reference to OECD and GLP-guidelines being made, but study is of good quality and considered valid.
	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537	0 and 20 – 5 000 µg/plate in DMSO	Negative ^(a)	(CIR, 1999)	Review.
	Chromosome aberration	Chinese hamster fibroblast cells V79	0 and 0.78 - 40 µg/ml in DMSO	Negative ^(a)	(CIR, 1999)	Review.
Cedrol [02.120]	Ames test	<i>S. typhimurium</i> , TA97a, TA98, TA102, TA1535	0 – 5 000 µg/plate	Negative ^(a)	(Scheerbaum, 2001)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.
1,2-Dihydrolinalool [02.140]	Ames test	<i>S. typhimurium</i> , TA97, TA98, TA100, TA102, TA1535	0 – 1 000 µg/plate	Negative ^(a)	(Gocke, 1999)	Research report according to OECD and GLP-guidelines, the study is considered valid.
2,6-Dimethyloct-7-en-2-ol [02.144]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA102, TA1535, TA1537	0 – 5 000 µg/plate	Negative ^(a)	(King, 2000)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol [02.197]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537 <i>E. coli</i> WP2 <i>uvrA</i>	0 - 250 µg/plate ^(c) 0 - 500 µg/plate ^(b)	Negative	(Watanabe, 2002)	Research report according to OECD guideline 471, the study is considered valid.

NR: Not Reported.

(a): With and without metabolic activation.

(b): With metabolic activation.

(c): Without metabolic activation.

(d): With and without pre-treatment with mitomycin C at 0.15 microM for 21 hours.

(e): With and without presence of beta-glucuronidase.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	Chromosomal Aberration assay	Male rats	Once via gavage	0.2xLD50	Positive ¹	(Barilyak and Kozachuk, 1988)	Validity questionable. This study was not in compliance with GLP and not in accordance with OECD Guideline No. 475 (1983). Some main details of method and results are not available ^(b) .
	Micronucleus assay	Mouse bone marrow erythrocytes	I.P. x 3 at 24 hours intervals (=72 hours)	312.5, 625, and 1250 mg/kg bw	Negative	(NTP, 1996)	Valid. It was not in compliance with GLP but in accordance with OECD Guideline No. 474 (1983/1997) except that only 5 male animals were tested. The study is published in the Web and the report contains sufficient details. Due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance has reached the bone marrow. Relevance of the result is limited.
	Micronucleus assay	Rat bone marrow cells	I.P. x 3 at 24 hours intervals (=72 h)	0, 39 – 1250 mg/kg bw,	Negative	(NTP, 1997)	
	Micronucleus assay	Mouse peripheral blood cells	Drinking water	3000 - 40000	Negative	(NTP, 1995)	
2-Methylbut-3-en-2-ol [02.123]	Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	500, 1000, 1500 mg/kg	Negative	(BASF, 1992)	Summary in IUCLID data set only. According to this summary, the assay was performed in compliance with GLP and in accordance with OECD guideline 474. One thousand PCEs were counted per animal. The unpublished study report is not available for re-evaluation.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Linalool [02.013])	Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	1500 mg/kg	Negative	(Meerts and van de Waart, 2001)	Valid. It was in compliance with GLP and in accordance with OECD Guideline 474 (1997). However, due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance reached the bone marrow. Thus, the relevance of the result is limited.
(Myrcene [01.008])	Micronucleus assay	Rat bone marrow cells	Gavage	0, 100, 500 or 1000 mg/kg bw	Negative	(Zamith et al., 1993)	
	Micronucleus assay	Mouse peripheral blood cells	Gavage	Up to 2000 mg/kg bw per day for 13 weeks	Negative	(NTP, 2001)	
(Menthol [02.015])	Host mediated mutation assay	<i>S. typhimurium</i> TA1530 and G46; <i>S. cerevisiae</i> D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Equivocal	(Food and Drug Research Laboratories, Inc., 1975)	Negative results, with exception of the combination <i>S. typhimurium</i> TA1530 – 5000 mg/kg bw and <i>S. cerevisiae</i> D3 – 1150 mg/kg bw per day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels.
	Cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	Oral DL ₅₀ was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
	Micronucleus assay	B6C3F1 male mouse bone marrow cells	I.P.	0, 250 - 1000 mg/kg bw per day, during 3 days	Negative	(Shelby et al., 1993)	d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	Dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	Valid.

- (a): Cytogenetic analysis indicated the following results for controls and 2-methylpropan-2-ol, respectively: % polyploid cells, 0.5 ± 0.3 / 0.8 ± 0.4 ; % cells with gaps 0.3 ± 0.2 / 0.4 ± 0.2 ; % cells with aberrations, 0 / 1.6 ± 0.5 . Statistical comparisons were not performed.
- (b): The authors report the results of tests on a series of monohydric alcohols (from C1 to C16, 18 compounds) in rat bone marrow cytogenetic tests. All compounds were claimed positive compared to the untreated control group, even though no statistics is shown. It is noted that a single control group, with 0.0 % of cells with aberrations was used throughout the study. Lacking historical control data, it is not possible to establish whether the alleged positive results were due to and uniformly positive response elicited by all chemicals, or rather by an incidental very low frequency of aberrations in the group of rats (8 animals) used as control. In this respect it is noted that the incidence of chromosomal aberrations observed with some “positive” compounds, including 2-methylpropan-2-ol (1.6 ± 0.5 %), are close to background incidences commonly observed. Even the lack of a concurrent raise in gaps in treated animals casts doubts on an induced genotoxic effect. Moreover, the lack of a positive control group in the study is noted. For these reasons, the results of this study are considered inconclusive.

ABBREVIATIONS

ADI	Acceptable Daily Intake
bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
CPN	Chronic progressive nephropathy
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EC	European Commission
EFFA	European Flavours and Fragrances Association
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practise
ID	Identity
IOFI	International Organization of the Flavor Industry
Ip	Intraperitoneal
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest Observed Adverse Effect Level
MSDI	Maximised Survey-derived Daily Intake

mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA Synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

GRASr2 Evaluation of Aliphatic Acyclic and Alicyclic Terpenoid Tertiary Alcohols and Structurally Related Substances Used as Flavoring Ingredients

Lawrence J. Marnett, Samuel M. Cohen, Shoji Fukushima, Nigel J. Gooderham, Stephen S. Hecht, Ivonne M.C.M. Rietjens, Robert L. Smith, Timothy B. Adams, Maria Bastaki, Christie L. Harman, Margaret M. McGowen, and Sean V. Taylor

Abstract: This publication is the 1st in a series of publications by the Expert Panel of the Flavor and Extract Manufacturers Assoc. summarizing the Panel's 3rd re-evaluation of Generally Recognized as Safe (GRAS) status referred to as the GRASr2 program. In 2011, the Panel initiated a comprehensive program to re-evaluate the safety of more than 2700 flavor ingredients that have previously met the criteria for GRAS status under conditions of intended use as flavor ingredients. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics, and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances as flavoring ingredients are evaluated. The group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances was reaffirmed as GRAS (GRASr2) based, in part, on their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavor use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic studies and the lack of significant genotoxic and mutagenic potential.

Keywords: FEMA GRAS, flavoring ingredients, terpene tertiary alcohols

Introduction

For over 50 y, the Expert Panel of the Flavor and Extract Manufacturers Assoc. (FEMA) has served as the primary, independent body evaluating the safety of flavor ingredients. A key part of the FEMA Generally Recognized as Safe (GRAS) program is the cyclical re-evaluation of the "generally recognized as safe" status of flavor ingredients determined to be GRAS by the FEMA Expert Panel during the course of its operations. The Panel has previously completed 2 re-evaluations of all FEMA GRAS flavor ingredients. This summary represents the 1st in a series of publications summarizing the Expert Panel's 3rd re-evaluation of GRAS status referred to as the GRASr2 program. While these summaries contain only critical data relevant to the safety evaluation, the Expert Panel has

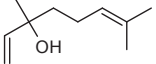
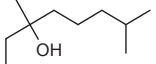
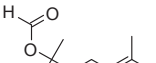
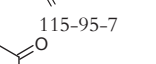
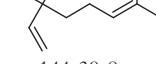
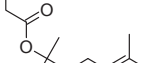
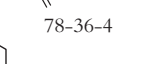
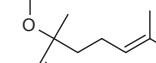
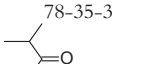
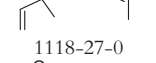
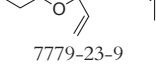
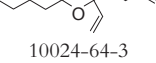
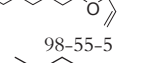
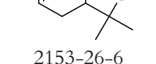
reviewed all of the available data during the course of its safety evaluation.

Currently, the FEMA Expert Panel has determined that over 2700 flavor ingredients have met the criteria for GRAS status under conditions of intended use as flavor ingredients. The FEMA GRAS program is ongoing; Panel decisions on individual flavor ingredients are provided to the U.S. Food and Drug Administration and are published on a regular basis on the FEMA Web site and in *Food Technology*. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics, and toxicology (Smith and others 2005a).

Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. The group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances was reaffirmed as GRAS (GRASr2) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic conversion, and excretion in humans and experimental animals; their low level of flavor use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels (NOAEL) determined from subchronic and chronic studies and the lack of genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

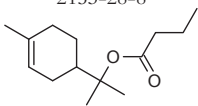
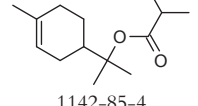
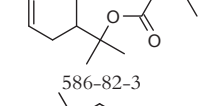
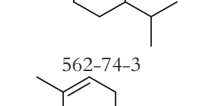
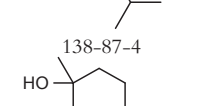
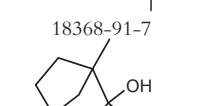
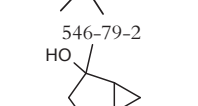
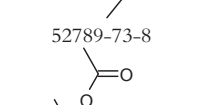
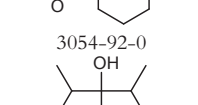
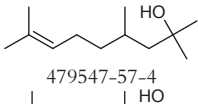
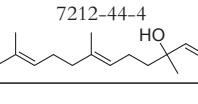
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Table 1—Identity and exposure data for aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances used as flavoring ingredients.

Flavoring ingredient	FEMA Nr.	DT class ^a	CAS Nr. and structure	Most recent annual volume, kg ^b	Daily per capita intake ^c (“eaters only”)		Annual volume in naturally occurring foods, kg ^d	Consumption ratio ^e
					μg/d	μg/kg bw/d		
1. Linalool	2635	I	78-70-6 	49000	5413	90	71619	1
2. Tetrahydrolinalool	3060	I	78-69-3 	0.4	0.04	0.001	— ^f	NA
3. Linalyl formate	2642	I	115-99-1 	24	3	0.04	+	NA
4. Linalyl acetate	2636	I	115-95-7 	21900	2419	40	18567	1
5. Linalyl propionate	2645	I	144-39-8 	100	11	0.2	+	NA
6. Linalyl butyrate	2639	I	78-36-4 	5	1	0.01	+	NA
7. Linalyl isobutyrate	2640	I	78-35-3 	73	8	0.1	—	NA
8. Linalyl isovalerate	2646	I	1118-27-0 	13	1	0.02	—	NA
9. Linalyl hexanoate	2643	I	7779-23-9 	4	0.4	0.007	—	NA
10. Linalyl octanoate	2644	I	10024-64-3 	0.5	0.06	0.0009	—	NA
11. α-Terpineol	3045	I	98-55-5 	6300	696	12	21451	3
12. Terpinyl formate	3052	I	2153-26-6 	0.1	0.01	0.0002	+	NA
13. Terpinyl acetate	3047	I	8007-38-0 	82	9	0.2	61	1
14. Terpinyl propionate	3053	I	80-27-3 	30	3	0.06	+	NA

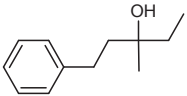
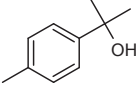
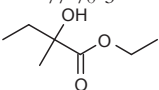
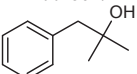
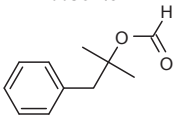
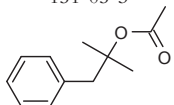
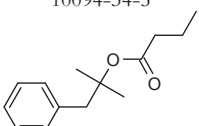
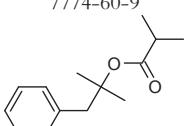
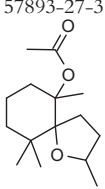
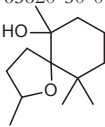
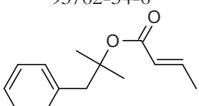
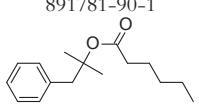
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Table 1—Continued.

Flavoring ingredient	FEMA Nr.	DT class ^a	CAS Nr. and structure	Most recent annual volume, kg ^b	Daily per capita intake ^c (“eaters only”)		Annual volume in naturally occurring foods, kg ^d	Consumption ratio ^e
					μg/d	μg/kg bw/d		
15. Terpinyl butyrate	3049	I	2153-28-8 	5	0.6	0.009	—	NA
16. Terpinyl isobutyrate	3050	I	7774-65-4 	5	0.5	0.008	—	NA
17. Terpinyl isovalerate	3054	I	1142-85-4 	5	0.7	0.01	—	NA
18. <i>p</i> -Menth-3-en-1-ol	3563	I	586-82-3 	2	0.3	0.005	3	2
19. 4-Carvomenthenol	2248	I	562-74-3 	640	71	1	38968	61
20. <i>p</i> -Menth-8-en-1-ol (<i>β</i> -Terpineol)	3564	I	138-87-4 	320	47	0.8	1792	6
21. 2-Ethyl-1,3,3-trimethyl-2-norbornanol	3491	II	18368-91-7 	0.1	0.01	0.0002	—	NA
22. 4-Thujaanol	3239	I	546-79-2 	34	4	0.06	9200	271
23. Methyl 1-acetoxycyclohexyl ketone ^g	3701	I	52789-73-8 	11	2	0.03	—	NA
24. 2,3,4-Trimethyl-3-pentanol	3903	I	3054-92-0 	5	0.7	0.01	—	NA
25. (+/-)-2,4,8-Trimethyl-7-nonen-2-ol	4212	I	437770-28-0 	1	0.1	0.002	—	NA
26. (E)- and (Z)-2,4,8-Trimethyl-3,7-nonadien-2-ol	4211	I	479547-57-4 	1	0.1	0.002	—	NA
27. Nerolidol	2772	I	7212-44-4 	181	20	0.3	300000	1657

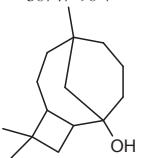
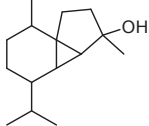
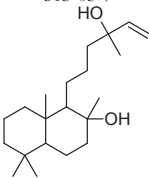
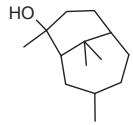
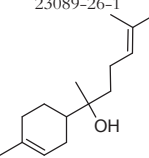
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Table 1–Continued.

Flavoring ingredient	FEMA Nr.	DT class ^a	CAS Nr. and structure	Most recent annual volume, kg ^b	Daily per capita intake ^c (“eaters only”)		Annual volume in naturally occurring foods, kg ^d	Consumption ratio ^e
					μg/d	μg/kg bw/d		
28. 1-Phenyl-3-methyl-3-pentanol	2883	I	10415-87-9 	1	0.1	0.002	—	NA
29. <i>p</i> -, α -Trimethylbenzyl alcohol	3242	I	1197-01-9 	0.1	0.01	0.0002	500000	5000000
30. (±)-Ethyl 2-hydroxy-2-methylbutyrate	4268	I	77-70-3 	50	7	0.117	+	NA
31. α,α -Dimethylphenethyl alcohol	2393	I	100-86-7 	95	10	0.2	+	NA
32. α,α -Dimethylphenethyl formate	2395	I	10058-43-2 	3	0.4	0.01	—	NA
33. α,α -Dimethylphenethyl acetate	2392	I	151-05-3 	4700	519	9	—	NA
34. α,α -Dimethylphenethyl butyrate	2394	I	10094-34-5 	8300	917	15	—	NA
35. α,α -Dimethylbenzyl isobutyrate	2388	I	7774-60-9 	0.4	0.04	0.0007	—	NA
36. 6-Acetoxydihydrotheaspirane	3651	II	57893-27-3 	5	0.7	0.0117	—	NA
37. 6-Hydroxydihydrotheaspirane	3549	II	65620-50-0 	0.4	0.04	0.0007	—	NA
38. Dimethylbenzyl carbonyl crotonate	4403	I	93762-34-6 	1	0.1	0.002	—	NA
39. Dimethylbenzyl carbonyl hexanoate	4404	I	891781-90-1 	0.2	0.03	0.0005	—	NA

(Continued)

Table 1–Continued.

Flavoring ingredient	FEMA Nr.	DT class ^a	CAS Nr. and structure	Most recent annual volume, kg ^b	Daily per capita intake ^c (“eaters only”)		Annual volume in naturally occurring foods, kg ^d	Consumption ratio ^e
					μg/d	μg/kg bw/d		
40. Caryophyllene alcohol	4410	I	56747-96-7 	5	0.7	0.0117	+	NA
41. Cubebol	4497	I	23445-02-5 	50	7	0.117	+	NA
42. (–)-Sclareol	4502	I	515-03-7 	39	6	0.1	+	NA
43. (+)-Cedrol	4503	I	77-53-2 	10	2	0.03	+	NA
44. α-Bisabolol	4666	I	23089-26-1 	227	33	0.55	+	NA

^aDT, Decision Tree; Cramer and others (1978).^bFrom NAS (1989); Lucas and others (1999); Gavin and others (2008). Values greater than zero but less than 0.1 kg were reported as 0.1 kg.^cMSDI (Tg/person/d) calculated as follows:

$[(\text{annual volume, kg}) \times (1 \times 10^9 \text{ Tg/kg})] / [\text{population} \times \text{survey correction factor} \times 365 \text{ d}]$, where population (10%, “eaters only”) = 31×10^6 for the United States, where the correction factor = 0.8 for the Gavin and others (2008), and Lucas and others (1999) and 0.6 for NAS (1989) and anticipated volumes, representing the assumption that only 80% or 60% of the annual flavor volume, respectively, was reported in the poundage surveys (NAS 1989; Lucas and others 1999; Gavin and others 2008; private communication to FEMA). $[(\text{Tg/person/d}) / \text{body weight}]$, where body weight = 60 kg. Slight variations may occur from rounding.

^dQuantitative data for the United States reported by Stofberg and Grundschober (1987).^eThe consumption ratio is calculated as follows:

$(\text{annual consumption via food, kg}) / (\text{most recent reported volume as a flavoring substance, kg})$.

^fNA, not available; +, reported to occur naturally in foods (TNO 2013), but no quantitative data; –, not reported to occur naturally in foods.

^gAt the 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives, methyl 1-acetoxycyclohexylketone (Nr. 23) was not supported by a relevant NOAEL. Data for structurally related substance, 6-acetoxycyclohexylketone (Nr. 36) provided a NOAEL of 3 mg/kg bw/d in a 90-d study in rats (Griffiths 1979) which is at least 100000 times the daily per capita intake of methyl 1-acetoxycyclohexyl ketone when used as a flavor ingredient.

and others 1969, 1974b; Horning and others 1976; Ventura and others 1985). Unsaturated terpenoid tertiary alcohols have the potential to undergo allylic oxidation to form polar diol metabolites, which may be excreted either free or conjugated. Those diols containing a primary alcohol function may undergo further oxidation to the corresponding carboxylic acid (Horning and others 1976; Ventura and others 1985; Madyastha and Srivatsan 1988).

The metabolic fate of the aliphatic tertiary alcohol linalool (Nr. 1) has been studied in mammals (Figure 1). Linalool undergoes rapid oxidation ($t_{1/2}$ for linalool = 11 min) by CYP-450 in a rat liver homogenate metabolic activation system (with added NADP and glucose-6-phosphate) (Buck and Renwick 1998c). In an earlier study, male Wistar rats orally administered a single dose of 500 mg/kg body weight (bw) ^{14}C -linalool excreted 55% of the radiolabel in the urine as the glucuronic acid conjugate, while 23% was excreted as CO_2 in expired air, and 15% was excreted

in the feces within 72 h of dose administration. After 72 h nearly all of the radiolabel had cleared the body with only 3% of the radioactivity detected in tissues (Parke and others 1974a). Reduction metabolites such as dihydro- and tetrahydrolinalool were also detected in significant amounts in the urine, either free or as their conjugates, after administration of a single dose of linalool to rats, although further details of this study have not been reported (Rahman 1974) (see Figure 1).

Weak induction of cytochrome P-450 (CYP-450) activity has been reported at relatively high doses of linalool. In a repeat-dose study, male rats (IISc strain) were administered 800 mg/kg bw/d of linalool for 20 d. A notable increase in allylic oxidation products 8-hydroxylinalool and 8-carboxylinalool was seen in the urine. A statistically significant but transient induction of 50% in CYP-450 activity in the liver microsomes was also noted after 3 d of treatment, followed by a return to control values after

6 d (Chadha and Madyastha 1984). In a similar study, induction of CYP-450 was not observed until the 30th d of administration of 500 mg linalool/kg bw/d to Wistar rats (Parke and others 1974b).

The data suggest that glucuronic acid conjugation and excretion is the primary route of metabolism of linalool. Allylic oxidation becomes an important pathway only after repeated dosing at relatively high doses. It has been suggested that the biotransformation of the diol metabolite of linalool to the corresponding aldehyde via the action of the NAD⁺-dependent enzyme alcohol dehydrogenase is inhibited due to the bulky nature of the neighboring alkyl substituents and the substrate specificity of the enzyme (Eder and others 1982).

In a repeat-dose study, male albino rats (IISc strain) were administered 600 mg/kg bw/d of α -terpineol for 20 d. Metabolite analysis showed oxidation at the allylic methyl group to the corresponding carboxylic acid, of which a small fraction was hydrogenated to yield the corresponding saturated carboxylic acid (Madyastha and Srivatsan 1988) (see Figure 2). In addition, α -terpineol resulted in a statistically significant induction of liver microsomal CYP-450 content, with 52% to 104% increase over the content in untreated rats that peaked on day 2 of treatment, and in a moderate increase (up to 43%, on day 2) in the activity of NADPH-cytochrome c reductase in treated rats (Madyastha and Srivatsan 1988), suggesting that oxidation is mediated by CYP-450.

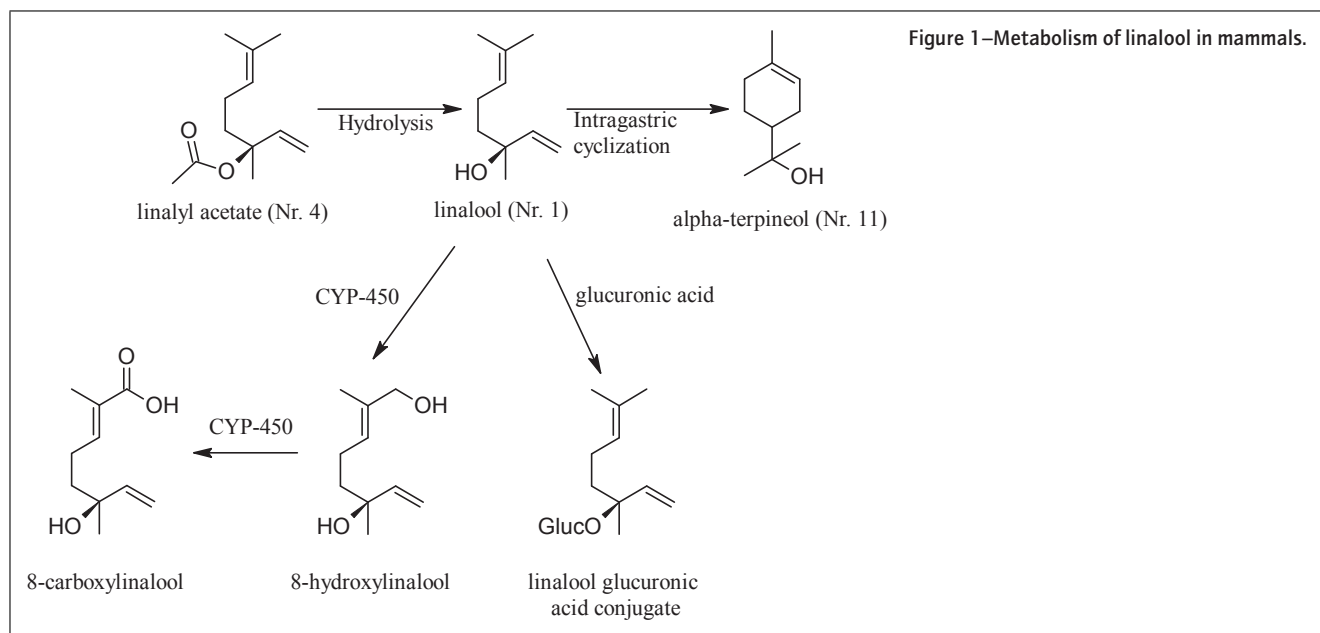
In a minor pathway, epoxidation of the alkene of α -terpineol is followed by hydrolysis to a triol metabolite, 1,2,8-trihydroxy-*p*-menthane, which also has been reported in humans following ingestion of a pine oil disinfectant containing α -terpineol (Horning and others 1976). It is expected that α -terpineol would undergo metabolism like linalool (Chadha and Madyastha 1984), primarily by glucuronic acid conjugation and excretion in the urine.

Bicyclic tertiary alcohols (Nr. 21, 22, 40, 42, and 43) are relatively stable *in vivo*, but are eventually conjugated with glucuronic acid and excreted (Williams 1959). In rabbits the structurally related bicyclic tertiary alcohol β -santenol (2,3,7-trimethyl bicyclo[2.2.1]-heptan-2-ol) was conjugated with glucuronic acid (Williams 1959).

In a metabolism study using the structurally related terpenoid tertiary alcohol *trans*-sobrerol in humans, dogs, and rats, 10 metabolites were isolated in urine, 8 of which were also detected in urine from humans exposed to *trans*-sobrerol. In this study, the 2 principle modes of metabolism observed were allylic oxidation of the ring positions and alkyl substituents, and conjugation of the tertiary alcohol functions with glucuronic acid. These are common pathways converting tertiary (Ventura and others 1985) and secondary (Yamaguchi and others 1994) terpenoid alcohols to polar metabolites that are easily excreted in the urine and feces (see Figure 3).

Potential pharmacokinetic outcomes in mammalian plasma were analyzed upon intravenous administration of sclareol (Nr. 42) at 100 mg/kg bw to 2 male Wistar rats. Plasma samples were collected at 5, 15, 30, 60, 180, 360 min, 12 and 24 h postadministration. At 5 min post-injection, plasma levels of sclareol were 84.9 μ g/mL. The sclareol plasma concentration dropped to 42.9 μ g/mL after 180 min and sclareol was not detectable at 360 min. The data indicate a rapid biphasic disappearance of sclareol from plasma following intravenous dosing. The authors suggest that sclareol may be distributed in fatty tissue due to its high lipophilicity (Kouzi and others 1993).

In a 2nd study, sclareol was administered to 2 male Wistar rats by intravenous injection at 100 mg/kg bw, and to male Wistar rats (*n* = unknown) by intragastric instillation at 1000 mg/kg bw. Urine and fecal samples were collected from all rats and bile samples were collected only from rats given intravenous injections. No unchanged sclareol was detected in urine (with or without β -glucuronidase treatment), or in fecal samples (only 9% of the initial dose was found in fecal samples of rats treated orally) and very low levels (0.02% over a 3-h period) of sclareol were found in bile samples from rats following intravenous injection. Very low levels (0.04%) of oxidized metabolites were found in bile only after 3 h, including 3- α -hydroxysclareol (0.24%), 3- β -hydroxysclareol (0.075%), 18-hydroxysclareol (0.056%), and 3-ketosclareol (0.03%). The low sensitivity of the assay and possible other metabolites were suggested as reasons for the very small percentage of intravenously injected sclareol (<0.05%) that was accounted for (Kouzi and others 1993).



In order to determine the phase I metabolism of cedrol (Nr. 43), Bang and Ourisson (1975) and Trifileff and others (1975) administered cedrol to rabbits and dogs, respectively. In rabbits, only metabolites formed by hydroxylation at C3 were identified, and the 2 resulting epimers, α -epiisobitol and α -isobitol, were subsequently dehydrated at C7/C8. The metabolic fate in dogs was far less regioselective and metabolites that were identified were

products of oxidation at multiple locations on the ring as well as at methyl groups. Additional Phase I functionalization must occur in humans, given that the initial glucuronidation (in Phase II) at the hydroxyl moiety of cedrol is inefficient because of steric hindrance. In the case of cedrol, the hydroxylation of a nonactivated saturated carbon atom is the most likely pathway (Bang and Ourisson 1975; Trifileff and others 1975; Ishida 2005).

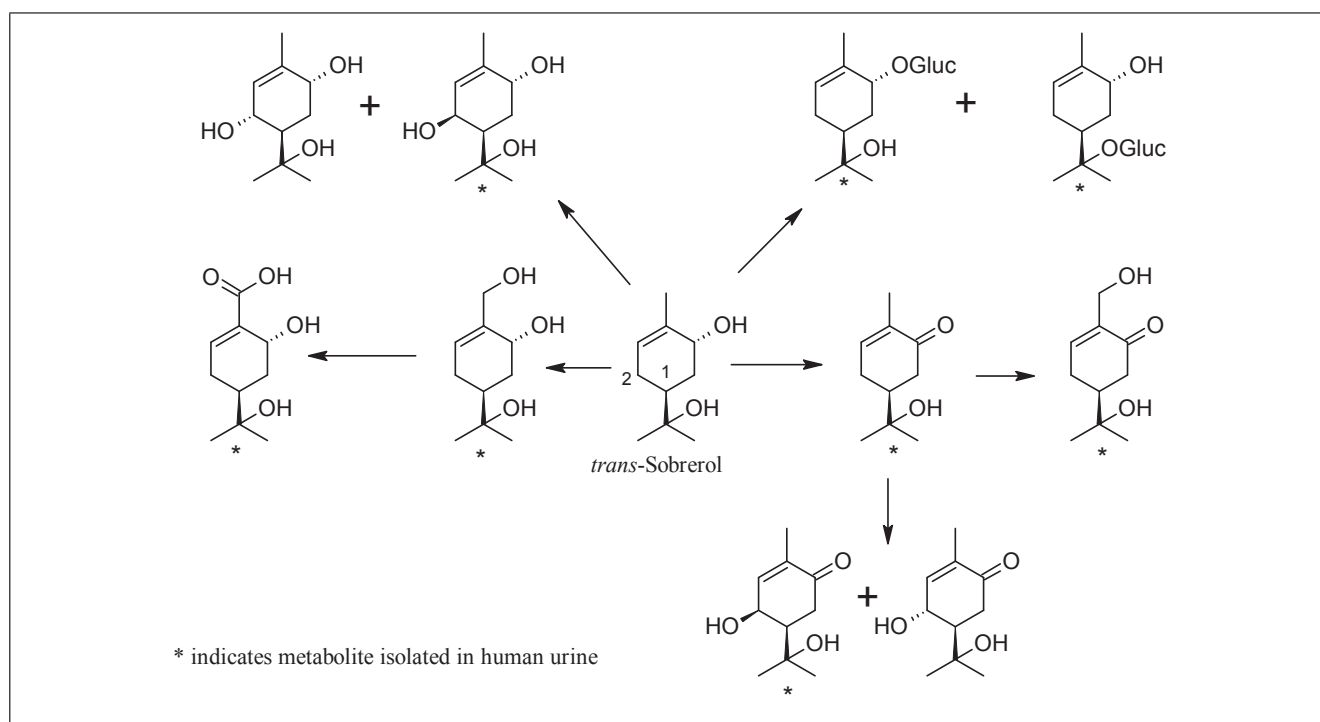
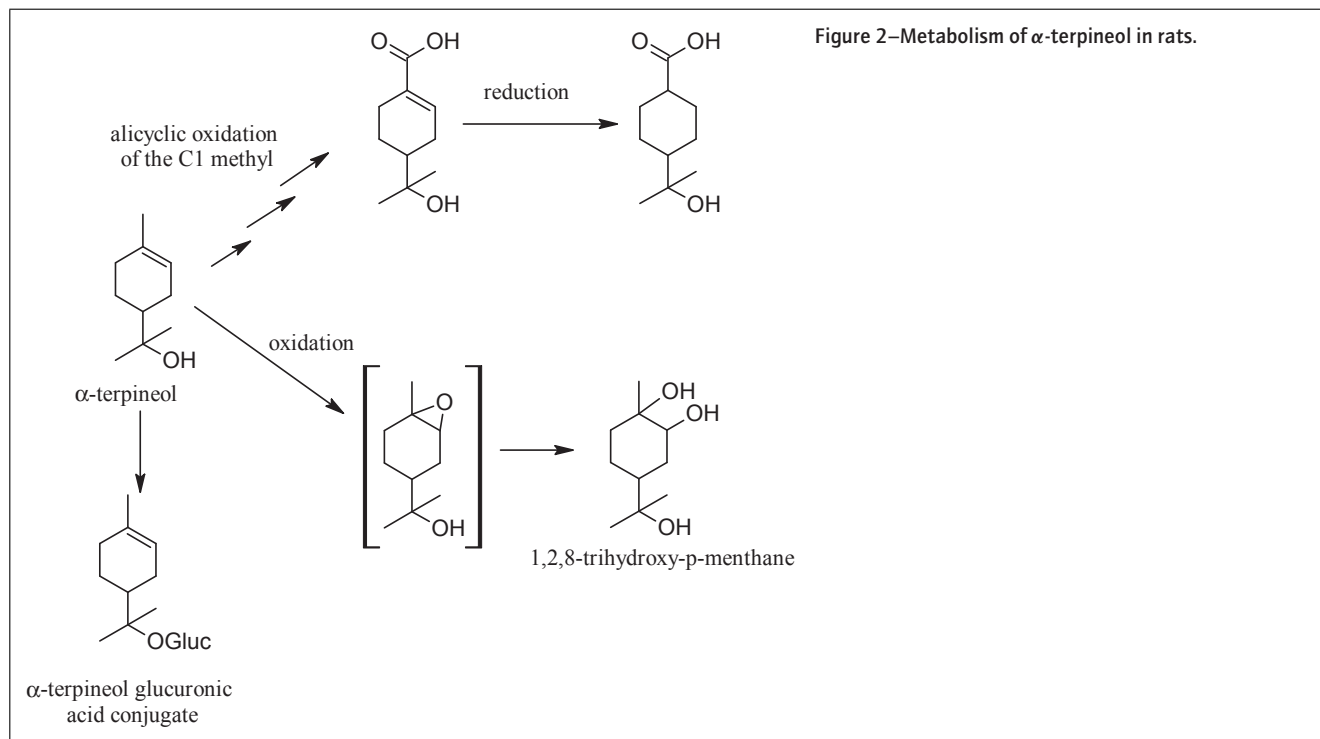


Figure 3–Metabolism of *trans*-sobrerol in rats, dogs, and humans.

Terpenoids are known to alter the activity of various drug-metabolizing hepatic enzymes (Parke and Rahman 1969). The inhibitory effect of chamomile essential oil and its major constituents (for example, α -bisabolol, Nr. 44) was studied on 4 selected human CYP-450 enzymes (CYP1A2, CYP2C9, CYP2D6, and CYP3A4). Increasing concentrations of the test compounds were incubated with individual, recombinant CYP isoforms and their effect on the conversion of surrogate substances was measured fluorometrically; enzyme inhibition was expressed as median inhibitory concentration (IC_{50}) and inhibition constant (K_i) values in relation to positive controls. α -Bisabolol ($IC_{50} = 2.18 \mu M$) produced a significant inhibition of CYP2C9 and CYP2D6. As indicated by these *in vitro* data, chamomile preparations contain constituents that can inhibit the activities of major human drug metabolizing enzymes; interactions with drugs whose route of elimination is mainly through CYP oxidation (especially CYP1A2) are therefore possible (Ganzer and others 2006).

Studies in humans, dogs, rabbits, and rats have shown that absorption of aliphatic acyclic and alicyclic terpenoid tertiary increases with increasing lipophilicity and are distributed primarily in adipose tissue. Oxidation to polar metabolites and/or conjugation with glucuronic acid, followed by excretion in the urine is expected for all of the flavoring ingredients in this group. Small amounts may be expired in exhaled air. The esters within this group (Nr. 3 to 10, 12 to 17, 23, 33 to 37, 39 and 40) are expected to be hydrolyzed in humans to their component tertiary alcohols and acids. The available data demonstrate that the aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances are rapidly absorbed, distributed, metabolized and excreted.

Toxicology

Acute toxicity

Oral median lethal dose (LD_{50}) values have been reported for 24 of the 43 substances in this group (Jenner and others 1964; Colaiaanni 1967; Moreno 1971, 1973, 1975, 1976, 1977, 1982; Russell 1973; Levenstein 1975; Griffiths 1979; Piccirillo and Hartman 1980; Yamahara and others 1985; Collinson 1989; Rhone-Poulenc 1992; Moore 2000). LD_{50} values range from 1300 to greater than 36300 mg/kg bw, demonstrating that the oral acute toxicity of tertiary alcohols and related esters is extremely low.

Short-term studies of toxicity

The results of short-term studies with representative acyclic and alicyclic tertiary terpenoid alcohols and related substances are summarized in Table 2 and key studies are described below.

A mixture of linalool (Nr. 1.) and citronellol (1:1) resulting in average daily intake of 50 mg/kg bw each, and a mixture of linalyl acetate (Nr. 4), linalyl isobutyrate (Nr. 7), and geranyl acetate at levels calculated to result in average daily intakes of 24, 27, or 48 mg/kg bw, respectively were incorporated into the feed of male and female rats (number and strain not specified) for 12 wk. A slight retardation of body weight gain was observed only in males fed the linalool mixture and in females fed the linalyl esters mixture. However, these effects were concluded by the authors to be biologically insignificant (Oser 1967).

Four groups of 10 male and 10 female Osborne–Mendel rats were fed the structurally related ester of linalool, linalyl cinnamate, at dietary concentrations of up to 10000 ppm for 17 wk, which is estimated to provide a daily intake of up to 1000 mg/kg

bw/d (FDA 1993). There were no differences between treated and control animals in the parameters evaluated (Hagan and others 1967).

Similarly, groups of 10 male and 10 female weanling Osborne–Mendel rats were fed terpinyl acetate (Nr. 13) in the diet for 20 wk at concentrations of 0, 1000, 2500 or 10000 ppm, calculated to result in daily intakes of 0, 100, 250, and 1000 mg/kg bw/d, respectively (FDA 1993). All animals were examined for growth, hematology, and macroscopic and microscopic changes in the tissues. No statistically significant adverse effects were reported at any dose level (Hagan and others 1967).

In the same study, groups of 5 weanling Osborne–Mendel rats per sex were housed individually in wire cages and administered 0 or 10000 ppm of α,α -dimethylphenethyl alcohol (Nr. 31) in the diet (approximately 1000 mg/kg bw/d) for 16 wk. In addition, groups of 10 rats per sex were administered 0 or 1000 ppm of α,α -dimethylphenethyl alcohol in the diet (approximately 100 mg/kg bw/d) for 28 wk. No effects were observed due to the administration of the test material at either dose level (Hagan and others 1967).

Groups of Sprague–Dawley rats (5/sex) were maintained on diets containing 2,3,4-trimethyl-3-pentanol (Nr. 24) at a dose level of 0 or 10 mg/kg bw/d for 14 d. Test and control groups showed no significant differences in relative or absolute kidney and liver weights (Madarasz 1997).

During its 51st meeting, the Joint FAO/WHO Expert Committee on Food Additives concluded that methyl 1-acetoxycyclohexylketone (Nr. 23) was not supported by a relevant no-observed-effect level (NOEL) (JECFA 2000). Data on the structurally related substance, 6-acetoxycyclohexylketone (Nr. 36) in a 90-d study in rats (Griffiths 1979), provides a NOAEL of 3 mg/kg bw/d which is at least 100000 times the daily per capita intake of methyl 1-acetoxycyclohexyl ketone when used as a flavor ingredient.

In studies using groups of 16 Sprague–Dawley rats per sex per dose, 6-acetoxycyclohexylketone (Nr. 36) and 6-hydroxydihydrotheaspiane (Nr. 37) were administered by gavage at doses of 3 mg/kg bw/d (Griffiths 1979) and 0.154 mg/kg bw/d, respectively (Griffiths 1976) for 91 d. No treatment-related clinical effects were observed in either study. In the acetoxycyclohexylketone study, treated females were reported to have significantly decreased body weights compared to controls at week 13. In comparison to controls, a significant increase in relative spleen weight was observed in treated males. Significant reductions in hemoglobin, hematocrit, and red blood cell count were observed in treated females at the end of the study relative to levels observed in controls. In the 6-hydroxydihydrotheaspiane study, 1 male death that occurred within 1 h of dosing and necropsy revealed oil in the lungs and trachea but not in the upper part of the gastrointestinal tract, indicating a gavage error. At week 12, treated males had significantly greater hemoglobin concentrations relative to controls. At week 6, but not at week 12, treated males and females had significantly greater total leukocyte counts compared to the controls (Griffiths 1976). These hematological changes were considered variations and not adverse effects because of the lack of reproducibility between sexes or a later time point, respectively. No other statistically significant adverse effects were reported (Griffiths 1976, 1979).

In a 28 d oral toxicity study, Sprague–Dawley rats (10/sex/ingredient) approximately 1 mo of age, were administered 10 mg/kg bw/d of sclareol (98.3% pure, Nr. 42) or 10 mg/kg

Table 2—Short-term toxicity studies on aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances.

Flavor ingredient	Species, sex ^a	Nr. test groups/Nr. per test group ^b	Route	Duration (days)	NOAEL (mg/kg bw/d)	Margin of safety	Reference
1. Linalool	Rat; M, F	1/— ^c	Diet ^d	84	50 ^{d,e}	>500	(Oser 1967)
1. Linalool	Rat, F	3/25	Gavage	11	500 ^f	>5000	(Lewis 2006)
4. Linalyl acetate	Rat; M, F	1/— ^c	Diet ^g	84	24 ^e	600	(Oser 1967)
7. Linalyl isobutyrate	Rat; M, F	1/— ^c	Diet ^h	84	27 ^e	270000	(Oser 1967)
13. Terpinyl acetate	Rat; M, F	3/20	Diet	140	500 ^e	2500000	(Hagan and others 1967)
24. 2,3,4-Trimethyl-3-pentanol	Rat; M, F	1/10	Diet	14	10 ^e	1000000	(Madarasz 1997)
31. α,α -Dimethylphenethyl alcohol	Rat; M, F	1/10	Diet	112	1000 ^{e,i}	5000000	(Hagan and others 1967)
31. α,α -Dimethylphenethyl alcohol	Rat; M, F	1/20	Diet	196	100 ^{e,j}	500000	(Hagan and others 1967)
36. 6-Acetoxydihydrotheaspirane	Rat; M, F	1/32	Gavage	91	3 ^c	256000	(Griffiths 1979)
37. 6-Hydroxydihydrotheaspirane	Rat; M, F	1/32	Gavage	91	0.154 ^c	220000	(Griffiths 1976)
42. (–)-Sclareol	Rat; M, F	1/20	Gavage	28	8.8 ^c	88000	(Merkel 2006)
43. (+)-Cedrol	Rat; M, F	1/20	Gavage	28	8.4 ^c	280000	(Merkel 2006)
44. α -Bisabolol	Rat; M, F	2/40	Gavage	28	<1860 ^k	3100000	(Habersang and others 1979)
44. α -Bisabolol	Dog; M, F	2/6	Gavage	28	<1860 ^{k,l}	3100,000	(Habersang and others 1979)
44. α -Bisabolol	Rat; F	4/— ^c	Gavage	10	931 ^f	>10000	(Habersang and others 1979)
44. α -Bisabolol	Rabbit; F	4/— ^c	Gavage	13	931 ^f	>10000	(Habersang and others 1979)

^aM, male; F, female.^bTotal number of test groups does not include control animals. Total number per test group includes both male and female animals.^cNot specified.^dAdministered with citronellol as part of a 1:1 mixture.^eThe study was performed at a single dose level or multiple dose levels that produced no adverse effects and, therefore, a NOAEL was not determined. The NOAEL is probably higher than the dose level reported here which is the highest dose level that produced no adverse effects.^fRefers to maternal NOAEL derived from a developmental study.^gAdministered with linalyl isobutyrate and geranyl acetate as part of a mixture.^hAdministered with linalyl acetate and geranyl acetate as part of a mixture.ⁱBased on a dietary concentration of 10000 ppm, which is equivalent to 1000 mg/kg bw.^jBased on a dietary concentration of 1000 ppm, which is equivalent to 100 mg/kg bw.^kAdverse effects seen in lowest dosage group; therefore, this is not a true NOAEL.^lCompared to controls, a significant increase was observed in relative liver weights in males; however, this organ weight variation was not accompanied by any histopathological abnormalities or any other adverse effects.

bw/d of cedrol (97% pure, Nr. 43) by intragastric instillation in carboxymethylcellulose with the control group receiving vehicle only. It was determined that the actual dose achieved was 8.8 mg/kg bw/d of sclareol and 8.4 mg/kg bw/d of cedrol. In rats dosed with sclareol, statistically significant increases in mean absolute and relative liver weight in males and liver to brain weight ratio in females were found not to correlate with evidence of macro- or microscopic alterations or associated enzyme activity. Minor clinical pathology and histopathological alterations were considered incidental, and therefore toxicologically nonadverse, according to the authors. Other statistically significant organ weight changes were isolated and not consistent between the sexes, and were therefore considered not to be related to administration of the test substance (Merkel 2006).

Groups of 40 (strain not specified) rats (20/sex/dose) or Beagle dogs (3/sex/dose) were administered α -bisabolol (Nr. 44) by intragastric instillation at 0, 2.0, or 3.0 mL/kg bw/d for 4 wk. These dose levels correspond to calculated daily intakes of 0, 1860 or 2790 mg/kg bw/d. In the rats, slight motor agitation and decreased body weight gain were noted in animals of both dose groups and the mortality rate was 20% in the 2790 mg/kg bw/d group. Decreased body weight gain was noted. Postmortem findings in the 2790 mg/kg bw/d group included inflammatory changes in the liver, trachea, spleen, thymus, and stomach (Habersang and others 1979). In the Beagle dog study, the high dose was increased to 3720 mg/kg bw/d after 2 wk. Loss of appetite, reduced feed intake, and vomiting were observed in 2 of the 6 dogs receiving 1860 mg/kg bw/d. At necropsy it was noted that the liver weight relative to the body weight was increased. Reactions and observations were more severe in the high dose group. No other changes were noted as compared to controls (Habersang and others 1979).

Developmental toxicity studies

Linalool was studied for potential developmental toxicity in rats. Four groups of presumed pregnant female Sprague–Dawley rats (25/group) were administered 0 (vehicle), 250, 500, or 1000 mg linalool/kg bw/d by intragastric instillation in corn oil on gestational days 7 to 17. There were no clinical signs of toxicity. No deaths related to the administration of linalool occurred. One dam in the low dose delivered early on the day of scheduled sacrifice. This delivery was considered unrelated to administration of linalool because the observation was not dose-dependent. There were no macroscopic lesions at necropsy in this dam and her litter consisted of 12 live-delivered pups and 1 early *in utero* resorption. All pups appeared normal for this dam and no external, soft tissue or skeletal alterations occurred in these pups. Body weight gains were reduced by 11% in the 1000 mg/kg bw/d dose group during the dosing period (in comparison with control) and this was accompanied by absolute and relative feed consumption values that were reduced and significantly reduced (by 7%), respectively, in the 1000 mg/kg bw/d dose group for the entire test period in comparison to the control group. The lower dose groups showed no difference in body weight gain or feed consumption when compared to controls. No Caesarean section or litter parameters were affected by dosages of linalool as high as 1000 mg/kg bw/d. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, percent resorbed conceptuses, percent live male fetuses and fetal body weights were similar across all test groups. No dam had a litter that consisted of all resorbed conceptuses. All placentas appeared normal. No macroscopic external, soft tissue or skeletal alterations appeared to be caused by dosages of linalool as high as 1000 mg/kg bw/d. There were no dose-dependent or significant differences in the litter or fetal incidences of any macroscopic external, soft tissues or skeletal

alterations. All ossification averages were comparable to vehicle control group values and did not significantly differ among the groups.

Based on these data, the maternal NOAEL of linalool is 500 mg/kg bw/d. The 1000 mg/kg bw/d dose caused nonsignificant reductions in body weight gain and also reduced absolute and relative feed consumption values during the dosage period. However, following the completion of the dosing period, these effects were reversed. The developmental NOAEL is at least 1000 mg/kg bw/d, since no embryo–fetal effects were observed at the highest dose tested (Lewis 2006).

A study was conducted in Sprague–Dawley and Wistar rats and in New Zealand white rabbits to determine the potential developmental toxicity of α -bisabolol (Nr. 44). In 2 separate experiments, groups of presumed pregnant rats or presumed pregnant rabbits were administered 0, 0.25, 0.50 or 1.0 mL/kg in the 1st experiment, and 3.0 mL/kg bw/d of α -bisabolol in the 2nd, by intragastric instillation on days 6 to 15 of pregnancy for rats and days 6 to 18 for rabbits. These dose levels correspond to calculated daily intakes of 0, 233, 466, 931 and 2793 mg/kg bw/d. At the highest dose of the test substance administered, there were reduced numbers of live fetuses in addition to reduced fetal weights and increased numbers of resorptions in both rats and rabbits. No adverse effects were observed in any other dose group. The NOAEL for reproductive effects in Sprague–Dawley and Wistar rats or New Zealand white rabbits is 931 mg/kg bw/d (Habersang and others 1979).

Genotoxicity

Genotoxicity testing has been performed on substances in this group. The results of these tests are summarized in Table 3 and are described below.

In Vitro

No increase in reverse mutations were observed in Ames assays in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537, TA1538, TA97a, TA92, and TA94 and *Escherichia coli* WP2 *uvrA* for linalool (Nr. 1), linalyl acetate (Nr. 4), linalyl propionate (Nr. 5), α -terpineol (Nr. 11), p-menth-8-en-1-ol- β -terpineol (Nr. 20), 1-phenyl-3-methyl-3-pentanol (Nr. 28), cedrol (Nr. 43), α,α -dimethylphenethyl formate (Nr. 32), and α -bisabolol (Nr. 44) at concentrations up to 5000 μ g/plate in the presence and absence of bioactivation systems (Rockwell and Raw 1979; Eder and others 1980; Florin and others 1980; Wild and others 1983; Ishidate and others 1984; Yoo 1986; Asquith 1989; Heck and others 1989; Scheerbaum 2001; King 2002; Sokolowski 2004; Gomes-Carneiro and others 2005). These results are summarized in detail in Table 3.

When incubated with *Bacillus subtilis* H17 (rec⁺) and M45 (rec⁻), linalool, linalyl acetate, and terpinyl acetate were negative in the rec assay at 17, 18, and 19 μ g, respectively (Oda and others 1978), but linalool was positive at 10 μ L/disk, corresponding to 8620 μ g (56 μ mol) (Yoo 1986). The positive rec assay results reported by Yoo were most likely due to cytotoxicity to *B. subtilis* rather than genotoxicity, given the high concentration.

In a mouse lymphoma assay, linalool was negative in L5178Y TK+/- cells with S-9 metabolic activation at 200 nL/mL, but weakly positive without S-9 activation at 150 nL/mL (Heck and others 1989). The authors of this study noted that the forward mutation assay was not run under conditions controlling for changes in osmolality and they interpreted the weak positive result not as evidence of genotoxicity but rather related to cytotoxicity (Heck

and others 1989). Negative results were obtained in this assay when cells were treated with 250 nL/mL or 300 nL/mL of α -terpineol, with and without S-9 metabolic activation, respectively (Heck and others 1989).

Linalool did not induce chromosomal aberrations when incubated with Chinese hamster fibroblast cells at a maximum concentration of 250 μ g/mL (Ishidate and others 1984), nor did it induce unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) in rat hepatocytes at concentrations up to 50 nL/mL (equivalent to 43.1 μ g/mL) (Heck and others 1989). No chromosomal aberrations were observed when 0, 10, 33, 56, 100, 130, or 180 μ g/mL of linalyl acetate (Nr. 4) were incubated with human peripheral lymphocytes for 3 h with 24- and 48-h fixation times, with and without metabolic activation (Bertens 2000). Linalyl acetate did not induce UDS in Fischer or SD rat hepatocytes at concentrations up to 300 nL/mL (Heck and others 1989).

In Vivo

The potential of 1-phenyl-3-methyl-3-pentanol (Nr. 28) to induce sex-linked recessive lethal mutations in adult *Drosophila melanogaster* was studied in the Basc test. Mutation frequency was unaffected when flies were exposed to a 0 or 20 mM (3565 μ g/mL) solution of 1-phenyl-3-methyl-3-pentanol for 3 d (Wild and others 1983).

In a mutagenicity study, 24-h urine of Sprague–Dawley rats administered 0.5 mL of linalool or β -terpineol was incubated with *S. typhimurium* strains TA98 and TA100 (Rockwell and Raw 1979). The following samples were tested: undiluted linalool and α -terpineol (with metabolic activation), aliquots of 24-h urine from rats fed linalool and β -terpineol (with and without metabolic activation), aliquots of the ether extract of 24-h urine (with and without metabolic activation), and aliquots of the aqueous phase of 24-h urine ether extract (with and without metabolic activation). A range of volumes was tested for each type of sample. Urines were diluted to 60 mL and incubated in the presence of chloroform and β -glucuronidase prior to ether extraction. All tests were negative except for the ether extract of urine from α -terpineol-treated rats.

Linalool (Nr. 1) was tested for *in vivo* mutagenicity in the bone marrow micronucleus assay in Swiss CD-1 mice. Four groups (5/sex/group) received a single dose of the test substance by intragastric instillation in corn oil; 2 of these groups were administered 1500 mg/kg bw of linalool and 1 group each was administered 500 or 1000 mg/kg bw of linalool. Vehicle controls received corn oil and positive controls received cyclophosphamide. Systemic toxicity signs were recorded at least once a day. The animals were terminated at 24 or 48 h after dosing, both femurs were removed, and bone marrow smears were prepared and analyzed for micronuclei. The animals of the groups dosed with linalool showed no decrease in the ratio of polychromatic to normochromatic erythrocytes, which reflects a lack of toxic effects of this compound on erythropoiesis. No increase in the frequency of micronucleated polychromatic erythrocytes was found in the linalool-dosed animals compared to the vehicle controls, whereas cyclophosphamide produced an increase. Therefore, linalool was not mutagenic in the micronucleus test under the experimental conditions used (Meerts 2001).

In a micronucleus test, groups of 4 male and female NMRI mice (number/sex not reported), administered single intraperitoneal doses of 0, 357, 624, 891, or 1416 mg 1-phenyl-3-methyl-3-pentanol (Nr. 27)/kg bw, demonstrated no increase in micronucleated erythrocytes in bone marrow samples obtained 30 h postadministration (Wild and others 1983).

Table 3—Studies of genotoxicity with aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances used as flavoring ingredients.

<i>In vitro</i>						
Nr.	Flavoring agent	End point	Test object	Concentration	Results	Reference
1	Linalool	Reverse mutation	<i>Salmonella typhimurium</i> TA100	0.01 to 3 μ L/2 mL incubation volume	Negative ^a	(Eder and others 1980)
1	Linalool	Reverse mutation	<i>Salmonella typhimurium</i> TA92, TA100, TA1535, TA1537, TA94, TA98	1 mg/plate	Negative ^a	(Ishidate and others 1984)
1	Linalool	Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100	0.05 to 100 μ L	Negative ^a	(Rockwell and Raw 1979)
1	Linalool	Reverse mutation	<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98	10000 nL/plate	Negative ^a	(Heck and others 1989)
1	Linalool	Reverse mutation	<i>Escherichia coli</i> WP2 <i>uvrA</i>	0.125 to 1 mg/plate	Negative	(Yoo 1986)
1	Linalool	Forward mutation	Mouse lymphoma L5178Y TK+/-	3.9 to 125 nL/mL 100 to 300 nL/mL 200 nL/mL 25 to 150 nL/mL	Negative ^b Weakly Positive ^c	(Heck and others 1989)
1	Linalool	Unscheduled DNA synthesis	Rat hepatocytes	50 nL/mL 43.1 μ g/mL	Negative	(Heck and others 1989)
1	Linalool	Chromosomal aberration	Chinese hamster fibroblasts	250 μ g/mL	Negative ^c	(Ishidate and others 1984)
1	Linalool	Mutation	<i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-)	17 μ g	Negative	(Oda and others 1978)
1	Linalool	Mutation	<i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-)	10 μ L/disk 8620 μ g/disc	Positive	(Yoo 1986)
4	Linalyl acetate	Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	25000 nL/plate	Negative ^a	(Heck and others 1989)
4	Linalyl acetate	Unscheduled DNA synthesis	Fischer or SD rat hepatocytes	300 nL/mL	Negative	(Heck and others 1989)
4	Linalyl acetate	Mutation	<i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-)	18 μ g	Negative	(Oda and others 1978)
4	Linalyl acetate	Chromosomal aberration	Human peripheral blood lymphocytes	180 μ g/mL	Negative	(Bertens 2000)
5	Linalyl propionate	Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	10 to 5000 μ g/plate	Negative	(Sokolowski 2004)
11	α -Terpineol	Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	10000 μ g/plate	Negative ^a	(Heck and others 1989)
11	α -Terpineol	Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA1537, TA98, TA100	Up to 3 μ mol/plate	Negative ^a	(Florin and others 1980)
11	α -Terpineol	Forward mutation	Mouse lymphoma L5178Y TK+/-	250 nL/mL 300 nL/mL	Negative ^b Negative ^c	(Heck and others 1989)
13	Terpinyl acetate	Mutation	<i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-)	19 μ g	Negative	(Oda and others 1978)
20	p-Menth-8-en-1-ol (β -terpineol)	Reverse mutation	<i>Salmonella typhimurium</i> TA100, TA98	0.05 to 100 μ L	Negative ^a	(Rockwell and Raw 1979)
	Terpineol	Mutation	<i>Bacillus subtilis</i> H17 (rec+) and M45 (rec-)	19 μ g	Negative ^a	(Oda and others 1978)
28	1-Phenyl-3-methyl-3-pentanol	Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA1537, TA1538, TA98, and TA100	Up to 3600 μ g/plate	Negative ^a	(Wild and others 1983)
32	α , α -Dimethylphenethyl formate	Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA1537, TA98, and TA100	Up to 1000 μ g/plate	Negative ^a	(Asquith 1989)
43	(+)-Cedrol	Reverse mutation	<i>Salmonella typhimurium</i> TA1535, TA97a, TA98, TA100 and TA102	0, 1.6, 5, 16, 50, 160, 500, 1600, and 5000 μ g/plate	Negative ^{a,d}	(Scheerbaum 2001)
44	α -Bisabolol	Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	0, 1.5, 5, 15, 50, 100, 150, and 500 μ g/plate	Negative ^{a,e,f}	(King 2002)
44	α -Bisabolol	Reverse mutation	<i>Salmonella typhimurium</i> TA100, TA97a, TA98 and TA1535	0, 1, 5, 10, 25, 50, 100, 200, 300, and 400 μ g/plate	Negative ^{a,d,g}	(Gomes-Carneiro and others 2005)
<i>In vivo</i>						
1	Linalool	Micronucleus assay	Male and female mice (Swiss CD-1)	0, 500, 1000, and 1500 mg/kg bw ^h	Negative	(Meerts 2001)
28	1-Phenyl-3-methyl-3-pentanol	Sex-linked recessive lethal mutation (Basc test)	<i>Drosophila melanogaster</i>	0 or 20 mM (3565 μ g/mL) ⁱ	Negative	(Wild and others 1983)
28	1-Phenyl-3-methyl-3-pentanol	Micronucleus induction	NMRI mice	0, 357, 624, 891, or 1416 mg/kg bw	Negative	(Wild and others 1983)

^aWith and without metabolic bioactivation system, S-9.

^bWith metabolic bioactivation system, S-9.

^cWithout metabolic bioactivation system, S-9.

^dThe test material was bacteriotoxic toward all strains at greater than or equal to 500 and 160 μ g/plate with and without S-9 activation.

^eThe test material was bacteriotoxic toward all strains at doses greater than or equal to 500 and 150 μ g/plate with and without S-9 activation.

^fStandard plate incorporation method.

^gThe test material was bacteriotoxic toward all strains at greater than 200 and 100 μ g/plate with and without S-9 activation.

^hAdministered via gavage.

ⁱCalculated using molecular weight of 1-phenyl-3-methyl-3-pentanol = 178.28.

Conclusion for Genotoxicity

The testing of these representative materials *in vitro* in bacterial test systems (Ames assay) and *in vivo* in mammalian test systems (micronucleus assay) showed no evidence of mutagenic or genotoxic potential. These results are further supported by the lack of positive findings in the Basc test in *D. melanogaster*. The 2 positive results reported in the literature are questionable at best and most likely attributable to cytotoxic mechanisms rather than genotoxic mechanisms. The *B. subtilis* rec Assay has since been superseded by the OECD-validated *S. typhimurium*/*E. coli* reverse mutation assay in the modern genotoxicity testing battery.

Conclusion

The group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances discussed here was determined to be GRAS under conditions of intended use as flavor ingredients by the FEMA Expert Panel in 1965 and several times thereafter (Hall and Oser 1965, 1970; Oser and Ford 1974, 1978; Oser and others 1984; Newberne and others 1998; Smith and others 2005b, 2009; Waddell and others 2007). In 1978, the Panel evaluated the available data and affirmed the GRAS status of these flavor ingredients (GRASa). In 1993, the Panel initiated a comprehensive program to reevaluate the status of all FEMA GRAS flavor ingredients concurrent with a systematic revision of the FEMA Scientific Literature Reviews and the Panel reaffirmed the status of this group in 1995 with GRASr status. In 2012, this group was again reaffirmed as GRAS (GRASr2) based on knowledge concerning their rapid absorption, metabolic conversion, and excretion in humans and animals; their low levels of use as flavors in food; the wide margins of safety between the conservative estimates of intake and the NOAEL or NOEL determined from subchronic studies and the lack of significant genotoxic and mutagenic potential. The consistency of the results obtained from subchronic studies in rodent models support the conclusion that consumption of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances as part of the food supply is not associated with any significant risk to human health.

Acknowledgments

This work was supported by the Flavor and Extract Manufacturers Assoc. The Expert Panel of the Flavor and Extract Manufacturers Assoc. is independent but is financially supported by the Flavor and Extract Manufacturers Assoc.

Author Contributions

Marnett, Cohen, Fukushima, Gooderham, Hecht, Rietjens, and Smith interpreted the results and drafted the manuscript. Adams, Bastaki, Harman, McGowen, and Taylor researched and compiled available study data and assisted in the drafting of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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**Summary of Evaluations Performed by the
Joint FAO/WHO Expert Committee on Food Additives**

LINALYL ACETATE

COE No.:	203
FEMA No.:	2636
JECFA No.:	359
Chemical names:	1,5-DIMETHYL-1-ETHENYLHEX-4-ENYL ACETATE
Synonyms:	3,7-DIMETHYLOCTA-1,6-DIEN-3-YL ACETATE; BERGAMOL; LICAREOL ACETATE; LINALOOL ACETATE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1998
ADI:	0-0.5 mg/kg bw (1979)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1979 group ADI of 0-0.5 mg/kg bw for citral, geranyl acetate, citronellol, linalool, and linalyl acetate, expressed as citral, was maintained at the fifty-first (TRS 891/90, 1998) and sixty-first (TRS for JECFA 61 in press) meetings.
Report:	TRS 891-JECFA 51/79
Specifications:	COMPENDIUM ADDENDUM 6/FNP 52 Add.6/180 (1998)
Tox monograph:	FAS 42-JECFA 51/293
Previous status:	1982, FNP 25-JECFA 26/105; COMPENDIUM/851. R 1979, TRS 648-JECFA 23/25, FNP 12-JECFA 23/63. 0-5 (GROUP ADI FOR CITRAL, GERANYL ACETATE, CITRONELLOL, LINALOOL, AND LINALYL ACETATE, EXPRESSED AS CITRAL). R 1967, NMRS 44/TRS 383-JECFA 11/12, FAS 69.31/NMRS 44B-JECFA 11/32,33, FAS 68.33/NMRS 44A-JECFA 11/54. 0-0.25 (CONDITIONAL). CO. N

27 Jan 04

See Also:

Toxicological Abbreviations

Linalyl acetate (FAO Nutrition Meetings Report Series 44a)

Linalyl acetate (SIDS)

*This report contains the collective views of an international group of experts and does not necessarily represent the decisions
or the stated policy of the World Health Organization or of the Food and Agriculture Organization of the United Nations*

WHO Technical Report Series

891

EVALUATION OF CERTAIN FOOD ADDITIVES

Fifty-first report of the
Joint FAO/WHO Expert Committee on
Food Additives



World Health Organization

Geneva 2000

WHO Library Cataloguing-in-Publication Data

Joint FAO/WHO Expert Committee on Food Additives (1998 : Geneva, Switzerland)
Evaluation of certain food additives : fifty-first report of the Joint FAO/WHO Expert
Committee on Food Additives.

(WHO technical report series ; 891)

1.Food additives – toxicity 2.Food additives – analysis 3.Food contamination
4.Flavoring agents – analysis I.Title II.Series

ISBN 92 4 120891 0
ISSN 0512-3054

(NLM Classification: WA 712)

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Typeset in Hong Kong

Printed in Singapore

99/12638 — Best-set/SNP — 6000

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Geneva, 9–18 June 1998

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 42, 1999.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, Addendum 6. FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.

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 Geneva from 9 to 18 June 1998. The meeting was opened by Dr M. Younes, Chief, Assessment of Risks and Methodology, International Programme on Chemical Safety, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr Younes noted the increasing importance in the work of the Committee of intake assessments, which are carried out in response to requests for advice by the Codex Committee on Food Additives and Contaminants in the course of its development of the General Standard for Food Additives and the General Standard for Contaminants and Toxins in Food.

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 50 previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the forty-ninth meeting (Annex 1, reference 131).

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 and flavouring agents (sections 3 and 4 and Annex 2);
- to assess the intake of certain food additives (section 5 and Annex 2); and
- to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 6 and Annex 2).

2.1 Modification of the agenda

Montanic acid esters were removed from the agenda because data were not submitted. Methylmercury was removed because some of the relevant studies needed for the evaluation had not yet been completed. Furfural was added to the agenda at the request of an international organization. (–)-Menthol ethylene glycol carbonate, a mixture of (–)-menthol 1- and 2-propylene glycol carbonate, (–)-menthone 1,2-glycerol ketal, (±)-menthone 1,2-glycerol ketal, mono-menthyl succinate and 1-ethylhexyl tiglate (octen-3-yl 2-methyl-2-butenate) were also added to the agenda at the present meeting.

On the basis of comments received at the Thirtieth Session of the Codex Committee on Food Additives and Contaminants (2), 11 substances were added to the agenda for review of the specifications only: citric acid, carthamus yellow, calcium propionate, sucrose esters of fatty acids, potassium sorbate, sodium sorbate, calcium sorbate, aluminium powder, talc, microcrystalline cellulose and gum arabic.

Six substances were added to the agenda for review of the specifications only at the initiative of FAO, by the Committee itself, or in response to requests of other interested parties: ferrous sulfate, furfuryl alcohol, polydextroses, sorbitan monolaurate, xanthan gum, and tartaric, acetic and fatty acid esters of glycerol, mixed (DATEM).

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 meetings of the Committee (Annex 1, references 77, 83, 88, 94, 101, 107, 116, 122 and 131), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference 76) embraces the major observations, comments and recommendations on the safety assessment of food additives and contaminants contained, up to the time of its publication, in the reports of the Committee and other associated bodies. The Committee noted that the document reaffirms the validity of recommendations that are still appropriate, and points out the problems associated with those that are no longer valid in the light of modern technical advances.

2.2.1 Prediction of metabolism of flavouring agents into innocuous products

Comprehensive metabolism data were not available for most of the flavouring agents evaluated at the present meeting. However, data were available for at least one member of each group of flavouring agents, and the response to question 2 of the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) "Can the substance be predicted to be metabolized to innocuous products?" was based on knowledge of the substrate specificities of the enzymes involved in the different metabolic pathways, and on the fates and toxicological profiles of structural analogues. The Committee recognized that the Procedure for the Safety Evaluation of Flavouring Agents requires scientific judgement of the predicted metabolic fates of the

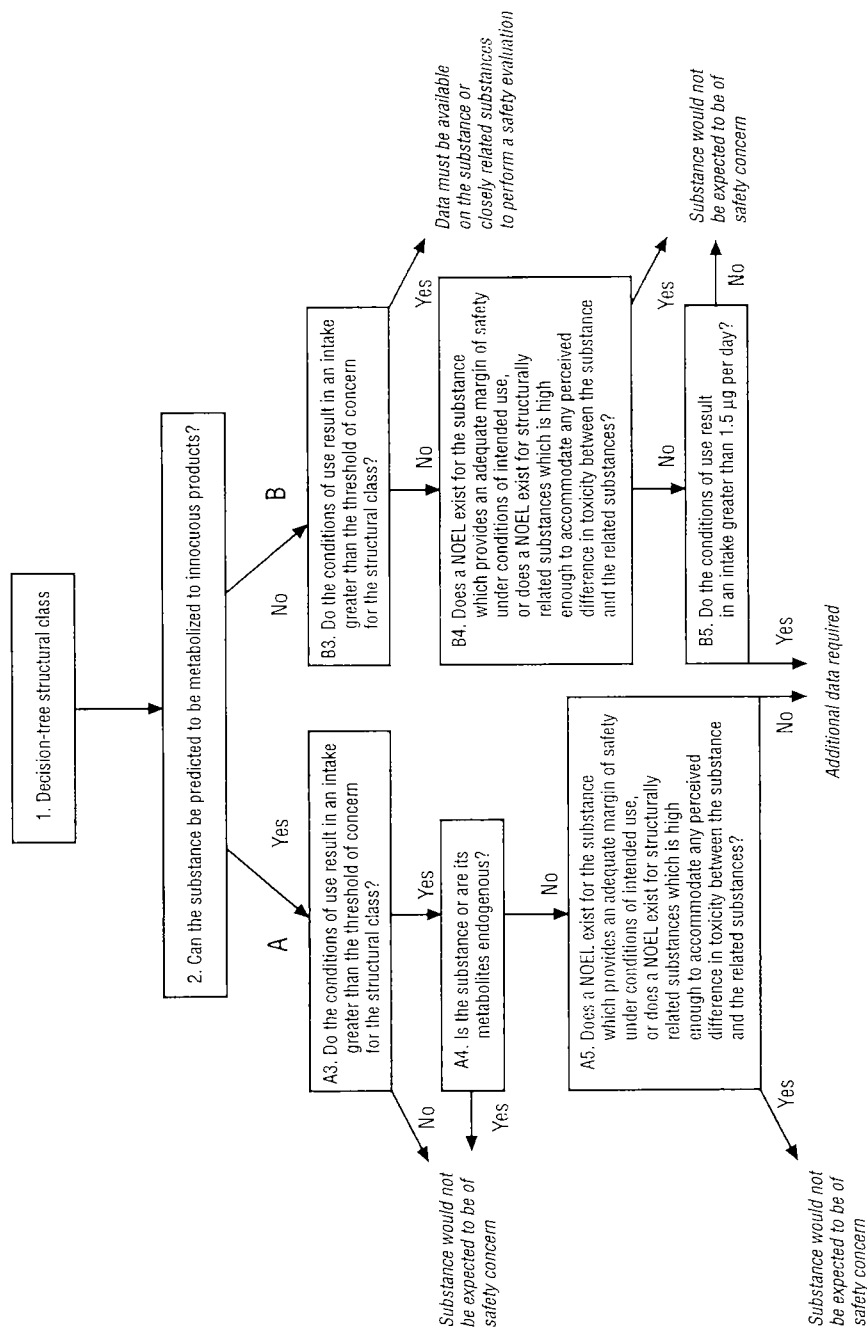


Figure 1
Procedure for the Safety Evaluation of Flavouring Agents

compounds under evaluation and the possible toxicological consequences. Such judgements are inherent in the adoption of the Procedure, and are considered acceptable only because of the low intakes of the flavouring agents, and the conservative assumptions made in defining the thresholds of concern for the different structural classes. In many cases, the prediction of the metabolic fates for individual members of the groups of flavouring agents considered at the present meeting was based on extrapolation from data on compounds with analogous structures, rather than from data on the individual flavouring agent *per se*. Accordingly, the Committee recognized that there are limitations in the extent to which reliable predictions of metabolic fate can be made. For instance, it is unlikely that the metabolism of pulegone into a menthofuran capable of oxidation to a hepatotoxic epoxide (3) would have been predicted. In consequence, steps B3, B4 and B5 of the Procedure should be used when the metabolic fate of a flavouring agent and its toxicological consequences cannot be predicted with confidence. Whenever flavouring agents are predicted to be metabolized to innocuous products, the validity of this conclusion should be checked by comparison with available toxicological data on the agent and/or its structural analogues.

All safety evaluations are based on the information available at the time. New data, including information on novel pathways of metabolic bioactivation, should be the stimulus for a re-evaluation of flavouring agents.

2.2.2 Literature surveys

The Committee expects sponsors of food additives to include in their dossiers relevant published papers that they have identified in literature surveys. However, the extent to which the literature has been surveyed is not always clear from the information submitted. Sponsors should perform comprehensive literature searches and indicate their origin and extent. They should specify the databases scanned, the years covered and the search strategy employed, including the key words used. This will permit authors of working papers and those doing independent literature surveys for the Committee to focus their own searches more productively.

2.3 Principles governing intake assessments

Important considerations underlie the procedures for evaluating assessments of intake of (or dietary exposure to) food additives and contaminants. Intake assessments may be part of an assessment of total exposure that includes water and other non-dietary sources of chemicals, where relevant. Exposure assessments are an integral part

of the risk assessment process which includes four stages: hazard identification, hazard characterization, exposure assessment and risk characterization (4).

Intake assessments can be requested by the Expert Committee, Member States, the Codex Committee on Food Additives and Contaminants, Codex commodity committees or international organizations. When substances are evaluated toxicologically by the Expert Committee, intake should also be assessed. All relevant information should be submitted and the purpose and scope of the intake assessment should be described.

To ensure that the submitted data are uniform, the Committee requests that data be collected using standardized methods as detailed in Table 1. Information about assessments of national intake is generally based on five methods: "poundage" (disappearance) data; food balance sheet data; household economic surveys or retail sales surveys; model diets; and individual dietary records. Countries are requested to submit information on dietary intake obtained using one or more of these methods, chosen on the basis of the availability of data.

General considerations

- Whenever a substance undergoes toxicological evaluation, the Committee should assess its intake so that the risk can be characterized.
- Intake assessments should be undertaken in a transparent and consistent manner.
- The budget method should be used at the international level as a screening tool to identify food additives requiring detailed intake assessments.
- Intake assessments should integrate data on food consumption with data on the level of chemicals in food to enable the intake of the chemical to be estimated.
- Estimates of chronic (long-term) intake should generally be derived for the population group to which the Acceptable Daily Intake (ADI) for a food additive or the Provisional Tolerable Weekly Intake (PTWI) for a contaminant applies.
- The estimate of chronic intake is adjusted for body weight and then compared with the ADI for a food additive or PTWI for a contaminant, where established by the Committee (5).
- Estimates of acute (short-term) intake can be derived when regarded as necessary because of potential acute adverse effects on health.

Table 1

Methods for screening for and assessing dietary intake of food additives

Method	Description	Disadvantages
For screening Budget method	Estimates a theoretical maximum additive level in the proportion of the food and/or beverage supply likely to contain the additive such that the ADI for the additive cannot be exceeded by the population (6). In cases where the permitted level of use of the additive exceeds the calculated maximum theoretical level, further intake assessments are required.	Used to screen for additives that require detailed intake assessment at an international level. Not intended for the assessment of intake.
For assessing dietary intake <i>Population-based methods</i> "Poundage" (disappearance) data	Estimates the amount of food additive available per capita for use in food manufacturing in a country during a given time period. Where appropriate, estimates can take into account the import or export of foods containing the additive, and non-food uses. Estimates may be adjusted for the proportion of the population likely to consume the additive.	Household wastage of the food containing the additive not usually accounted for. Will underestimate the intake of an additive for individuals with high intakes if the number of consumers of foods containing the additive is not considered.
Food balance sheet (FBS) data	Estimates the amount of food available for use per capita per day based on food (raw and certain semi-processed commodities) available for consumption (food available = food production + imports – exports – non-food use). For use in estimating food additive intake, the amount of commodity likely to contain the additive must be predicted by estimating the percentage of the commodity that is processed and the percentage of this processed food that contains the additive.	Household wastage of the food containing the food chemical not usually accounted for. May overestimate per capita intakes, but underestimate the intake of a food chemical for individuals with high intakes. Difficult to obtain accurate estimates of the percentage of food that is processed and of the percentage of processed food containing the additive.

Household-based methods
Household economic survey

Estimates the daily per capita intake of a food chemical based on household surveys of food bought by households over a given time period.

Household wastage of the food containing the food chemical not usually accounted for.

May underestimate the intake of a food chemical for individuals with high intakes.

Retail sales survey

Estimates the daily per capita intake of a food chemical based on sales of food items in shops (food sold at retail level over a given time period).

Household wastage of the food containing the food chemical not usually accounted for.

May underestimate the intake of a food chemical for individuals with high intakes.

Individual-based methods
Model diets

Estimates are based on model diets constructed from available information on food consumption to represent a typical diet of a specified population subgroup or for individuals with high intakes of foods containing the food chemical.

Model diets can be extremely useful in estimating intakes of food chemicals, but are only as good as the underlying data and assumptions.

Interpretation of intake estimates made using model diets requires that the assumptions made in constructing each model diet are stated.

Individual dietary records

Estimates are based on data on food consumption by individuals. Individual intakes of additives or contaminants can be adjusted according to individual body weights, if available, before deriving population statistics from the distribution of additive or contaminant intakes.

Duration of the survey can influence intake estimates, especially for assessments of chronic intake.

Interpretation of intake estimates requires that the assumptions made are stated.

- Additional assessments of intake for a specific population subgroup can be undertaken when the Committee has identified the groups that are potentially susceptible or at risk for toxicological reasons.
- When a number of compounds have a group ADI, the intake assessment should cover the whole group of compounds.
- A body weight of 60kg for entire populations is used for intake assessments unless alternative body weights are provided by countries for use with national data.

Data used in evaluations

- The Committee uses the data on intake assessments submitted by countries to undertake additional calculations, where appropriate.
- Data on food consumption are collected at the national, household or individual level (Table 1).
- Data on food consumption should be representative of the population group to which the ADI or PTWI refers.
- Intake assessments are based on three types of data: maximum levels of use from the proposed draft General Standard for Food Additives or the proposed draft General Standard for Contaminants and Toxins in Food, national food standards, and measured levels, where available.
- Intake assessments based on national data on food consumption and maximum levels of use specified in the draft General Standard for Food Additives should state whether all the food categories proposed in the General Standard have been considered or only those food categories permitted in national standards.
- Food intakes reported on an “as consumed” basis should be grouped for assessments of intake of a food additive according to the food classification system used in the draft General Standard for Food Additives (Annex 4), and for assessments of intake of contaminants according to the General Standard for Contaminants and Toxins in Food.

Evaluations

- Evaluations of national estimates of intake should include comments on the quality, uncertainty and variability of the data. All data sources should be documented.
- Assessments of chronic intake should be presented as means, percentiles or distributions for the entire population studied and for actual consumers of foods containing the food chemical of

interest. Assessments of chronic intake should be expressed as a percentage of the ADI or PTWI.

- Intake estimates represent the intake of the additive or contaminant from the total diet. They include both average and high intakes of the additive or contaminant.
- Food groups that account for a high proportion of the total estimated mean intake of a food chemical by consumers should be identified in the evaluation, where possible.

Variability and uncertainty

It is important to distinguish between the relative contributions of variability and uncertainty to the accuracy and precision of intake estimates (5). In evaluating intake estimates based on “poundage” data, food balance sheet data, household economic surveys or retail sales surveys, model diets or individual dietary records, it is important to note that the data may not be strictly comparable even among countries using any one approach to estimate intakes due to differences in the following:

- duration of the survey;
- number of individuals surveyed;
- population groups selected, such as age/sex groupings;
- average body weights;
- definition of “high-intake consumers”;
- chemical levels (depending on whether the maximum levels specified in the draft General Standard for Food Additives, the draft General Standard for Contaminants and Toxins in Food, or national food standards are used, or levels are measured); and
- assumptions made about how to assign foods reported on an “as consumed” basis in the food classification systems used in the draft General Standard for Food Additives, the draft General Standard for Contaminants and Toxins in Food or national standards.

In addition, the use of certain methods of data collection, data sets and assumptions in estimating consumption of food and/or food chemicals may result in different estimates of the intake of additives or contaminants. Potential sources of uncertainty and variability are detailed in Table 2.

Recommendations

In making recommendations based on the evaluation of national assessments of intake, it is essential that information on the reliability, variability and uncertainty of data used in the assessments be provided. In cases where data are insufficient for the purpose of the

Table 2

Uncertainty and variability associated with methods for estimating food consumption and intakes of food additives and contaminants

Method	Uncertainty and variability
For estimating food consumption	
<i>“Poundage” data and household-based methods</i>	
<i>Short-term surveys of food consumption</i>	
	May underestimate intakes of food additives and contaminants.
	The assumption that data on short-term food consumption (1–14 days) across several age groups (cross-sectional data) represents a lifetime of consumption will tend to overestimate average intakes of food chemicals over a lifetime, especially for foods that are not staples.
	Short-term surveys of food consumption tend to underestimate the number of consumers of a selected food over a longer time period.
One-day dietary surveys versus surveys of consumption averaged over several days	One-day surveys of food consumption tend to overestimate the consumption of specific foods or food groups and result in higher levels of estimated consumption for consumers with high intakes compared with surveys in which average food consumption over several days is recorded.
Dietary recall versus methods in which the weight of food consumed is recorded	Dietary recall methods may result in over- or underestimates of consumption compared with methods in which the weight of food consumed is recorded. The extent of uncertainty may depend on the selected food item and the sex and body weight of respondents.
Data on mean food consumption in a population versus individual dietary records	Data on mean food consumption in a population tend to overestimate daily food consumption when compared with data from individual dietary records (in most cases mean food consumption is higher than median food consumption).
<i>Surveys of chronic food consumption (food frequency surveys)</i>	Data on the frequency of consumption of a particular food is combined with data on portion size derived from short-term surveys. Such estimates of food consumption will generally be lower than estimates derived from short-term surveys, resulting in lower estimates of intake of food chemicals.

For estimating food additive intake

Assumption that the maximum permitted additive level is present in relevant food groups

Assumption that all foods in any one food group contain a permitted additive

Assigning the maximum permitted additive level to a wide food group

Combining two or more food categories into one and assigning the maximum permitted additive level to all foods in the new single category

Inclusion of foods from two or more categories (prepared foods) in intake estimates

Inclusion of corrections for concentrates, dried mixes or dehydrated foods or fat content for fat-soluble chemicals

For estimating contaminant intake

Assumption that the maximum permitted level of a contaminant is present in relevant food groups

Assumption that all foods in any one food group contain a contaminant at the maximum permitted level

Assigning a contaminant level to a wide food group

Combining two or more food categories into one and assigning the highest level of a contaminant to all foods in the new single category

Inclusion of foods from two or more categories (prepared foods) in intake estimates

Will overestimate food additive intakes compared with estimates based on the mean or median level of an additive derived from data on reported use.

Will generally overestimate food additive intakes because not all manufacturers of foods in a group will use the additive.

Will tend to overestimate food additive intakes when the maximum level applies to a minor component of the food group.

Will tend to overestimate food additive intakes.

Will tend to improve intake estimates when the weighted average of food additive levels in each food category is used, based on standard recipes.

Will tend to improve intake estimates compared with the assignment of a maximum additive level for concentrates or fat to the food "as consumed".

Will overestimate contaminant intakes.

Will generally overestimate contaminant intakes because not all foods in the food group may contain the contaminant at the same level.

Will tend to overestimate contaminant intakes when the contaminant level applies to a minor component of the food group.

Will tend to overestimate contaminant intakes.

Will tend to improve intake estimates when the weighted average of contaminant levels in each food category is used, based on standard recipes.

evaluation, recommendations will be made for the submission of additional data.

Recommendations will be accompanied by relevant supporting information that may be important for Codex committees when considering various options for risk management. For example, information may be included on alternative food additives that serve the same technological function. For contaminants, information may be included on the feasibility of reducing contaminant levels in specific foods, monitoring levels in foods and the availability of suitable methods of analysis.

2.4 Principles governing the establishment and revision of specifications

2.4.1 *Specifications and the ADI*

For easy reference the Committee decided to indicate in all specifications the status of the ADI of the substance.

2.4.2 *Microbiological criteria in specifications monographs for food additives*

In some instances, particularly for products of natural origin, the Committee has included microbiological criteria in the specifications monograph. At its present meeting, the Committee agreed on the appropriateness of establishing a policy for setting microbiological criteria. The Committee therefore established the following policy.

Policy on microbiological criteria

In general, manufacturers of food additives are expected to use good manufacturing practices and to establish microbiological controls in production processes as necessary. This is to ensure that food additives are not contaminated with pathogenic or other undesirable organisms or with microbial metabolites and that the food additive is suitable for its intended use. Such a requirement will be included in the specifications monograph when the Committee recognizes the need for microbiological criteria for an individual substance.

The Committee will consider, on a case-by-case basis, the following factors when developing microbiological criteria for food additives:

- the origin of the food additive (plant, animal or natural mineral source or microbially derived via fermentation);
- evidence of a health hazard or potential health hazard based on epidemiological data, hazard analysis or specific populations that may be at risk;

- the nature of the natural and commonly acquired microorganisms that contaminate the food additive and the ability of the food additive to support their growth;
- the effect of further processing on the microorganisms that contaminate the food additive;
- the potential for microbial contamination and/or growth in a food additive during its procurement, processing, handling, storage and distribution;
- the state in which the food additive is packaged, stored and distributed (e.g. frozen, refrigerated, heat-processed); and
- the potential for direct use by consumers.

Any one of these factors may signal the need to consider establishing microbiological criteria for a food additive.

2.4.3 ***Specifications for flavouring agents***

The Committee considered the specifications for 232 flavouring agents at the present meeting. These included 224 substances that were on the original agenda (nos 139 and 219–442) and a further eight that were added to the agenda at the meeting (nos 443–448, 450 and 451). No information was provided on substance no. 436 (3-(L-menthoxy)propane-1,2-diol), which was not considered further. The specifications considered included a number of substances that were previously described in separate monographs. The new specifications replace these monographs for flavouring uses of the substances.

Sixty-eight specifications were classified as tentative because not all the necessary relevant information had been provided. Many of the flavouring agents examined at the present meeting were more complex than those considered previously, and the sponsors were asked to examine specifications carefully in the future to ensure that all relevant information is available and to correct any information that does not accurately represent flavouring agents that are on the market.

As at previous meetings, identification of most of the flavouring agents was based on infrared spectra. However, this may not always be the method of choice because the infrared spectra of closely related substances may be indistinguishable, even in the fingerprint region. The Committee would welcome further comments on this point, in particular on the possible merits of using more sophisticated methods such as mass spectrometry and/or nuclear magnetic resonance.

2.4.4 **Specifications for vitamins and minerals**

At its present meeting, the Committee was requested by FAO to develop food-grade specifications for ferrous sulfate for use in food fortification. Vitamins and minerals do not fall within the scope of the Committee's traditional definition of the term "food additive";¹ nonetheless, FAO receives repeated requests for food-grade specifications for substances used in food fortification.

Although the Committee has prepared specifications for approximately 40 traditional food additives that also have incidental uses as vitamins and minerals, more than 60 substances remain for which internationally recognized food-grade specifications are lacking (7). It was agreed that such substances would normally be on the agenda for the development of specifications only. In certain cases, however, it may be necessary to undertake toxicological evaluations as well, for example when novel forms of nutrients are involved.²

2.4.5 **Enzyme preparations derived from genetically modified microorganisms**

At the thirty-seventh meeting of the Committee (Annex 1, reference 94), an addendum to the "General specifications for enzymes used in food processing" (Annex 1, reference 96) was prepared. This addendum addressed issues relating to enzyme preparations derived from genetically modified microorganisms. The text is published in Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference 96).

At its present meeting, the Committee concluded that the first part of Appendix B (General considerations) should be revised as follows.

For the proper evaluation of enzyme preparations derived from genetically modified microorganisms, information should be provided on the host microorganism, the genetic material introduced into the

¹ At its first meeting in 1956, the Committee defined the term *food additives* as "non-nutritive substances added intentionally to food, generally in small quantities, to improve its appearance, flavour, texture or storage properties" (Annex 1, reference 1). The Committee later expanded its scope to include food contaminants as well as certain nutritive substances consumed in high amounts (Annex 1, reference 76).

² Such a case occurred at the forty-first meeting when the Committee evaluated the safety of the use of sodium iron EDTA (ethylenediamine tetraacetate or edetate) in supervised food fortification programmes in populations in which iron-deficiency anaemia is endemic (Annex 1, reference 107). The Committee expressed concern about the potential for over-fortification of food because of the enhanced bioavailability of iron in this form.

host microorganism and the recombinant production microorganism. Annex 1 of the *Compendium of food additive specifications* (Annex 1, reference 96) addresses factors relevant to all microbial sources (conventional and recombinant) used in the production of enzyme preparations and to fermentation and recovery procedures. The following points need emphasis when the production of enzyme preparations derived from genetically modified microorganisms is considered:

- The host microorganism should be taxonomically and genetically characterized.
- Documentation demonstrating that the host microorganism is non-pathogenic and non-toxicogenic should be provided.
- The genetic material (i.e. the expression vector or plasmid) intended for introduction into the host microorganism should be characterized and a description of its construction provided. As appropriate, it should be demonstrated that the genetic material does not contain genes coding for virulence factors, protein toxins or enzymes that may be involved in the synthesis of mycotoxins or any other toxic or undesirable substances. The source of the DNA encoding the enzyme of interest should be identified where possible.
- The production microorganism should be characterized with respect to the introduced DNA, its genetic stability and its growth properties.
- If the production microorganism is capable of producing proteins that inactivate clinically useful antibiotics, documentation should be provided that the finished enzyme preparation contains neither antibiotic-inactivating proteins at concentrations that would interfere with antibiotic treatment nor DNA that is capable of transforming microorganisms, which potentially could lead to the spread of antibiotic resistance.
- All enzyme preparations should be evaluated for their potential to elicit allergic reactions. As a general rule, if a food is known to cause an allergic reaction in humans, its use as a source of DNA encoding the enzyme of interest should be avoided. In exceptional cases, where there is a demonstrated need to use an allergenic source of DNA, documentation should be provided indicating that the enzyme is not associated with the allergic reaction. Worldwide the most common allergenic foods are fish, crustaceans, peanuts, tree nuts, soybeans, milk, eggs and wheat.

The above points cover the major issues pertinent to the development of enzyme preparations derived from genetically modified microorganisms. These points emphasize and supplement the issues that must be considered in the safety evaluation of such enzyme preparations which in general relate to the avoidance of undesirable impurities.

The following properties might be useful in characterizing enzymes from recombinant microorganisms: relative molecular mass, isoelectric point, substrate specificity, reaction kinetics, activity as a function of pH and temperature, amino acid composition, amino acid sequence, a peptide map and the sequence of DNA bases coding for the enzyme.

In revising the second part of Appendix B (Specifications for enzymes from genetically manipulated microorganisms), the Committee removed the tentative designation. The revised section on specifications to be published in the specifications monograph focuses on the section of the monographs that deals with the host microorganism for enzymes from genetically modified microorganisms. It notes that any microbial strain that meets the general considerations for enzymes specified in Annex 1 of the *Compendium of food additive specifications* (Annex 1, reference 96) should be a safe and suitable host for the introduced DNA. Citation of the genus and species of the host and donor organisms is usually adequate for microorganisms that have been determined to be safe and suitable. The citation of the strain is appropriate where a non-pathogenic and non-toxicogenic strain belongs to a species that also encompasses pathogenic and toxicogenic strains. Citation of the specific expression plasmids is generally unnecessary where the plasmid vector is well characterized and documentation on the production microorganism, including the introduced DNA, can be used to verify the appropriateness of the expression plasmid selected.

The Committee requested comments on the revised text of Appendix B, which will be published as an Annex in the specifications monograph.

The Committee also amended the "General notices applying to the standards, tests and assays of the specifications prepared by the Joint FAO/WHO Expert Committee on Food Additives" in the *Compendium of food additive specifications* (Annex 1, reference 96) to align the text on the source of enzyme preparations with the revision of Appendix B to Annex 1, as described above.

2.4.6 **Limit test for heavy metals**

The Committee reaffirmed the decision taken at its forty-ninth meeting (Annex 1, reference 131) to replace, as appropriate, the general test for heavy metals in the specifications with tests for specific metals, particularly for lead, cadmium, mercury and arsenic.

In order to confirm the limits for heavy metals, the Committee would require confirmation of the actual levels determined in the food additives in question from sponsors.

The Committee will endeavour to establish guidelines for setting limits for specific heavy metals. At its present meeting, the Committee confirmed that it would aim to set limits that were as low as practicable for those elements of concern.

The Committee adopted a general limit for lead of 2 mg/kg. When an additive was known to be used in substantial amounts, a limit of 1 mg/kg was chosen. In a few cases where there was evidence that the lead content of the product could not be reduced to these levels, a limit of 5 mg/kg was specified.

3. **Specific food additives**

The Committee evaluated four food additives for the first time and re-evaluated 12 food additives considered at previous meetings. In addition, the Committee evaluated a large number of flavouring agents using the Procedure for the Safety Assessment of Flavouring Agents (see section 4). Information on the 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 **Enzyme preparations**

3.1.1 ***α-Acetolactate decarboxylase***

α-Acetolactate decarboxylase derived from a genetically modified organism was previously reviewed by the Committee at its forty-ninth meeting (Annex 1, reference 131), when a temporary ADI “not specified” was established. The ADI was designated as “temporary” because the specifications were tentative.

At its present meeting, the Committee prepared specifications that are consistent with the specifications for the product that was tested toxicologically. Therefore, the Committee removed the temporary qualification and established an ADI “not specified”.

A toxicological monograph was not prepared. The existing specifications were revised in accordance with the revised Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference 96) as described in section 2.4.5 and the “tentative” designation was deleted.

3.1.2 **Maltogenic amylase**

Maltogenic amylase derived from a genetically modified organism was previously reviewed by the Committee at its forty-ninth meeting (Annex 1, reference 131), when a temporary ADI “not specified” was established. The ADI was designated as “temporary” because the specifications were tentative.

At its present meeting, the Committee prepared specifications that are consistent with the specifications for the product that was tested toxicologically. Therefore, the Committee removed the temporary qualification and established an ADI “not specified”.

A toxicological monograph was not prepared. The existing specifications were revised in accordance with the revised Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference 96) as described in section 2.4.5 and the “tentative” designation was deleted.

3.2 **Flavouring agents**

3.2.1 ***trans*-Anethole**

trans-Anethole was first evaluated by the Committee at its eleventh meeting (Annex 1, reference 14) and re-evaluated at its twenty-third meeting (Annex 1, reference 50), when a temporary ADI of 0–2.5 mg/kg of body weight was allocated. At the thirty-third meeting (Annex 1, reference 83), the temporary ADI was reduced to 0–1.2 mg/kg of body weight on account of the tumorigenic effects observed in the liver of female rats in a 27-month study. At that time, the Committee requested further details of the long-term study in rats and the results of metabolic studies, and further noted that a long-term study in mice and an epidemiological study might be required. At the thirty-seventh meeting (Annex 1, reference 94), the temporary ADI for *trans*-anethole was reduced to 0–0.6 mg/kg of body weight after consideration of the results of three independent reviews of the histological changes in the liver of rats in the long-term study. At that time, a

recommendation was made for further metabolic and pharmacokinetic studies, chromosomal aberration studies and *in vitro* tests for gene mutations in mammalian cells. At its thirty-ninth and forty-ninth meetings (Annex 1, references 101 and 131), the Committee further extended the temporary ADI of 0–0.6 mg/kg of body weight, pending completion of the recommended studies.

At its present meeting, the Committee evaluated the results of new 90-day studies in rats and mice, together with those of studies on the metabolism of *trans*-anethole in mice and rats, studies on the effects on hepatic enzyme induction in these species of short-term administration of *trans*-anethole in the diet, *in vitro* cytotoxicity and genotoxicity studies and some epidemiological data which had become available since the thirty-seventh meeting. These studies provided information on the significance to humans of the results of the long-term study in rats. In addition, studies on reproductive toxicity and immunotoxicity were reviewed. A long-term study in mice, which had been requested at the thirty-ninth meeting, was not available.

The no-observed-effect level (NOEL) from the previously reviewed long-term study in rats was 2.5 g/kg (0.25%) in the diet (equivalent to 125 mg/kg of body weight per day), based on an increased incidence of hepatic focal nodular hyperplasia in animals of both sexes at higher doses. Administration of *trans*-anethole to 30 female CD-1 mice for 12 months at 4.6 g/kg (0.46%) in the diet (equivalent to 700 mg/kg of body weight per day) did not result in the induction of hepatomas in any of the animals up to 18 months after the feeding of *trans*-anethole had been initiated; the related alkenylbenzenes, safrole and estragole, induced hepatomas in the same assay.

Most of the evidence on genotoxicity and DNA interactions of *trans*-anethole reviewed at the present meeting suggested that it is not genotoxic. Under standard assay conditions, *trans*-anethole was not mutagenic in *Salmonella typhimurium*, and negative results were obtained in tests for induction of chromosomal aberrations in Chinese hamster ovary cells *in vitro*, induction of micronuclei in mice *in vivo* and unscheduled DNA synthesis *in vitro* and *in vivo*. The only positive results were obtained for mutation at the *tk* locus in mouse lymphoma cells in both studies performed and in three assays in *S. typhimurium* when a microsomal activation system with enhanced protein content or when the co-factor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) was added. The rate of formation of adducts in hepatic DNA from adult and weanling mice exposed to *trans*-anethole was very low in comparison with the rate after exposure to

the hepatocarcinogenic alkylbenzenes, safrole, estragole and methyl-eugenol.

The potential of metabolites of *trans*-anethole to induce unscheduled DNA synthesis in rat hepatocytes *in vitro* was also investigated. Neither the initial ω -oxidation product, 3'-hydroxy anethole, nor the epoxide products, anethole 1,2-oxide and its metabolite, anethole 1,2-diol, induced unscheduled DNA synthesis. Anethole 1,2-oxide was cytotoxic, while anethole 1,2-diol exhibited little cytotoxicity. Agents that prevented further metabolism of the epoxide markedly enhanced the cytotoxicity of *trans*-anethole.

The metabolism of *trans*-anethole proceeds via *O*-demethylation, side-chain ω -oxidation and epoxidation of the side chain. These pathways of metabolism occur in rats, mice and humans, but the proportion of the dose metabolized by different routes is species- and dose-dependent. *O*-Demethylation and ω -oxidation are the major pathways in rats and mice receiving single doses of 200–300 mg/kg of body weight (similar to those administered in the toxicity studies), and also in humans receiving a single dose of 1 mg (equivalent to 0.015 mg/kg of body weight). The formation of the cytotoxic epoxide metabolite has been deduced from the urinary excretion of the isomeric diols and of the *N*-acetylcysteine metabolite derived from conjugation with glutathione. The total amounts of these metabolites excreted by rats (about 15–20%) were higher than those excreted by mice (about 5–10%) or humans (about 3%). However, in this respect, the human data have been described adequately for only two subjects given [^{14}C] *trans*-anethole. The available data indicate that rats given single doses of 200–300 mg/kg of body weight metabolize proportionally more *trans*-anethole to the cytotoxic metabolite than do humans given a single dose of 1 mg. The metabolic data from humans were considered by the Committee to be too limited to permit accurate quantitative comparisons of formation of anethole epoxide in rats and humans.

Recently conducted 90-day feeding studies in CD-1 mice and Sprague–Dawley rats were reviewed, together with preliminary 28-day range-finding studies. Most of the observed effects were related to inanition resulting from decreased food consumption by the treated animals. The effects of diet rejection were much more apparent in mice and were reflected in the much lower dietary levels tolerated. In contrast, in studies in which single doses were given by gavage, mice appeared to tolerate higher doses of *trans*-anethole than did rats. The doses used in the 90-day studies were 30–240 mg/kg of body weight per day for mice and 150–900 mg/kg of body weight per

day for rats. In both species, the major indications of inanition were large decrements in body weight, depletion of hepatic glycogen and decreased organ weights, while enlarged livers and hypertrophy of the centrilobular hepatocytes were suggestive of hepatic enzyme induction. The NOEL in the study in mice was 120mg/kg of body weight per day, based on decrements in body weight of greater than 10% in males at the highest dose.

Toxicologically significant effects were apparent from the results of the 28-day and 90-day studies in rats. In both male and female rats a hepatotoxic effect of *trans*-anethole was suggested by elevated serum activity of γ -glutamyl transferase in the 90-day study at doses of 600 or 900mg/kg of body weight per day, and by a reduction of serum protein and albumin concentrations at a dose of 900mg/kg of body weight per day. However, no proliferative or other toxicologically significant lesions were apparent on histopathological examination of the livers. There was no evidence of a similar effect in mice over the lower dose range necessitated by their lower tolerance to *trans*-anethole in the diet. The NOEL was 300mg/kg of body weight per day, based on the elevated γ -glutamyl transferase activity in both male and female rats and decrements in body weight exceeding 10% in the males at higher doses.

Studies of reproductive toxicity in rats given *trans*-anethole were conducted over one or four generations. In the 1-generation study, litter effects (reduced viability of pups, pup survival and pup body weights) were noted only at doses that were toxic to the dams. The NOEL for maternal toxicity was 175mg/kg of body weight per day, based on reduced food consumption and body-weight gain and overall poor condition in the dams. In the 4-generation study, in which treated groups received *trans*-anethole at a dose of 10g/kg (1%) in the diet (equal to 700–1400mg/kg of body weight per day), the only effect seen in the pups was a reduction in body-weight gain. A cross-fostering experiment conducted as part of the 4-generation dietary study indicated that the reduced body-weight gain observed in pups in the treated groups was related to exposure during lactation, rather than during the gestation period.

trans-Anethole was not immunotoxic when tested in the sheep erythrocyte plaque-forming assay or a challenge assay with *Listeria monocytogenes*.

The data reviewed at the present meeting indicated that *trans*-anethole and its metabolites are unlikely to be genotoxic *in vivo* and suggested that a cytotoxic metabolite, anethole epoxide, is the possible causative agent of the hepatotoxic effects noted in the liver of

rats. Since *trans*-anethole is metabolized along the same three major pathways in mice, rats and humans, hepatotoxicity in rats was considered an appropriate end-point on which to base the ADI. Because the 90-day study in rats was well conducted by current standards and the hepatic effects observed were consistent with the hepatotoxicity observed in the long-term study, it was used to derive the ADI. The NOEL was 300mg/kg of body weight per day on the basis of alterations in serum parameters considered to be indicators of hepatotoxicity. Because of the low tolerance of mice for *trans*-anethole administered in the diet, they could not be fed doses comparable to those that were toxic to rats. It is therefore unlikely that a new long-term study in mice could include sufficiently high doses to induce effects in the liver uncomplicated by inanition.

Because of limitations in the long-term studies in rats and mice, the Committee concluded that the recent 90-day study in rats provided the most reliable data for the determination of the NOEL for adverse effects in the liver. Application of the usual safety factor of 100 to the NOEL from the 90-day study was considered inappropriate because of the limitations in the long-term studies. An overall safety factor of 200 was considered adequate to allow for deficiencies in the long-term studies and to provide a suitable safety margin for the maternal toxicity observed in reproductive toxicity studies as well as for the effects seen in the 90-day study in mice.

The Committee allocated an ADI of 0–2mg/kg of body weight on the basis of the NOEL of 300mg/kg of body weight per day in the 90-day study in rats, to which a safety factor of 200 was applied. The ADI was rounded to one significant figure, in accordance with usual practice.

An addendum to the toxicological monograph was prepared. The existing specifications included in the list of flavouring agents in the *Compendium of food additive specifications, addendum 5* (Annex 1, reference 133) were maintained.

3.2.2 **Furfural**

Furfural was previously evaluated at the thirty-ninth meeting of the Committee (Annex 1, reference 101). An ADI was not established because of evidence available to the Committee regarding the genotoxicity and carcinogenicity of furfural. At that time, the Committee considered that the direct addition of furfural as a flavour was not appropriate, and that its use as a solvent should be restricted to situations in which alternatives were not available. The Committee also considered that transfer of furfural into food should be reduced

to the lowest extent technically feasible. At its present meeting, the Committee considered furfural as a flavouring agent.

Furfural is used as a flavouring agent in a variety of food products and alcoholic and non-alcoholic beverages. Furfural and many of its derivatives occur widely as natural constituents of food.

Since the last evaluation additional data, including those from studies of the metabolism of furfural, its potential genotoxicity and hepatotoxicity, have become available.

In both humans and rodents, furfural is efficiently metabolized by oxidation of the aldehyde functional group to furoic acid, most of which is conjugated with glycine and excreted. A minor proportion (<5%) of furoic acid is condensed with acetyl coenzyme A to form 2-furanacryloyl coenzyme A, which is conjugated with glycine and excreted in the urine. About 5% of [¹⁴COOH]furfural is eliminated by rats and mice as radiolabelled carbon dioxide. The metabolic pathway, which could involve either direct decarboxylation or epoxidation and opening of the carbon ring, has not been defined.

In short-term studies, furfural was clearly hepatotoxic at doses at and above 90mg/kg of body weight per day and 150mg/kg of body weight per day in male rats and male mice, respectively. Minor changes were reported in the liver of male rats given furfural at lower doses in corn oil by gavage for 13 weeks and were also present to a lesser extent in controls given corn oil by gavage. In a study of developmental toxicity furfural was not toxic in rats at doses of up to 150mg/kg of body weight per day, the highest dose tolerated.

Furfural, like other aldehydes such as endogenous acetaldehyde, is a reactive aldehyde; it is reported to bind to soluble proteins and protein components of cell membranes. Various metabolic processes (i.e. oxidation, conjugation and condensation) will effectively eliminate the reactive aldehyde functional group when these metabolic pathways are not saturated by high, non-physiological doses.

Furfural was not genotoxic in most tests *in vitro*. Positive results were reported at relatively high concentrations in only three of 16 reverse mutation assays in *Salmonella typhimurium* and in one of three *rec* assays in *Bacillus subtilis*. A few chromosomal aberrations were seen in Chinese hamster ovary cells exposed to relatively high concentrations. Sister chromatid exchanges and forward mutations were induced in mouse lymphoma cells. This weak activity of furfural *in vitro* in some tests for genotoxicity might be explained by the reactivity of its aldehyde group. Recent studies of unscheduled DNA

synthesis in hepatocytes of rats treated *in vivo* and in human liver treated *in vitro* gave negative results. Negative results were obtained in tests for genotoxicity *in vivo*, except in *Drosophila melanogaster* injected with furfural.

In a 2-year study of carcinogenicity in rats given furfural in corn oil by gavage at doses of 30 or 60 mg/kg of body weight per day, hyperplasia of the bile duct and cholangiofibrosis were seen in essentially all rats in all groups (treatment and control); the incidence was highest in the control groups. Mild hepatocellular necrosis was seen in all groups; however, the rates in males were higher in all treated groups. Cholangiocarcinomas were observed in two of 50 males at the highest dose, but the incidence was not statistically significant.

In mice, the combined incidence of adenomas and carcinomas of the liver (64%) was significantly increased ($p < 0.01$) in males given the highest dose (175 mg/kg of body weight per day), but not in females. Hepatocellular adenomas and carcinomas also occurred in the controls and in animals that received the lowest dose (50 mg/kg of body weight per day) and in those given the intermediate dose (100 mg/kg of body weight per day), at incidences of 34–44% in males and 6–14% in females. The incidences in historical controls were 38% (14–50%) in males and 6.2% (0–30%) in females. Hepatotoxicity, manifested by features such as focal and multifocal necrosis and chronic inflammation, was seen in all groups, including the controls, but was considerably more frequent in mice given the highest dose.

Studies of oncogene activation in samples of liver tumours in this study revealed some differences in the pattern of mutations in the oncogenes observed in treated mice and controls. As it was not possible to identify the animals from which the tumours originated and since hepatotoxicity was seen at the intermediate and highest doses, it was not possible to determine whether a direct genotoxic event or a secondary genotoxic pathway was involved.

Because of concern about the tumours observed in male mice given furfural and the fact that no NOEL was identified for hepatotoxicity in rats, the Committee was unable to allocate an ADI. Before reviewing this substance again, the Committee would wish to review the results of studies of DNA binding or adduct formation *in vivo* to clarify whether furfural interacts with DNA in the liver of mice. The Committee also requested the results of a 90-day study of toxicity in rats to identify a NOEL for hepatotoxicity.

A toxicological monograph was prepared. The existing specifications for furfural were revised and, as the only direct food-related use of

furfural is as a flavouring agent, they were transferred to the list of flavouring agents in the specifications monograph.

3.2.3 **Menthol**

Menthol was first evaluated at the eleventh meeting of the Committee (Annex 1, reference 14), when it was allocated an unconditional ADI of 0–0.2 mg/kg of body weight and a conditional ADI of 0.2–2 mg/kg of body weight. At the eighteenth meeting, an ADI of 0–0.2 mg/kg of body weight was established (Annex 1, reference 35). Menthol was re-evaluated at the twentieth meeting of the Committee (Annex 1, reference 41), when the previous ADI was maintained. At that meeting, the Committee considered it desirable to evaluate the results of long-term studies of toxicity and carcinogenicity in rats, information on the average and likely maximum levels of intake of menthol, clinical observations on subjects with a higher than average intake of menthol and the results of studies on metabolism (Annex 1, reference 42). Since that time, new studies have become available, principally, 2-year studies of carcinogenicity in mice and rats.

Menthol exists as two optical isomers: (+)-menthol, (–)-menthol and the racemic mixture ((±)-menthol). The (–)- and (±)-forms are used as flavouring agents.

Menthol is readily absorbed. Up to 100% of an ingested dose appears to be absorbed as measured by the elimination of menthol metabolites in the faeces and urine. Absorbed menthol is known to be eliminated largely as glucuronides; 70–80% of an oral dose is eliminated in the urine and faeces within 48 hours of ingestion. Metabolic studies indicate that oral doses of menthol are metabolized mainly in the liver and excreted via the kidneys and in the bile. Menthol is efficiently metabolized by normal metabolic pathways. The metabolites are simple glucuronic acid conjugates and oxidation products. Mammals can efficiently metabolize menthol by pathways that do not create hazardous metabolites.

In 13-week toxicity studies in which (±)-menthol was given in the diet, the NOEL was 560 mg/kg of body weight per day in mice and 750 mg/kg of body weight per day in rats on the basis of slightly increased incidences of interstitial nephritis at higher doses.

In a 2-year study of toxicity and carcinogenicity in mice, (±)-menthol was administered in the diet at levels equivalent to 300 or 600 mg/kg of body weight per day. The incidences of hepatocellular tumours in males and lung tumours in females at the higher dose level were not significantly different from those in concurrent or historical controls.

The survival rate was decreased in female mice but remained within the range of historical controls. The NOEL was 600mg/kg of body weight per day. In a 2-year toxicity/carcinogenicity study in rats given (±)-menthol in the diet at concentrations equivalent to 190 or 380mg/kg of body weight per day, the NOEL was 380mg/kg of body weight per day.

Neither menthol nor its metabolites were genotoxic in *in vitro* or *in vivo* studies.

While no reproductive toxicity studies were available for (±)-menthol, (–)-menthol was tested at doses of up to 190–430mg/kg of body weight per day for teratogenicity in mice, rats, hamsters and rabbits; no teratogenic effects were observed.

The limited data available on metabolism and toxicity provided no indication of a difference in the toxicity of (–)-menthol and (±)-menthol. Therefore, the Committee concluded that an ADI could be established for the two optical isomers of menthol.

The Committee noted that the highest dose of (±)-menthol used in the long-term studies in mice and rats caused no specific toxic effects. As survival was reduced in mice given a dose of 600mg/kg of body weight per day, the Committee allocated an ADI for menthol of 0–4mg/kg of body weight, based on the NOEL of 380mg/kg of body weight per day in the long-term study in rats and a safety factor of 100. The ADI was rounded to one significant figure, in accordance with usual practice.

A toxicological monograph was prepared. The existing specifications for (+)-menthol and (–)-menthol were revised and replaced by a single set of specifications for menthol, which was added to the list of flavouring agents in the specifications monograph.

3.3 Food colours

3.3.1 Curcumin

Turmeric oleoresin and curcumin (the main colouring component of turmeric oleoresin) were evaluated previously by the Committee at its thirteenth, eighteenth, twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth, thirty-ninth and forty-fourth meetings (Annex 1, references 19, 35, 47, 53, 59, 73, 88, 101 and 116). At the eighteenth meeting, a temporary ADI of 0–0.1mg/kg of body weight was established for curcumin, based on the ADI for turmeric oleoresin (0–2.5mg/kg of body weight per day) 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. At the thirty-ninth meeting, the Committee requested the results of carcinogenicity studies in mice and rats fed turmeric oleoresin (which were known to have been completed) and the results of a study of reproductive toxicity and teratogenicity with curcumin.

At its forty-fourth meeting, the Committee evaluated the results of the carcinogenicity studies on turmeric oleoresin in rats and mice, together with new biochemical and genotoxicity data. The Committee concluded that teratogenicity data on curcumin were no longer required but reiterated the request for a reproductive toxicity study. On the basis of a NOEL of 220mg/kg of body weight per day in the carcinogenicity study 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 the submission of the results of a reproductive toxicity study with curcumin for review in 1998.

At its present meeting, the Committee evaluated the results of fertility studies with turmeric oleoresin given in the diet at levels equal to 0, 120, 650 or 3300mg/kg of body weight per day in female rats; 0, 80, 430 or 2200mg/kg of body weight per day in male rats; 0, 220, 1100 or 5500mg/kg of body weight per day in female mice; and 0, 210, 1100 or 5500mg/kg of body weight per day in male mice. The curcumin content of the turmeric oleoresin was 68–76.5%. In mice low rates of survival of pups (44% or less) were seen in all treated groups, the lowest rate occurring at the highest dose. The highest-dose group also showed a decrease in mean litter size. In rats there were no effects on the survival of parents or their F₁ offspring, but pregnancy rates were decreased in all groups, and were lower in treated than in control groups. Decreased body-weight gain of pups was observed at the intermediate and highest doses on days 0–14 postpartum. Because of the low survival rate of pups in the study in mice, and the low rates of pregnancy observed in rats, the Committee was unable to evaluate with confidence the significance of the reproductive effects observed in the two studies.

The Committee concluded that the studies of fertility in rats and mice provided very limited data on the effects of curcumin on this endpoint and that these studies did not provide assurance that the potential reproductive effects of curcumin have been adequately investigated. The Committee again requested the results of a reproductive toxicity study on a substance complying with the specifications for curcumin.

The temporary ADI of 0–1mg/kg of body weight per day for curcumin was extended until 2001, pending the submission of data from a reproductive toxicity study.

A toxicological monograph was not prepared. The existing specifications were revised and designated as “tentative”, pending the receipt of information on the need and technological justification for alternative solvents for use in the current manufacturing processes of curcumin, which was required for evaluation in 2001.

3.3.2 **Riboflavin from genetically modified *Bacillus subtilis***

Riboflavin derived from a fermentation process using a genetically modified strain of *Bacillus subtilis* has not been previously evaluated by the Committee. Synthetic riboflavin was evaluated previously by the Committee at its thirteenth meeting (Annex 1, reference 19), when an ADI of 0–0.5 mg/kg of body weight was allocated on the basis of a limited database. It was re-evaluated at the twenty-fifth meeting (Annex 1, reference 56), when a group ADI of 0–0.5 mg/kg of body weight was allocated to riboflavin and riboflavin-5'-phosphate, expressed as riboflavin. The data reviewed at that time included information on reproductive toxicity.

The strain of *B. subtilis* used in riboflavin production has been genetically modified to overproduce riboflavin by amplification of the chromosomal region of the riboflavin operon containing suitable promoters and flanked by pUC19 and antibiotic resistance marker genes. The lack of pathogenicity and toxicity of the strain of *B. subtilis* from which the genetic information encoding riboflavin was cloned is extensively and well documented, and the methods for genetic modification have been well described. The production strain of *B. subtilis* was shown to be genetically stable during fermentation. It was convincingly established that the final product is riboflavin of a purity comparable to, or greater than, that produced by conventional methods and is free of DNA from the production organism. Thus, antibiotic resistance marker genes are not present in the final product.

In a 90-day study of toxicity, rats were fed diets containing either 98% pure food-grade riboflavin produced by fermentation from genetically modified *B. subtilis* or 98% pure riboflavin derived by chemical synthesis. The doses tested were 0, 20, 50 or 200 mg/kg of body weight per day. Some animals in the highest-dose groups had reduced weight gain, but the reduction was generally less than 10%, and the efficiency of food conversion was not affected. In the group fed the highest dose of fermentation-derived riboflavin some minor changes in red blood cell parameters were observed, but they were not considered relevant. The NOEL for 98% pure riboflavin produced by fermentation was 200 mg/kg of body weight per day, which was the same as that for chemically synthesized riboflavin.

Fermentation-derived riboflavin and the degradation product 8 α -hydroxyriboflavin did not induce gene mutations in bacteria *in vitro* in either the presence or absence of metabolic activation.

The Committee concluded that the recombinant DNA techniques used to derive the production strain of *B. subtilis* were well characterized, providing assurance that no DNA is present in the end-product. On the basis of molecular biological data and chemical analytical research, it can be concluded that fermentation-derived riboflavin from genetically modified *B. subtilis* is substantially equivalent to synthetic riboflavin.

For 98% pure riboflavin produced by fermentation for use in food, the NOEL in the 90-day toxicity study in rats was 200mg/kg of body weight per day, the highest dose tested. Riboflavin produced by fermentation was evaluated on the basis of its substantial equivalence to synthetic riboflavin. Therefore, the Committee included riboflavin derived from a production strain of genetically modified *B. subtilis* with the previously established group ADI of 0–0.5mg/kg of body weight for synthetic riboflavin and riboflavin-5'-phosphate.

A toxicological monograph and new specifications were prepared.

3.4 **Glazing agents: mineral oils (medium- and low-viscosity)**

Mineral oils¹ were previously considered by the Committee at its thirty-seventh and forty-fourth meetings (Annex 1, references 94 and 116). At the latter meeting, the Committee allocated a temporary group ADI of 0–0.01mg/kg of body weight to class II mineral oils (including the medium-viscosity oils N70(H) and N70(A)) and to class III mineral oils (including the low-viscosity oils P15(H), N15(H) and N10(A)) and a temporary ADI of 0–1mg/kg of body weight to class I mineral oils (including the medium-viscosity oil P70(H)), based on the results of 90-day toxicity studies in Fischer 344 (F344) rats.

At its forty-fourth meeting, the Committee requested more information about the compositional factors in mineral oils that influence their absorption and toxicity for review in 1998. It also requested a study of at least 1 year's duration of one of these materials in F344

¹ Oils may be obtained from crude oil sources of naphthenic (N) or paraffinic (P) origin and by either the conventional acid- (o/eum) treatment process (A) or the hydrogenation or hydrotreatment process (H). Their viscosity ranges from 10 to 100 centistokes (cSt) (10–100 mm²/s). Thus a P100(H) oil refers to a paraffinic oil with a viscosity of 100 cSt produced by the hydrogenation process and an N10(A) oil to a naphthenic oil with a viscosity of 10 cSt produced by the acid-treatment process.

rats, including an assessment of immune function at appropriate time periods (with a reversal period of 1 year), and an investigation of the kinetics of accumulation of the material, and particularly whether a plateau is reached.

Only some of the requested information was available at the present meeting. The new information indicated that Fischer rats are more sensitive to mineral oils with respect to granuloma formation than other strains and species. An adequate long-term bioassay in Fischer rats given mineral oils at concentrations of 25 or 50 g/kg (2.5 or 5%) in the diet (equal to 1000 or 2000 mg/kg of body weight per day) did not show any carcinogenic potential for the materials studied, which were comparable to the medium-viscosity oils P70(H) and N70(H). The Committee understood that research on the pharmacokinetics of white mineral oils and their potential effects on immune function was still in progress, and that the requested long-term feeding study with a 1-year reversal period would not be available before January 2001.

The additional data did not provide a basis for changing the previously established temporary ADIs, which were extended to 2002, pending the review of reports of the requested studies.

A toxicological monograph was not prepared. The existing specifications were revised, with minor changes.

3.5 Preservatives: sulfur dioxide and sulfites

Sulfur dioxide and sulfites were evaluated at the sixth, eighth, ninth and seventeenth meetings of the Committee (Annex 1, references 6, 8, 11 and 32). An ADI of 0–0.7 mg/kg of body weight was allocated at the seventeenth meeting to sulfur dioxide and to sulfur dioxide equivalents arising from sodium and potassium metabisulfite, sodium sulfite and sodium hydrogen sulfite. At subsequent meetings, potassium and calcium hydrogen sulfite and sodium thiosulfate were included in the group ADI (Annex 1, references 41, 47 and 62).

At its thirtieth meeting, the Committee retained the ADI of 0–0.7 mg/kg of body weight allocated to this group of compounds (Annex 1, reference 73). The ADI was based on long-term studies in rats, including a 3-generation study of reproductive toxicity, with a NOEL of 2.5 g/kg (0.25%) sodium metabisulfite in the diet (supplemented with thiamine, as treatment of foods with sulfites reduces their thiamine content), equivalent to 70 mg/kg of body weight per day of sulfur dioxide equivalents. At doses at or above 10 g/kg (1%) in the diet, local irritation of the stomach was observed, with inflammatory changes and hyperplasia, and occult blood was detected in the faeces.

The histopathological changes were limited to the stomach; the incidence of neoplasms was not increased at any site or at any dose. A safety factor of 100 was used. Similar local changes in the stomach were observed in pigs fed thiamine-supplemented diets to which sodium metabisulfite was added. The Committee also reviewed case studies and challenge tests for idiosyncratic sensitivity to sulfiting agents, and noted the life-threatening nature of the adverse effects in some cases. It recommended that, where a suitable alternative method of preservation exists, its use should be encouraged, particularly in those applications in which the use of sulfites may lead to high levels of acute intake. The Committee also reiterated the view expressed at its twenty-seventh meeting (Annex 1, reference 62, section 2.4) that appropriate labelling was the only feasible means of protecting individuals who cannot tolerate certain food additives. At its thirtieth meeting, the Committee recommended that the situation with regard to the frequency of idiosyncratic adverse reactions and the relative toxic effects of free and bound sulfur dioxide should be kept under review. It also requested information on the chemical forms of sulfur dioxide in food.

At its present meeting, the Committee identified two issues pertaining to the toxicological evaluation of sulfites: general toxicity and idiosyncratic intolerance. Idiosyncratic intolerance did not appear to be related to deficiency of sulfite oxidase in humans. Since idiosyncratic intolerance and general toxicity appeared to be unrelated they were considered separately.

General toxicity

Studies performed since the thirtieth meeting of the Committee have demonstrated the quantitative conversion of sulfite ions to sulfate ions. The release of reduced glutathione by both isolated hepatocytes and perfused liver is probably due to sulfitolysis of oxidized glutathione by sulfite ions. The other product of sulfitolysis, glutathione *S*-sulfonate, was found to be a strong competitive inhibitor of both microsomal and cytosolic glutathione *S*-transferase in rat lung and liver cells and tumour cells from human lungs. These results suggested that sulfite ions could interfere with the glutathione pathway through their reaction with oxidized glutathione. In a study in rats, administration of 25 g/kg (2.5%) metabisulfite in the diet for 5 weeks increased the level of three disaccharidases involved in carbohydrate metabolism and of alkaline phosphatase in the brush-border membrane of cells of the small intestine.

The toxicity of bound and free forms of sulfite (acetaldehyde hydroxysulfonate and sodium metabisulfite, respectively) administered

in drinking-water was assessed in normal rats and rats made deficient in sulfite oxidase by administration of tungstate ions in drinking-water for 8 weeks. The rats deficient in sulfite oxidase were considered to be a better model for humans than the conventional rat model, which has an estimated 10–20 times higher concentration of this enzyme in the liver. Acetaldehyde hydroxysulfonate is a major bound form of sulfite in wines and other fermented foods and beverages and is considered to be a very stable form of bound sulfite. The doses of bound and free sulfite chosen were calculated to deliver the same dose in terms of sulfur dioxide equivalents (7, 70 or 350 mg/kg of body weight per day, the highest dose being reduced to 175 mg/kg of body weight per day after 3 weeks). The effects on body weight, food consumption and water intake were most marked in rats that received free rather than bound sulfite and in enzyme-deficient rats as compared with normal rats. The concentrations of sulfite and thiosulfate in urine and of S-sulfonates in plasma were elevated in untreated rats deficient in sulfite oxidase. Administration of bound sulfite to these rats resulted in increased excretion of sulfite in the urine, while administration of free sulfite was associated with increases in the concentrations of urinary thiosulfate and plasma S-sulfonates. The measured concentrations were variable, and were not clearly related to the dose. Histopathological lesions of the forestomach and glandular stomach were detected in all groups of rats treated at the highest dose. The severity and frequency of the gastric lesions were greater in the rats deficient in sulfite oxidase. Hepatic lesions were observed in animals receiving acetaldehyde hydroxysulfonate, and the NOEL was lower in the rats deficient in sulfite oxidase than in normal rats (7 mg and 70 mg of sulfur dioxide equivalents/kg of body weight per day, respectively). The effect may be due to the acetaldehyde that was formed after dissociation. The results showed that the gastric toxicity of bound sulfites, in the form of acetaldehyde hydroxysulfonate, was equivalent to that of free sulfite. Although the enzyme-deficient rats were more sensitive at doses above the NOEL, the NOEL was the same as that in normal rats.

Three assays of *in vitro* genotoxicity were reviewed; the results confirmed the previously noted observation that sulfites are genotoxic *in vitro* but not *in vivo*. This conclusion is consistent with the high reactivity of sulfite and its rapid inactivation in mammals. Two studies of teratogenicity in which sulfite was fed in the diet were available: neither was conducted in a completely satisfactory manner, as treatment did not cover the entire period of organogenesis. Maternal toxicity was demonstrated in both studies at doses equivalent to 840 mg of sulfur dioxide equivalents/kg of body weight per day and

above. No teratogenic effects were noted at intakes below this concentration.

The results of a study of the effects of ingested sulfite on the rat kidney demonstrated that administration of sodium metabisulfite by gavage at a dose of 5 mg/kg of body weight per day (3.4 mg of sulfur dioxide equivalents/kg of body weight per day) for up to 2 weeks reduced alkaline phosphatase activity in kidney tissue and concomitantly increased the concentrations of the enzyme in serum and urine; these effects occurred after the first dose. The activity of lactate dehydrogenase was likewise decreased in kidney tissue and increased in urine but not in serum. Urinary excretion of protein was increased 10-fold by the end of 2 weeks. These effects appear not to be related to treatment, as sulfite is quickly and quantitatively metabolized to sulfate and no sulfite was detected in urine nor were lesions of the kidney detected in normal rats after 8 weeks of treatment with a 20–50 times higher dose of sulfite in drinking-water.

Thus, the previous NOEL in studies of animals that ingested sulfite was confirmed in an 8-week study in rats in which the NOEL for gastric lesions was 70 mg of sulfur dioxide equivalents/kg of body weight per day in both normal rats and those deficient in sulfite oxidase, whether they were fed free sulfite or acetaldehyde hydroxy-sulfonate, which is a stable and prevalent form of bound sulfite. These gastric effects of sulfite, which were reported in rats and pigs, arise from local irritant effects. These effects would therefore be dependent on concentration rather than dose and a numerical ADI might not be appropriate. The Committee considered that the role of acetaldehyde in the reported effects of acetaldehyde hydroxy-sulfonate on the liver needed to be resolved before a re-evaluation of the safety of sulfur dioxide and sulfites could be completed. In view of these reservations, the previously established ADI of 0–0.7 mg/kg of body weight was maintained.

Idiosyncratic reactions in humans

A number of case studies in humans were available in which suspected hypersensitivity to sulfites based on adverse reactions to foods was confirmed by administration of single- or double-blind oral challenges with sulfites in solution or capsule form. While many of the cases involved individuals with chronic asthma whose response was primarily respiratory, a number of reports described adverse allergic-type responses in people without asthma, which did not involve respiratory symptoms. Some of these individuals had chronic allergic conditions. These responses were consistent with self-reported reactions documented by the Adverse Reaction Monitoring System

of the United States Food and Drug Administration. Evidence for an allergic basis for the adverse reactions to free and bound sulfites was provided in a number of the case reports. In studies in which only skin-prick testing had been attempted, allergy could not be ruled out, as further testing had not been done. Challenges with various food commodities containing sulfites also resulted in positive responses. In a double-blind study, lettuce treated with sulfites elicited the most consistent responses; sulfites in sulfite-treated lettuce are considered to be present mostly as free sulfite. While the mechanism by which sulfite ingestion precipitates idiosyncratic adverse reactions has not been established, there was some evidence of a mechanism involving the action of nitric oxide on parasympathetic receptors.

The prevalence of sulfite sensitivity was determined in groups of asthmatic adults and children who were either dependent or not dependent on steroids. In adults, the prevalence among those dependent on steroids was 4–8% and appeared to be less than 1% in those who were not dependent on steroids. The prevalence in the total population of asthmatic adults has been estimated to be 4%. The prevalence in asthmatic children was higher, approximately 20–30%, following double-blind challenges to children who were either dependent or not dependent on steroids.

The Committee reiterated the recommendation made at its thirtieth meeting (Annex 1, reference 73) that, where a suitable alternative method of preservation exists, its use should be encouraged, particularly in those applications (e.g. control of enzymic browning in fresh salad vegetables) in which the use of sulfites may lead to high levels of acute intake and which have most commonly been associated with life-threatening adverse reactions. The Committee considered that appropriate labelling would help to alert individuals who cannot tolerate sulfites.

The intake of sulfites is assessed in section 5.4.

An addendum to the toxicological monograph was prepared. The existing specifications for potassium metabisulfite, potassium sulfite, sodium hydrogen sulfite, sodium metabisulfite, sodium sulfite and sodium thiosulfate were revised and designated as “tentative”, pending the receipt of information regarding the test for selenium. The existing specifications include a limit for selenium for which the test method is no longer feasible because of a lack of availability of the required reagents. The Committee also questioned the need for a limit for selenium because new processes by which sulfur is produced as a by-product of the oil industry have replaced extraction methods from sulfur mines. The Committee noted that the limit for

selenium may no longer be necessary and requested information in this regard.

The existing specifications for calcium hydrogen sulfite were maintained and designated as “tentative”. Information was requested regarding a modified name for calcium hydrogen sulfite that would be more reflective of its physical properties, the extent and functionality of its use as a food additive, and the need for limits for selenium and arsenic and associated analytical methods.

The existing specifications for sulfur dioxide were revised.

No information was made available to the Committee regarding calcium metabisulfite, calcium sulfite and potassium hydrogen sulfite. No specifications were prepared for these substances.

3.6 Sweetening agent: stevioside

Stevioside is a sweet glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-18-oic acid). It is a natural constituent of the plant *Stevia rebaudiana* Bertoni, belonging to the Compositae family. Stevioside has not been previously evaluated by the Committee.

After oral administration to rats, stevioside is not readily absorbed from the upper small intestine but is hydrolysed to the aglycone, steviol, before absorption from the gut. Steviol is completely absorbed and is excreted in the bile as conjugates; only a very small fraction is detectable in urine. After biliary excretion, the conjugates are hydrolysed, and steviol undergoes enterohepatic circulation; its elimination half-life is 24 hours. Steviol is the only faecal metabolite of stevioside that has been identified, and excretion in the faeces is the major route. After intravenous administration, stevioside is rapidly distributed throughout the body, partially secreted by the renal tubular epithelium, and excreted in the urine.

At high concentrations, stevioside affected a variety of biochemical parameters in rat tissues *in vitro*. It weakly inhibited oxidative phosphorylation; steviol was about 30 times more potent in this respect. The most likely mechanism for this effect is inhibition of the mitochondrial translocation of adenine nucleotides. Steviol also inhibited glucose absorption from rat gut by reducing the ATP content of the intestinal mucosa. Stevioside may also act as a calcium antagonist, showing a hypotensive effect and inducing diuresis, natriuresis and a fall in reabsorption of glucose in the renal tubules. Stevioside may not, however, be able to penetrate cell membranes. Although most of these studies were performed after intravenous

injection of stevioside, oral administration of extracts of *S. rebaudiana* to rats caused similar effects (hypotension and diuresis).

Stevioside has very low acute oral toxicity. Oral administration of stevioside at a concentration of 25 g/kg (2.5%) in the diet to rats for 2 years, equal to 970 and 1100 mg/kg of body weight per day in males and females, respectively, had no significant effect. Reduced body-weight gain and survival rate were observed at a level of 50 g/kg (5%) in the diet. There was no indication of carcinogenic potential in the long-term study and no evidence of potential to promote tumours of the urinary bladder in a separate bioassay.

In reproductive toxicity studies, administration of stevioside at doses of up to 2500 mg/kg of body weight per day to hamsters and 3000 mg/kg of body weight per day to rats had no effect. Although an aqueous infusion of *S. rebaudiana* administered orally to female rats was reported to cause a severe, long-lasting reduction in fertility, the contraceptive effect was probably not due to stevioside. Neither teratogenic nor embryotoxic effects were observed in rats given stevioside at doses of up to 1000 mg/kg of body weight per day by gavage.

The results of genotoxicity tests with stevioside in various *in vitro* systems were uniformly negative.

The aglycone, steviol, exhibited greater acute toxicity than stevioside in hamsters but not in rats. Steviol was clearly genotoxic after metabolic activation, inducing forward mutations in bacteria and gene mutations and chromosomal aberrations in lung fibroblasts of Chinese hamsters. Several mechanistic studies indicated that the structural features necessary for the expression of mutagenic activity include a hydroxyl group at position C13 and an unsaturated bond joining the C16 and C17 carbon atoms of steviol. The fact that stevioside is glycosylated at position C13 could explain the absence of mutagenicity. The active metabolite of steviol responsible for its mutagenic activity is not known. While some data suggest that epoxidation may be involved in the metabolic activation of steviol, other data indicate that the active metabolite is not an epoxide. Preliminary data indicate that steviol may be activated to a mutagenic metabolite by human liver microsomes.

The Committee noted a number of shortcomings in the information available on stevioside. In several studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of metabolism of stevioside and steviol in humans were available. In addition, data on long-term toxicity and

carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.

In view of the absence of information for the elaboration of specifications for stevioside and since the evaluation of the available toxicological data revealed several limitations, the Committee was unable to relate the results of the toxicological investigations to the commercial product and could not allocate an ADI to stevioside.

Before reviewing stevioside again, the Committee considered that it would be necessary to develop specifications to ensure that the material tested was representative of the commercial product. Further information on the nature of the substance that was tested, data on the metabolism of stevioside in humans and the results of suitable *in vivo* genotoxicity studies with steviol would also be necessary.

A toxicological monograph was prepared. No specifications were prepared as no information was forthcoming.

3.7 Thickening agents

3.7.1 Carrageenan

Carrageenan is a sulfated polygalactan with an average relative molecular mass well above 100000 derived from a number of seaweeds of the class Rhodophyceae. It has no nutritive 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 generalized differences in the composition and degree of sulfation at specific locations in the polymer.

Carrageenan was previously reviewed by the Committee at its thirteenth, seventeenth and twenty-eighth meetings (Annex 1, references 19, 32 and 66). At the twenty-eighth meeting, an ADI "not specified" was allocated on the basis of the results of a number of toxicological studies on carrageenans obtained from various species of seaweed. The studies included a 3-generation study of reproductive toxicity, short-term and long-term toxicity studies in rats at levels of up to 50 g/kg (5%) in the diet, and short- and long-term toxicity studies in hamsters, guinea-pigs and monkeys. In general, the only effect observed was soft stools or diarrhoea at high doses, except in two studies in which material, identified as being ι -carrageenan, administered at 10 g/l (1%) in the drinking-water or 50 g/kg (5%) in

the diet, produced ulceration in the gastrointestinal tract of guinea-pigs. Degraded carrageenans can produce this effect; they are not, however, used as food additives. At its twenty-eighth meeting, the Committee pointed out that degraded carrageenans and “semi-refined carrageenan” (or “processed *Eucheuma* seaweed”) were not included in the specifications for the food-grade material. At its forty-fourth meeting, in reviewing the data on processed *Eucheuma* seaweed obtained from *E. cottonii*, the Committee concluded that a complete review of data on carrageenan should be undertaken in 1998, particular attention being paid to the identity of the source materials and to the specifications of the products that have been tested (Annex 1, reference 116).

At its present meeting, the Committee considered studies published since the review at the twenty-eighth meeting and, for earlier studies, indicated the identity of the source material and the type of carrageenan, when these could be identified.

Most of the toxicological studies of the carrageenans in which an identifiable type of carrageenan and an identifiable seaweed species were used were undertaken with κ - or κ/λ -carrageenan from *Chondrus crispus*. There have been a few parallel studies, the results of which do not suggest that there are large differences between the effects of various forms of carrageenan or between the effects of carrageenans prepared from different species of seaweed.

The carrageenans are dietary fibres, generally of a high relative molecular mass, which are not broken down to very small molecules in the gastrointestinal tract. At high levels of intake, these properties can cause adverse effects through their physical action on the gastrointestinal tract. As mentioned above, ulceration has been observed in the gastrointestinal tract of guinea-pigs given high levels of ι -carrageenan. These effects were not observed in a recent well conducted 90-day study in which rats were fed diets containing 50 g/kg (5.0%) of conventionally processed ι -carrageenan from *E. spinosum* or κ -carrageenan from *E. cottonii*. The changes that occurred during the study, most notably an increase in the relative weight of the full and empty caecum, were considered to be the consequence of the accumulation of poorly absorbed material in the caecum and to be without toxicological significance. The partial reversal of the caecal weight changes during the 28-day recovery phase of the study and the absence of histopathological changes support this conclusion.

Studies of the carcinogenicity of carrageenan in rats have failed to demonstrate any effect. In addition, assays of the genotoxicity of

carrageenan have been negative. A proliferative response of the mucosa of the gastrointestinal tract of rats fed two forms of carrageenan at 26 g/kg (2.6%) or 50 g/kg (5.0%) in the diet has been reported; the response was reversible in the study in which carrageenan was given at 50 g/kg in the diet. This response might explain the promotion of the action of known experimental colon carcinogens administered to rats given carrageenans at 25 g/kg (2.5%) or 60 g/kg (6%) in the diet. The Committee was aware of an abstract of a conference report which indicated that tumour promotion does not occur in rats in which the intestinal microflora are derived from human donors who have been “adapted” to the presence of carrageenan in the gastrointestinal tract. This would suggest that promotion of colon carcinogenesis in the rat is dependent on the presence of the normal microflora of the gastrointestinal tract.

Early reports of the detection of carrageenan in parenteral tissues after dietary intake are probably unreliable. The presence of carrageenans in the macrophages found in the walls of the caecum and colon may reflect the relative molecular mass distribution of the preparation used in the study. Maintenance of a restriction on the relative molecular mass distribution in the specifications for carrageenan for food use provides protection against the adverse effects of carrageenans of low relative molecular mass.

Some data suggest that carrageenan affects the immune response of the gastrointestinal tract; however, no validated tests for assessing the nature and potential consequences of such an effect were available. A short communication relating to an epidemiological study did not indicate that carrageenan was immunotoxic in neonates receiving milk preparations containing carrageenan. The Committee reiterated its previous statement that the ADI should not be considered applicable to neonates and young infants below the age of 12 weeks.

The Committee extended the previous ADI “not specified” for carrageenan to include processed *Eucheuma* seaweed in a group ADI “not specified”. 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. The Committee requires this information for evaluation in 2001.

An addendum to the toxicological monograph was prepared. The existing specifications were revised in order to improve both the description and the definition of the materials covered. The test methods were modified by introduction of the methods initially

prepared for processed *Eucheuma* seaweed. Microbiological criteria were also added.

3.7.2 **Processed *Eucheuma* seaweed**

Processed *Eucheuma* seaweed was previously considered by the Committee at its thirtieth, thirty-ninth, forty-first and forty-fourth meetings (Annex 1, references 73, 101, 107 and 116). At its thirtieth and thirty-ninth meetings, the Committee was unable to evaluate the use of processed *Eucheuma* seaweed in food because no relevant toxicological data were available. At its forty-first meeting, the Committee considered a 90-day feeding study in rats, for which complete details were not available, and a series of genotoxicity studies on processed *Eucheuma* seaweed from *E. cottonii*. The Committee allocated a temporary ADI of 0–20 mg/kg of body weight to processed *Eucheuma* seaweed from *E. cottonii*, pending submission of the complete details from the 90-day study, including histopathological data for individual animals. At its forty-fourth meeting, the Committee reviewed these data and the results of new genotoxicity and cytotoxicity assays. Because of the chemical relationship between processed *Eucheuma* seaweed and traditionally refined carrageenan, the Committee considered that toxicological data on the latter 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. Although the data for individual animals in the 90-day feeding study in rats confirmed the accuracy of the summary data and the conclusions derived from them, the Committee expressed reservations about the design, conduct and documentation of the study. Additionally, none of the available genotoxicity studies was considered to be adequate because of deficiencies in conduct or reporting. The Committee therefore extended the temporary ADI, pending submission of the results of a new 90-day feeding study in rats and an appropriate battery of genotoxicity studies, all meeting present-day standards, for processed *Eucheuma* seaweed derived from *E. cottonii*.

At the forty-fourth meeting, a request was made to amend the specifications to include *E. spinosum* as a source material for processed *Eucheuma* seaweed. In recognition of the fact that ulcerative lesions in the caecum had been noted in toxicity studies in guinea-pigs given ι-carrageenan, the major component of *E. spinosum*, the Committee concluded that a separate 90-day feeding study in rodents and a separate battery of genotoxicity studies were required for processed *Eucheuma* seaweed derived from *E. spinosum*,

if the specifications were to be expanded to include processed *Eucheuma* seaweed derived from this species. The Committee also concluded that a complete review of all data on carrageenan should be undertaken in 1998, particular attention being paid to the identity of the source materials and to the specifications of the products that have been tested.

At its present meeting, the Committee reviewed the results of a new 90-day toxicity study in rats fed processed *Eucheuma* seaweed from two sources, *E. cottonii* and *E. spinosum*, at concentrations of 0 g/kg, 5 g/kg (0.5%), 15 g/kg (1.5%) or 50 g/kg (5%) in the diet, or conventionally processed carrageenan from these two sources at 50 g/kg in the diet. At the highest concentrations, the intake of the processed *Eucheuma* seaweeds was equal to 4300 and 5000 mg/kg of body weight per day, respectively, for male and female rats fed the material derived from *E. cottonii* and to 4500 and 5100 mg/kg of body weight per day, respectively, for male and female rats fed the material from *E. spinosum*. No adverse effects were noted in the study. The changes observed in rats receiving the highest concentrations of processed *Eucheuma* seaweed from these two sources, most notably an increase in the relative weight of the full and empty caecum, were considered to be the consequence of the accumulation of poorly absorbed material in the caecum and to be without toxicological significance. There was no indication that the effects of the processed *Eucheuma* seaweeds differ from those of conventionally prepared carrageenans from the same seaweed species.

Processed *Eucheuma* seaweed derived from either *E. cottonii* or *E. spinosum* was not mutagenic in well conducted assays for reverse mutation in *Salmonella typhimurium* strains. The Committee considered that no further studies of genotoxicity were required.

In view of the lack of toxicity of processed *Eucheuma* seaweeds derived from either *E. cottonii* or *E. spinosum*, the Committee determined that both these species could be included in the specifications for processed *Eucheuma* seaweeds. Additionally, because of the similarities in the nature of the processed *Eucheuma* seaweeds and the conventionally processed carrageenans and in the effects that they caused in the recent 90-day toxicity study in rats, the Committee included carrageenans and processed *Eucheuma* seaweed in a temporary group ADI "not specified", to be reviewed in 2001 (see section 3.7.1).

An addendum to the toxicological monograph was prepared. The existing specifications were revised and *E. spinosum* was included as a source material.

3.7.3 **Sodium carboxymethyl cellulose, enzymatically hydrolysed**

Sodium carboxymethyl cellulose, enzymatically hydrolysed, is a water-soluble dietary fibre with a relative molecular mass in the range of about 800–10000. It acts mainly as a stabilizer with fat-extending properties. It can be used in formulating low-fat and reduced-fat foods and soft drinks.

Sodium carboxymethyl cellulose, enzymatically hydrolysed, is prepared from regular, food-grade sodium carboxymethyl cellulose by partial enzymatic hydrolysis under mildly acidic conditions using a food-grade cellulase enzyme from *Trichoderma longibrachiatum*.

Sodium carboxymethyl cellulose was allocated an ADI “not specified” at the thirty-fifth meeting of the Committee (Annex 1, reference 88). The cellulase enzyme was allocated an ADI “not specified” at the thirty-ninth meeting (Annex 1, reference 101). Sodium carboxymethyl cellulose, enzymatically hydrolysed, has not been previously evaluated by the Committee.

At its present meeting, the Committee reviewed two studies in which the properties of carboxymethyl cellulose, enzymatically hydrolysed, were compared with those of the parent material, carboxymethyl cellulose. The first was a 90-day study of toxicity in which male and female rats received diets containing 0g/kg, 25g/kg (2.5%), 50g/kg (5.0%) or 100g/kg (10%) of either sodium carboxymethyl cellulose, enzymatically hydrolysed, or sodium carboxymethyl cellulose. The second was a comparative study of the absorption, distribution and excretion of these two materials in rats.

In the 90-day study in rats receiving either sodium carboxymethyl cellulose, enzymatically hydrolysed, or carboxymethyl cellulose histopathological changes in the kidney and urinary bladder were reported. These were associated with changes in kidney weight, increased urine volume and increased excretion of some ionic substances in the urine. Caecal enlargement was also reported. For comparison, the Committee considered the toxicity studies on sodium carboxymethyl cellulose that had been reviewed at its thirty-fifth meeting (Annex 1, reference 88). It noted that, in early studies that appeared to be comparable with regard to species, dietary concentration of sodium carboxymethyl cellulose and duration of exposure, no changes of organ weight or histopathological appearance were found that were comparable to those reported in the 90-day study reviewed at the present meeting.

Since the morphological changes in the kidney occurred only in males receiving the highest dose of sodium carboxymethyl cellulose, enzymatically hydrolysed, and the lowest dose of carboxymethyl

cellulose, the Committee was not convinced that the response was of toxicological concern. The observed epithelial hyperplasia of the urinary bladder was generally graded as slight or very slight and occurred in male rats given either sodium carboxymethyl cellulose, enzymatically hydrolysed, or carboxymethyl cellulose at 100 g/kg (10%) in the diet. The Committee considered that this represented a response to a high intake of sodium ions by rats of the strain used in the new study. Administration in the diet of additional sodium (approximately four times that available in the diet of the control group) could have led to the observed histopathological changes and changes in urinary excretion. Also, the caecal enlargement, diarrhoea and other secondary changes were considered to be the consequence of the accumulation of poorly absorbed, water-soluble material in the caecum and colon and to be without toxicological significance. The NOELs for sodium carboxymethyl cellulose, enzymatically hydrolysed, were equal to 6000 and 6600 mg/kg of body weight per day in male and female rats, respectively.

The metabolic study showed that passage of carboxymethyl cellulose through the gastrointestinal tract results in partial breakdown of the bonds between monomeric units to result in a material with a relative molecular mass equivalent to that of carboxymethyl cellulose, enzymatically hydrolysed. The amounts of radioactivity excreted in the faeces, urine and expired air after administration of radiolabelled carboxymethyl cellulose and sodium carboxymethyl cellulose, enzymatically hydrolysed, were quantitatively almost identical, providing additional evidence that in rats, the end-products of digestion of the two products are similar.

The Committee concluded that these similarities are consistent with the absence of any toxicologically significant difference between carboxymethyl cellulose and carboxymethyl cellulose, enzymatically hydrolysed. Therefore, the Committee included carboxymethyl cellulose, enzymatically hydrolysed, in the group ADI “not specified” with ethyl cellulose, ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, methyl ethyl cellulose and sodium carboxymethyl cellulose.

A toxicological monograph was prepared. The existing tentative specifications were revised and the “tentative” designation was deleted.

3.8 Miscellaneous substances

3.8.1 γ -Cyclodextrin

γ -Cyclodextrin is a ring-shaped molecule made up of eight glucose units linked by α -1,4 bonds. It is produced by the action of the enzyme

cyclomaltodextrin glucanotransferase (cyclodextrin-glycosyl transferase; EC 2.4.1.19) on hydrolysed starch syrups. The circular structure of γ -cyclodextrin provides a hydrophobic cavity that allows complexes to be formed with a variety of organic molecules, while the hydrophilic outer surface makes γ -cyclodextrin water-soluble. Because of these properties, γ -cyclodextrin can be used in a variety of ways in food with potential intakes of the order of grams per person per day.

γ -Cyclodextrin has not been previously evaluated by the Committee; however, the structurally related β -cyclodextrin (seven glucose units) was evaluated at the forty-first and forty-fourth meetings of the Committee (Annex 1, references 107 and 116). The Committee noted that the close structural similarity between γ -cyclodextrin and β -cyclodextrin allows some comparisons to be made of the metabolism and the toxicity of these two compounds.

The metabolism of γ -cyclodextrin is different from that of β -cyclodextrin, as demonstrated in both *in vitro* and *in vivo* studies. In *in vivo* studies in rats, γ -cyclodextrin (at single doses of up to 1000 mg/kg of body weight) was rapidly metabolized to glucose, presumably by the luminal and/or epithelial enzymes of the gastrointestinal tract. In contrast to the metabolism of β -cyclodextrin, there was little involvement of the gastrointestinal microflora. Similarly, in contrast to β -cyclodextrin, much less γ -cyclodextrin was absorbed and only very low levels could be detected in the urine. After intravenous injection, γ -cyclodextrin (at single doses of up to 600 mg/kg of body weight) was rapidly cleared from the blood and was excreted, largely unchanged, in the urine. Although no studies of metabolism in humans *in vivo* were available, γ -cyclodextrin, unlike β -cyclodextrin, can be readily hydrolysed by human salivary and pancreatic amylases *in vitro*.

Short-term (28- and 90-day) toxicity studies indicate that γ -cyclodextrin has little toxicity when given intravenously or orally to rats or orally to dogs. After administration of a very high concentration (200 g/kg or 20%) in the diet, caecal enlargement and associated changes were seen in both species. This effect is likely to result from the presence of a high concentration of an osmotically active substance in the large intestine. This result suggests that the metabolism of γ -cyclodextrin is less efficient at doses higher than those used in the studies of metabolism. The results of the studies in rats treated intravenously indicate that γ -cyclodextrin is well tolerated when given systemically, and may be less toxic than β -cyclodextrin.

Studies conducted in rats and rabbits with γ -cyclodextrin at doses of up to 200 g/kg (20%) in the diet did not indicate any teratogenic effects. Similarly, the results of a battery of genotoxicity studies were

negative. No long-term studies of toxicity, carcinogenicity or reproductive toxicity have been conducted, but, given the rapid metabolism of γ -cyclodextrin to glucose and its lack of genotoxicity, the Committee concluded that such studies were not required for an evaluation of this substance.

In *in vitro* studies, γ -cyclodextrin, like β -cyclodextrin, sequestered components of the membranes of erythrocytes, causing haemolysis. The threshold concentration for this effect was, however, higher than that of β -cyclodextrin. Furthermore, γ -cyclodextrin was not detected in blood after dietary administration of high doses to animals.

It was considered unlikely that interaction of γ -cyclodextrin with lipophilic vitamins would impair their bioavailability because of the rapid metabolism of γ -cyclodextrin *in vivo*. Also, there was no evidence that vitamin deficiency was induced in experimental animals given high doses of γ -cyclodextrin.

The Committee considered studies on the short-term toxicity and genotoxicity of the enzyme, cyclomaltodextrin glucanotransferase, used in the production of cyclodextrins, and of the complexant, γ -cyclohexadecen-1-one, used to optimize formation of γ -cyclodextrin. The toxicological data indicated that these substances are unlikely to be of toxicological concern in the final preparation of γ -cyclodextrin complying with the specifications. The Committee also reviewed information on the genetic modification of the organism used to produce the enzyme, which did not raise any concerns.

Although no study of human tolerance to γ -cyclodextrin was submitted before the meeting, the Committee was aware that such a study was available. Although it was unable to review the data, the Committee noted that the toxicity of this compound is relatively low in animals and that it is less toxic than β -cyclodextrin, for which studies of human tolerance were available. Also, its rapid metabolism to maltose, maltotriose and glucose *in vitro* by human salivary and pancreatic amylases supports the conclusion that, as in laboratory animals, it would be metabolized to innocuous metabolites before absorption.

The Committee concluded that there were sufficient data to allocate a temporary ADI "not specified", but that the study of human tolerance known to have been conducted should be reviewed in order to confirm the absence of adverse effects on the gastrointestinal tract at normal levels of intake. These data should be submitted for consideration by the Committee by 1999.

A toxicological monograph and new specifications for γ -cyclodextrin were prepared.

3.8.2 ***Glucono- δ -lactone, and the calcium, magnesium, potassium and sodium salts of gluconic acid***

Glucono- δ -lactone was previously evaluated by the Committee at its tenth, eighteenth and thirtieth meetings (Annex 1, references 13, 35 and 73). At its eighteenth meeting, the Committee established an ADI of 0–50mg/kg of body weight for glucono- δ -lactone. At the thirtieth meeting, the Committee changed the ADI for glucono- δ -lactone to an ADI “not specified” on the basis of biochemical and metabolic data on glucono- δ -lactone and gluconic acid, noting that in an aqueous medium glucono- δ -lactone exists in equilibrium with D-gluconic acid. These compounds are intermediates in the oxidation of glucose through the pentose phosphate cycle. Data from studies that were evaluated previously by the Committee showed no evidence of carcinogenicity, teratogenicity or genotoxicity caused by glucono- δ -lactone.

Since the last toxicological evaluation of glucono- δ -lactone, a new acute toxicity study and two new 28-day oral toxicity studies on sodium gluconate in rats have become available. These studies were evaluated in order to determine whether the current ADI “not specified” for glucono- δ -lactone could be extended to a group ADI “not specified” for glucono- δ -lactone and the calcium, magnesium, potassium and sodium salts of gluconic acid.

Calcium, magnesium, potassium and sodium gluconate were previously evaluated by the Committee as individual compounds or in other group categories as inorganic salts and salts of organic acids (Annex 1, references 32, 50 and 70). The Committee concluded that they were freely ionizable and that it was appropriate to allocate ADIs on the basis of data on their corresponding anion (gluconic acid).

The results of the new acute toxicity study showed no evidence of toxicity in rats that were given single doses of 500, 1000 or 2000mg of sodium gluconate/kg of body weight by gavage.

In the two new 28-day studies in rats, sodium gluconate was administered orally either by gavage at doses of 0, 500, 1000 or 2000mg/kg of body weight per day or in the feed at doses of 0g/kg, 12.5g/kg (1.25%), 25g/kg (2.5%) or 50g/kg (5.0%) (equal to 0, 1000, 2000 and 4100mg/kg of body weight per day, respectively). A control group received sodium chloride at 13.5g/kg (1.35%) in the feed (equal to 1100mg/kg of body weight per day), which was equivalent to the concentration of sodium in the sodium gluconate given to the highest-dose group.

In the gavage study, there was a significant increase in the relative weight of the kidneys (unilateral) in the males that received 1000 and 2000 mg of sodium gluconate/kg of body weight per day. No treatment-related or dose-related effects were observed on any of the other parameters examined in this study.

The effects observed in the feeding study, i.e. increased water intake, prothrombin time and relative kidney weights, were not dose-related.

Qualitative analysis of urine revealed effects in both 28-day studies that were considered by the Committee to be related to the high sodium intake arising from the sodium gluconate.

On the basis of a re-evaluation of the data previously considered by the Committee and the new short-term toxicity data on sodium gluconate, the Committee extended the previous ADI “not specified” for glucono- δ -lactone to a group ADI for glucono- δ -lactone and the calcium, magnesium, potassium and sodium salts of gluconic acid.

An addendum to the toxicological monograph was prepared. The existing specifications for glucono- δ -lactone, calcium gluconate, potassium gluconate and sodium gluconate were revised. The specifications for magnesium gluconate were revised and designated as “tentative”, with a request for information on the need for maintaining the microbiological criteria included in the specifications, and for introducing maximum limits for sulfate and chloride.

3.8.3 ***Polyglycitol syrup***

Polyglycitol syrup has not previously been evaluated by the Committee. Since its components are the same as those of maltitol syrup, differing only in the relative proportions of sorbitol, maltitol and higher-order polyols, the evaluation conducted for maltitol syrup at the forty-ninth meeting (Annex 1, reference 131) in order to accommodate a wide range of starch hydrogenation products would also apply to polyglycitol syrup.

At the forty-ninth meeting, toxicological data in support of new specifications for maltitol syrup were reviewed. Since an ADI “not specified” had been established for both sorbitol and maltitol, the toxicological review concentrated only on the consequences of the higher-order hydrogenated saccharides. The Committee concluded at its forty-sixth meeting (Annex 1, reference 122, section 2.2.4) that the occurrence of proliferative lesions of the adrenal medulla in rats fed polyols (including sorbitol and maltitol) and lactose was a species-specific response and was not relevant to the toxicological evaluation of these substances for humans. Several short-term studies in which

rats and dogs were given materials with a higher-order polyol content exceeding 80% of the syrup were reviewed at the forty-ninth meeting, as were data on the metabolic fate of higher-order polyols.

At its present meeting, the Committee reviewed new data relating to digestibility *in vitro*, a short-term toxicity study in rats given material with a higher-order polyol content of 78%, and a study on the effects of hydrogenated starch hydrolysates on the glycaemic response in diabetic and non-diabetic individuals.

Hydrolysis of a polyglycitol syrup composed of 14% sorbitol, 8% maltitol and 78% higher-order polyols resulted in the production of more glucose and less sorbitol than did hydrolysis of products conforming to the specifications for maltitol syrup. This is consistent with the relative proportion of glucose released on hydrolysis of each component. The greater digestibility of the hydrogenated starch hydrolysates with the highest content of highly polymerized saccharides was considered to be the consequence of a greater activity of digestive enzymes or a higher affinity for glucose–glucose bonds than for glucose–sorbitol bonds. Data on the disposition of maltitol syrup reviewed at the forty-ninth meeting indicated that the higher-order polyols would be completely hydrolysed to glucose and maltitol, with a considerable portion undergoing fermentation in the lower gut. Bacterial fermentation was demonstrated by detection of an increased amount of hydrogen in the air expired by human subjects in studies reviewed at the present meeting.

Inclusion of a polyglycitol syrup (composed of 14% sorbitol, 8% maltitol and 78% higher-order polyols) and two maltitol syrups (one containing 7% sorbitol, 60% maltitol and 33% higher-order polyols and the other 7% sorbitol, 52% maltitol and 41% higher-order polyols) in the diet of rats at doses of up to 200g/kg (20%), equal to 13g/kg of body weight per day, for 13 weeks, was not associated with adverse effects. The only effects observed — increased weight of the empty caecum and increased excretion of calcium in the urine in the absence of elevated levels of serum calcium — were considered to be the consequence of the accumulation of poorly absorbed material in the caecum and were without toxicological significance.

In studies of diabetic and non-diabetic human subjects, ingestion of maltitol syrup resulted in a lower glycaemic response than with polyglycitol syrup, which in turn was lower than the response observed with glucose. These results reflect the relative proportion of glucose released following hydrolysis of each material.

On the basis of the data on hydrogenated oligo- and polysaccharides reviewed at the forty-ninth meeting and the present one, the

Committee allocated a group ADI “not specified” to materials conforming to the specifications for polyglycitol syrup and maltitol syrup. A toxicological monograph and new specifications were prepared.

4. **Substances evaluated using the Procedure for the Safety Evaluation of Flavouring Agents**

Seven groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Fig. 1 (see section 2.2.1; Annex 1, references 116, 122 and 131).

The Committee noted that in applying the Procedure, the substance is first assigned to a structural class as identified at the forty-sixth meeting of the Committee (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 oral toxicity.
- 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, the Committee used the following definitions, adapted from the report of its forty-sixth meeting:

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

Intake estimates were derived from surveys in Europe and the USA. Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. In the USA, a series of surveys was conducted between 1970 and 1987 by the National Academy of Sciences National Research Council (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 in a number of broad food categories. 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 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 using these survey data to estimate intakes of flavouring agents, it was assumed that only 60% of the total amount used is reported and that the total amount used in food is consumed by only 10% of the population.

$$\text{Intake} \left(\frac{\mu\text{g}}{\text{person per day}} \right) = \frac{\text{Annual volume of production (kg)} \times 10^9 \left(\frac{\mu\text{g}}{\text{kg}} \right)}{\text{Population of consumers} \times 0.6 \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe and 24×10^6 in the USA.

4.1 General aspects of metabolism

The flavouring agents evaluated at the present meeting share a number of functional groups, e.g. linear, branched, alicyclic and unsaturated alkyl chains, and alcohol, ester and ketone groups. Therefore, they have a number of common and interrelated metabolic features, and data on one or more members of a group under evaluation could be used to predict the metabolic fate of analogues for which there were no data. Metabolic pathways common to the groups of flavouring agents evaluated at the present meeting are:

- hydrolysis of esters;
- oxidation of alcohols and aldehydes;
- conjugation of alcohols;
- reduction of ketones;
- reduction of double bonds;
- oxidation of side-chains;

- oxidation of alicyclics; and
- conjugation with glutathione.

4.1.1 **Hydrolysis of esters**

The hydrolysis of ethyl, isoamyl and allyl esters was considered at the forty-sixth meeting of the Committee (Annex 1, reference 122). Most esters are hydrolysed rapidly by enzymes present in the gut lumen, intestinal wall and liver to yield the corresponding acids and alcohols.

A wide range of enzymes (carboxyesterases and carboxylic acid hydrolases) is capable of hydrolysing esters into their constituent alcohols and acids. Carboxyesterases are found in many tissues, and there is high esterase activity in the intestinal tract, blood, liver, kidney, lung, brain and pancreas. The multiplicity of esterases and their wide distribution in the tissues result in the rapid hydrolysis of esters *in vivo*. This extensive metabolic activity is the basis for the development by the pharmaceutical industry of prodrugs, which are carboxylic acid esters of therapeutically active agents. The esters considered at the present meeting contain larger and more complex alkyl constituents in both the alcohol and acid moieties, and there were only limited data on their *in vitro* hydrolysis, but the data were comparable to those considered at the forty-sixth meeting of the Committee. Isopropyl phenylacetate and isopropyl butyrate (isopropyl butanoate) underwent 40% hydrolysis after 2 hours' incubation with pancreatin, a value similar to that for some of the esters considered at the forty-sixth meeting. Essentially complete hydrolysis of isopropyl palmitate (isopropyl hexadecanoate), isopropyl oleate or isopropyl stearate (isopropyl octadecanoate) radiolabelled in the fatty acid moiety was demonstrated *in vivo* in rats by the detection of over 95% of the radioactivity in the triglyceride fraction of blood. *n*-Octyl esters would be expected to undergo very rapid hydrolysis on the basis of the relationship between an increase in chain length (in the range C1–C8) and an increase in $V_{\max}$ (maximal rate of reaction) and a decrease in K_m (Michaelis constant), as discussed at the forty-sixth meeting. The hydrolysis of branched-chain octyl esters and esters of longer chain length would probably be slower than that of the corresponding *n*-alkyl analogues, and absorption of the intact ester is possible.

The hydrolysis of esters with larger alcohol substituents, such as are present in the linalyl, terpinyl, carvyl and menthyl esters evaluated at the present meeting, has been the subject of only limited study. Linalyl acetate was hydrolysed rapidly in water and in artificial gastric and pancreatic fluids, and the mean half-lives for hydrolysis were

5.5 minutes in artificial gastric fluid and 52.5 minutes in artificial pancreatic fluid, values comparable to those considered at the forty-sixth meeting. Allyl tiglate and benzyl tiglate were not hydrolysed by artificial gastric or pancreatic fluid, but were hydrolysed extensively on incubation with intestinal tissue and would be predicted to be completely hydrolysed by the liver, both *in vitro* and *in vivo*. (–)-Menthol ethylene glycol carbonate and (–)-menthol propylene glycol carbonate were hydrolysed slowly on incubation with rat liver homogenate for 4 hours, with 85% hydrolysis to menthol for (–)-menthol ethylene glycol carbonate and 75% for (–)-menthol propylene glycol carbonate. The structurally related flavouring agent bornyl acetate was excreted largely as the glucuronic acid conjugate of borneol after oral administration to rabbits, which is consistent with extensive hydrolysis *in vivo*. More than 80% of radiolabelled cyclandelate, a trimethyl cyclohexyl ester, was hydrolysed after 20 minutes of incubation with rat hepatic microsomes. In an *in vivo* study in which methyl cinnamate was administered orally to rats, it was hydrolysed only slowly in the intestinal lumen, but underwent essentially complete first-pass hydrolysis, because none of the parent compound could be detected in the systemic circulation. In contrast, propyl anthranilate was not completely hydrolysed during absorption, and the intact ester was detected in the peripheral blood and urine. These findings are similar to the hydrolysis data considered at the forty-sixth meeting.

These results indicate that hydrolysis would be the major pathway for all of the esters evaluated at the present meeting; however, first-pass metabolism may be incomplete for a few of the esters in some groups. In general, esters that reach the systemic circulation intact would be expected to be hydrolysed extensively by tissue esterases into their constituent acids and alcohols.

4.1.2 **Oxidation of alcohols and aldehydes**

Primary alcohols and aldehydes

Primary aliphatic alcohols (attached to linear, branched or unsaturated alkyl chains) and their corresponding aldehydes are efficiently oxidized to the corresponding carboxylic acids by high-capacity enzyme pathways. NAD⁺/NADH-dependent alcohol dehydrogenase catalyses the oxidation of primary aliphatic saturated and unsaturated alcohols to their corresponding aldehydes. A comparison of the alcohol structure with the enzyme-binding affinity of alcohol dehydrogenase indicates that increased binding (lower K_m) occurs with increasing chain length (C1–C6) of the substrate and the presence of unsaturation. Maximum rates of oxidation were

essentially constant regardless of the alcohol structure, suggesting that alcohol–enzyme binding is not the rate-limiting step for oxidation; the activity of the enzyme appears to be dependent on the lipophilic character of the alcohol substrate. The three classes of alcohol dehydrogenase present in human liver show a decrease in K_m with an increase in chain length from C2 to C8; the alcohol groups of 12-hydroxydodecanoic acid and 16-hydroxyhexadecanoic acid show K_m values similar to that of hexanol, indicating rapid oxidation of alcohols arising from ω -oxidation reactions (see p. 57, section 4.1.6).

Similarly, aldehyde dehydrogenase, which is present predominantly in liver cytosol, has a broad substrate specificity for the oxidation of aldehydes to carboxylic acids. Aldehyde dehydrogenase is more active for aldehydes of high relative molecular mass. Three isoenzymes of aldehyde dehydrogenase, with overlapping substrate specificities, are present in human liver. They oxidize a range of naturally occurring aldehydes, such as γ -butyraldehyde, 3,4-dihydroxyphenylacetaldehyde, 5-hydroxyindoleacetaldehyde and acrolein. Alkyl aldehydes (C1–C6) have K_m values which are similar to those for these naturally occurring aldehydes, and the K_m decreases with an increase in chain length. Xanthine oxidase and aldehyde oxidase also catalyse oxidation of a wide range of aldehydes to their corresponding unsaturated acids. Before oxidation to the corresponding acid, the aldehyde may be conjugated with sulfhydryl groups such as glutathione to yield thiohemiacetals. Oxidation of aldehydes with low relative molecular masses requires glutathione, which implies that the substrate for aldehyde dehydrogenase-mediated oxidation may be the thiohemiacetal.

Branched-chain aliphatic primary alcohols and aldehydes are substrates for alcohol dehydrogenase and aldehyde dehydrogenase, although the rates of oxidation are lower than those for linear primary alcohols and aldehydes. Unsaturated, linear and branched-chain primary alcohols are better substrates than the saturated analogues, but the reverse is true of aldehydes. Citral, a mixture of two unsaturated branched-chain aldehydes (neral and geranial), was not oxidized to the corresponding acid by aldehyde dehydrogenase in rat liver preparations *in vitro* but was reduced by alcohol dehydrogenase in the presence of NADH; oxidation is a major metabolic pathway *in vivo*, producing acid metabolites.

Secondary and tertiary alcohols

Secondary alcohol groups (such as those present in carveol and β -ionol) undergo reversible oxidation to the corresponding ketone (see

section 4.1.4); the alcohol is the more important form *in vivo* because of its removal from the equilibrium by conjugation with glucuronic acid. Tertiary alcohol groups (such as those present in linalool and terpineol) do not undergo oxidative metabolism, and tertiary alcohols are eliminated by conjugation.

4.1.3 **Conjugation of alcohols**

Conjugation represents the major pathway of metabolism for both secondary and tertiary alcohols. Secondary and tertiary alcohols are metabolized extensively by conjugation with glucuronic acid followed by excretion primarily in the urine or the bile, depending on the relative molecular mass and the animal species. Both the liver and the intestinal wall have a high capacity for glucuronic acid conjugation with a wide range of substrates. Sulfate conjugation of alcohols occurs in many tissues, especially the liver, and results in the formation of highly polar, water-soluble excretory products. The urine is the main route of elimination of sulfate conjugates of alcohols resulting from the metabolism of the flavouring agents evaluated at the present meeting.

Conjugation with glucuronic acid followed by excretion in the urine and faeces represents the primary route of elimination of linalool. Conjugation with glucuronic acid represents the major pathway of metabolism of menthol. In rodents, the glucuronic acid conjugates of linalool and menthol are excreted primarily via the bile into the intestine, where they may be hydrolysed to yield the free alcohol, which undergoes reabsorption and subsequent oxidative metabolism. The metabolites of menthol are eliminated in the urine and faeces either unchanged or conjugated with glucuronic acid. Bicyclic tertiary alcohols are relatively stable *in vivo*, and in rabbits thujyl alcohol and β -santenol (2,3,7-trimethyl bicyclo[2.2.1]-heptan-2-ol) are extensively conjugated with glucuronic acid.

4.1.4 **Reduction of ketones**

Aliphatic ketones are metabolized primarily via reduction to the corresponding secondary alcohol. The reduction of aliphatic ketones is mediated by alcohol dehydrogenases and NADH/NADPH-dependent cytosolic carbonyl reductases. The reaction catalysed by carbonyl reductase is stereoselective and favours formation of the *S*-enantiomer of the alcohol. The reaction is reversible under physiological conditions, and oxidation of the secondary alcohol may lead to the formation of the corresponding ketone *in vivo*. Ketones have been shown to undergo reduction to alcohols in human hepatic

microsomes. Three reductases have been purified from human liver cytosol: two aldehyde reductases, which can reduce aliphatic aldehydes, alicyclic ketones and α -diketones, and a carbonyl reductase that is active with a broad range of aldehyde and ketone substrates. The activity of carbonyl reductase in human liver showed limited variability between individuals, and the enzyme reduced both 4-nitrobenzaldehyde and 4-nitroacetophenone.

Alicyclic monoketones, such as cyclohexanone, are reduced to their corresponding alcohols or undergo α -hydroxylation and reduction to yield diols, which are excreted as the glucuronic acid conjugates.

Unsaturated ketones, such as menthone, dihydrocarvone and carvone, undergo extensive reduction of the ketone functional group to yield the corresponding secondary alcohols (pulegone, dihydrocarveol and carveol), which are conjugated mainly with glucuronic acid and then excreted in the urine or bile. In rodents, but probably not in humans, the conjugate is excreted primarily in the bile, following which it may be hydrolysed to yield the free alcohol, which may then undergo enterohepatic recirculation and finally be excreted by the kidney. Conjugates with relative molecular masses below about 500, such as the glucuronides of menthan-2-ol, dihydrocarveol and carveol, would be eliminated by humans in the urine; the absence of significant biliary excretion would reduce the extent of secondary oxidative metabolism.

In general, alicyclic α -diketones are metabolized via the reduction pathway. Methyl-substituted diketones may be reduced to the corresponding hydroxyketones and diols, which are excreted in the urine as glucuronic acid conjugates. α -Hydroxyketones or their diol metabolites may be excreted as glucuronic acid conjugates. Excretion as glucuronic acid conjugates is favoured at elevated concentrations of α -hydroxyketones and diols *in vivo*, especially for ketones of long chain length. If the carbonyl functional group is located elsewhere on the chain, reduction is the predominant pathway of detoxification.

4.1.5 **Reduction of double bonds**

Reduction of endocyclic and exocyclic double bonds occurs with some of the flavouring agents evaluated at the present meeting. For example, the endocyclic double bond of carvone is reduced to form dihydrocarvone and dihydrocarveol and the exocyclic double bond in β -ionone is reduced to give dihydro- β -ionol. The enzymes involved in these reactions have not been characterized, and the intestinal microflora may be involved either prior to absorption or following biliary excretion.

4.1.6 Oxidation of side-chains

The position and size of substituents in carboxylic acids influences the route of metabolism. As chain length and lipophilicity increase, ω -oxidation becomes more important than β -oxidative cleavage.

β -Oxidation

Linear and branched-chain saturated carboxylic acids are substrates for β -oxidative cleavage to yield acetyl coenzyme A (acetyl CoA) and a new CoA thioester of the carboxylic acid, which has been reduced by two carbon atoms. This process continues to give complete oxidation or until a branch point is reached. Acids with an even number of carbon atoms continue to be cleaved to yield acetyl CoA, while acids with an odd number of carbon atoms yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly, whereas propionyl CoA is transformed into succinyl CoA which then enters the citric acid cycle. Acids with a methyl substituent located at an even-numbered carbon (e.g. 2-methylpentanoic acid and 4-methyldecanoic acid) are metabolized to carbon dioxide via β -oxidative cleavage in the fatty acid pathway. If the methyl group is located at the C3 position, β -oxidation is inhibited and ω -oxidation predominates, leading primarily to polar diacid metabolites that are excreted in the urine. Ethyl or propyl substituents located at the α - or β -position in relation to the carboxylic acid group inhibit metabolism to carbon dioxide, in which case there is direct conjugation of the acid with glucuronic acid or ω -oxidation followed by conjugation.

Linear unsaturated carboxylic acids participate in normal fatty acid metabolism. In this pathway, the saturated fatty acid is condensed with CoA, which then undergoes catalytic dehydrogenation mediated by acyl-CoA dehydrogenase. The resulting *trans*-2,3-unsaturated ester (*trans*- Δ^2 -enoyl CoA) is converted to the 3-ketothioester, which undergoes β -oxidative cleavage to yield an acetyl CoA fragment and a new thioester reduced by two carbons. Cleavage of acetyl CoA units continues along the carbon chain until the position of unsaturation is reached. If unsaturation begins at an even-numbered carbon, fragmentation of acetyl CoA units yields a Δ^2 -enoyl CoA product which is a substrate for further fatty acid oxidation. If unsaturation begins at an odd-numbered carbon, fragmentation of acetyl CoA units will eventually yield Δ^3 -enoyl CoA, which cannot enter the fatty acid cycle until it is isomerized to the *trans*- Δ^2 -enoyl CoA by enoyl CoA isomerase. If the stereochemistry of the double bond is *cis*, it is isomerized to the *trans* double bond by the action of 3-hydroxyacyl CoA epimerase before entering the fatty acid oxidation pathway. Short-chain acids containing terminal unsaturation may be

metabolized via desaturation to yield a substrate which may participate in the fatty acid pathway. For example, 4-pentenoic acid is converted into the CoA thioester, which is dehydrogenated to yield *trans*-2,4-pentadienoyl CoA, which undergoes NADPH-dependent enzyme-catalysed reduction of the Δ^2 -alkene to *trans*-2-pentenoic acid, which then participates in normal fatty acid oxidation. A second but minor pathway involves β -oxidation to yield 3-keto-4-pentenoyl CoA.

ω -Oxidation and (ω -1)-oxidation

ω -Oxidation and (ω -1)-oxidation are important in the elimination of compounds with long and/or complex alkyl chains, because they eventually yield polar acid metabolites that are eliminated in the urine. ω -Oxidation results in the formation of a primary alcohol, which may undergo further oxidation to the corresponding carboxylic acid, which may then be excreted in the urine. For example, linalool undergoes cytochrome P450-mediated oxidation to form 8-hydroxylinalool and 8-carboxylinalool, whereas geraniol is oxidized to 8-hydroxygeraniol, 8-carboxygeraniol and the dibasic acid, Hilderand's acid (3,7-dimethyl-2,6-octadienedioic acid). Citral, the aldehyde analogue of geraniol, undergoes oxidation of the aldehyde group *in vivo* followed by ω -oxidation to yield a mixture of diacids and hydroxy acids. Menthol undergoes ω -oxidation of the side-chain substituents to yield various polyols and hydroxyacids; the (–)-isomer undergoes more extensive oxidation than the (+)-isomer.

Short-chain (containing four or fewer carbon atoms) ketones that contain a carbonyl functional group at the C2-position (e.g. butanone) and α -diketones, such as acetoin, may undergo ω -oxidation of the terminal methyl group to give a diketocarboxylic acid, which would undergo decarboxylation to yield an α -ketocarboxylic acid. As intermediary metabolites, α -ketoacids undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which may be completely metabolized via the fatty acid pathway and citric acid cycle. Almost one-half of a dose of radiolabelled acetoin, administered by intravenous injection to rats, was eliminated as carbon dioxide in the expired air and about 20% was eliminated as unidentified urinary metabolites. *trans*-Methyl styryl ketone undergoes essentially complete metabolism of the methyl group after oral administration to rats; the glycine conjugates of phenylacetic and benzoic acid were the major metabolites in the urine.

ω -Oxidation of ketones yields hydroxyketones that may be further reduced to diols and excreted in the urine as glucuronic acid conjugates. Participation in these pathways depends on chain length,

the position of the carbonyl functional group, and the dose. Longer-chain aliphatic ketones (containing five or more carbon atoms) are metabolized primarily via reduction, but ω -oxidation and/or (ω -1)-oxidation are competing pathways at high aliphatic ketone concentrations, which yield ketoacids and diketones, respectively.

A number of the flavouring agents evaluated at the present meeting are alicyclic ketones containing an alkyl or alkenyl side-chain; these undergo oxidation of the side-chain (probably by cytochrome P450) to form polar metabolites that are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces.

Oxidation of double bonds

Oxidation of double bonds, via an epoxide intermediate, to form diols is probably only a minor pathway of metabolism for the flavouring agents considered at the present meeting. For example, α -terpineol is oxidized by cytochrome P450 in rats, via an epoxide intermediate, to a 1,2-dihydroxy metabolite; this diol metabolite has been detected in humans following ingestion of α -terpineol.

4.1.7 Oxidation of alicyclics

Cyclohexane and cyclohexene rings, which are present in the carvone and ionone groups of flavouring agents considered at the present meeting, are rapidly oxidized *in vivo* by ring hydroxylation, even when other pathways of elimination are present. For example, the main pathways of elimination of cyclohexane are in the expired air (as the unchanged substance) and in the urine as glucuronic acid conjugates of cyclohexanol and cyclohexane-1,2-diol. Cyclohexylamine is highly water-soluble, and is excreted in the urine as both the parent compound and as the glucuronic acid conjugates formed via ring hydroxylation, together with small amounts of cyclohexane-1,2-diol formed via deamination and ring hydroxylation. Ring oxidation, which is catalysed by cytochrome P450 (isoenzyme CYP3A4), is a major pathway of metabolism for the substituted cyclohexene ring moieties that are present in vitamin A and its metabolites such as 13-*cis*- and all-*trans*-retinoic acid, as well as the ionone group of flavouring agents. Oxidation of the substituted cyclohexene rings present in retinoids and ionones occurs on the carbon atoms adjacent to the double bond.

4.1.8 Conjugation with glutathione

The vast majority of the flavouring agents evaluated at the present meeting are metabolized by the pathways described in sections 4.1.1–4.1.7. The possible exceptions are carvone, carveol (and its esters) and

some members of the ionone group in which a ketone group, or a precursor secondary alcohol group, are adjacent to a double bond or conjugated double bonds (α,β -unsaturated ketone). Glutathione conjugation is an important pathway for detoxification, especially for reactive compounds. The products of conjugation with glutathione are usually eliminated in the bile as the glutathione conjugate *per se*, and in the urine as the *N*-acetylcysteine metabolite produced from the glutathione conjugate. Glutathione conjugates may also be split by C–S (carbon–sulfur) lyases (β -lyases) in the kidney and intestinal microflora to give a thiol compound: this reaction represents a bioactivation process for some chemicals. Only limited data on metabolism were available on the ionone group of flavouring agents, and glutathione conjugation products have not been reported as urinary metabolites of ionones or of carvone. Oral administration of carvone by gavage in corn oil to mice at a dose of 20 mg per animal for 3 successive days caused a reduction in the concentration of glutathione in the liver, and an increase in glutathione transferase activity. However, these effects may not have been due to the α,β -unsaturated ketone moiety, because the decrease in glutathione was not found with α,β -unsaturated carvone analogues lacking the exocyclic allyl group, while the increase in glutathione transferase was detected with compounds such as *p*-menthan-2-one, which contain neither an α,β -unsaturated ketone moiety nor an exocyclic allyl group.

The potential for non-enzymatic interaction with glutathione has been shown by *in vitro* studies. The greatest reactivity with glutathione was found with the simplest α,β -unsaturated ketone (butenone), which showed a rate of reaction about 1000 times that of oct-2-ene-4-one. Damasconone, the 1,3-endocyclic diene analogue of damascone and carvone, which were evaluated at the present meeting, showed rates of reaction that were 17 000, 11 000 and 320 000 times slower than butenone, which indicates a low reactivity.

Overall, the flavouring agents with an α,β -unsaturated ketone moiety which were evaluated at the present meeting showed a low reactivity, and conjugation with glutathione would not be a major route of metabolism. The low reactivity predicted is supported by the toxicological data on the ionones and carvone.

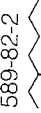
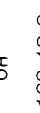
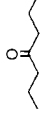
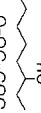
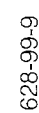
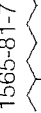
4.2 **Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters**

The Committee evaluated 39 saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters (Table 3) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Table 3

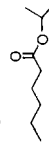
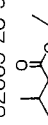
Summary of the results of safety evaluations of 39 saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters

Substance	No.	CAS no. and structure	Step 2 Metabolized to innocuous products?	Step A3/B3 ^a Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step B4 Adequate NOEL for substance or structurally related substance?	Conclusion based on current intake
Structural class I							
Acetone	139	67-64-1 	Yes	Yes Europe: 7100 USA: 36 000	Yes	NA	No safety concern
Isopropyl alcohol	277	67-63-0 	Yes	Yes Europe: 99 000 USA: 9900	Yes	NA	
2-Butanone	278	78-93-3 	Yes	No Europe: 110 USA: 36	NA	NA	
2-Pentanone	279	107-87-9 	Yes	No Europe: 140 USA: 42	NA	NA	
2-Pentanol	280	6032-29-7 	Yes	No Europe: 6 USA: 0.04	NA	NA	
3-Hexanone	281	589-38-8 	Yes	No Europe: 0.4 USA: 1	NA	NA	
3-Hexanol	282	623-37-0 	Yes	No Europe: 13 USA: 11	NA	NA	

2-Heptanol	284	543-49-7 	Yes	No Europe: 8 USA: 1	NA	NA
3-Heptanol	286	589-82-2 	No	No Europe: 0.2 USA: 0.6	NA	Yes
4-Heptanone	287	123-19-3 	Yes	No Europe: 2 USA: 2	NA	NA
2-Octanol	289	123-96-6 	Yes	No Europe: 13 USA: 4	NA	NA
3-Octanol	291	589-98-0 	Yes	No Europe: 6 USA: 320	NA	NA
2-Nonanol	293	628-99-9 	Yes	No Europe: 1 USA: 1	NA	NA
3-Decanol	295	1565-81-7 	Yes	No Europe: ND USA: 16	NA	NA
2-Undecanol	297	1653-30-1 	Yes	No Europe: 0.2 USA: 0.04	NA	NA
3-Methyl-2-butanol	300	598-75-4 	Yes	No Europe: 1 USA: 0.2	NA	NA
2,6-Dimethyl-4-heptanol	303	108-82-7 	Yes	No Europe: ND USA: 5	NA	NA

No safety concern

Table 3 (continued)

Substance	No.	CAS no. and structure	Step 2 Metabolized to innocuous products?	Step A3/B3 ^a Does intake exceed the threshold for human intake?	Step A4 Is the substance or its metabolites endogenous?	Step B4 Adequate NOEL for substance or structurally related substance?	Conclusion based on current intake
Isopropyl formate	304	625-55-8 	Yes	No Europe: 0.5 USA: 0.02	NA	NA	No safety concern
Isopropyl acetate	305	108-21-4 	Yes	No Europe: 41 USA: 9	NA	NA	
Isopropyl propionate	306	637-78-5 	Yes	No Europe: 0.01 USA: 0.02	NA	NA	
Isopropyl butyrate	307	638-11-9 	Yes	No Europe: 7 USA: 0.08	NA	NA	
Isopropyl hexanoate	308	2311-46-8 	Yes	No Europe: 4 USA: 0.02	NA	NA	
Isopropyl isobutyrate	309	617-50-5 	Yes	No Europe: 0.6 USA: 0.06	NA	NA	
Isopropyl isovalerate	310	32665-23-9 	Yes	No Europe: 0.3 USA: 0.2	NA	NA	

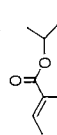
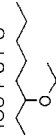
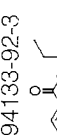
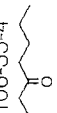
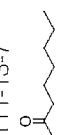
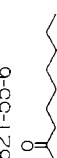
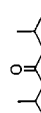
Isopropyl myristate	311	110-27-0		Yes	No Europe: 23 USA: 0.02	NA	NA	No safety concern
Isopropyl tiglate	312	6284-46-4		Yes	No Europe: 0.01 USA: 0.1	NA	NA	
3-Octyl acetate	313	4864-61-3		Yes	No Europe: 0.7 USA: 30	NA	NA	
1-Ethylhexyl tiglate	448	94133-92-3		Yes	No Europe: 0.01 USA: 29	NA	NA	
Structural class II								
2-Heptanone	283	110-43-0		Yes	No Europe: 110 USA: 48	NA	NA	No safety concern
3-Heptanone	285	106-35-4		No	No Europe: 4 USA: 10	NA	Yes	
2-Octanone	288	111-13-7		Yes	No Europe: 110 USA: 67	NA	NA	
3-Octanone	290	106-68-3		Yes	No Europe: 3 USA: 2	NA	NA	
2-Nonanone	292	821-55-6		Yes	No Europe: 380 USA: 27	NA	NA	

Table 3 (continued)

Substance	No.	CAS no. and structure	Step 2 Metabolized to innocuous products?	Step A3/B3 ^a Does intake exceed the threshold for human intake?	Step A4 Is the substance or its metabolites endogenous?	Step B4 Adequate NOEL for substance or structurally related substance?	Conclusion based on current intake
3-Nonanone	294	925-78-0 	Yes	No Europe: 0.1 USA: 0.1	NA	NA	No safety concern
2-Undecanone	296	112-12-9 	Yes	No Europe: 380 USA: 21	NA	NA	
2-Tridecanone	298	593-08-8 	Yes	No Europe: 73 USA: 30	NA	NA	
2-Pentadecanone	299	2345-28-0 	Yes	No Europe: 21 USA: 430	NA	NA	
4-Methyl-2-pentanone	301	108-10-1 	Yes	No Europe: 7 USA: 2	NA	NA	
2,6-Dimethyl-4-heptanone	302	108-83-8 	Yes	No Europe: 0.2 USA: 0.06	NA	NA	

CAS: Chemical Abstracts Service; NA: not applicable; ND: no intake data reported.

^a The thresholds for human intake are 1800 µg/day for Class I and 540 µg/day for Class II. All intake values are expressed in µg per day.

The Committee had evaluated five members of this group previously. Acetone (propan-2-one) was evaluated as an extraction solvent at the fourteenth meeting (Annex 1, reference 22), when the Committee considered that, with good manufacturing practice, the residues in food would be toxicologically insignificant. The evaluation was tentative owing to a lack of relevant data. Isopropyl alcohol (2-propanol) was evaluated at the fourteenth and twenty-fifth meetings (Annex 1, references 22 and 56); an ADI was not allocated because of a lack of data. Isopropyl acetate was evaluated at the twenty-third meeting (Annex 1, reference 50); the Committee was unable to establish an ADI because of a lack of data on hydrolysis and other toxicological end-points. Isopropyl myristate (isopropyl tetradecanoate), evaluated at the twenty-third meeting (Annex 1, reference 50), was not allocated an ADI owing to a lack of data. 2-Butanone (ethyl methyl ketone) was evaluated at the twenty-third and twenty-fifth meetings (Annex 1, references 50 and 56), when the Committee concluded that the data available were not sufficient for evaluation of the substance, and no ADI was allocated.

Estimated daily per capita intake

The total annual volume of production of the 39 saturated aliphatic acyclic secondary alcohols, ketones and related esters in this group is approximately 750 tonnes in Europe and 240 tonnes in the USA. In Europe, 92% of the total annual volume is accounted for by isopropyl alcohol (no. 277) alone. In the USA, 98% of the total annual volume is accounted for by acetone (no. 139) and isopropyl alcohol. On the basis of the reported annual volumes of production, the estimated daily per capita intake of acetone resulting from its use as a flavouring agent is approximately 7.1 mg in Europe and 36 mg in the USA. Similarly, the total estimated daily per capita intake of isopropyl alcohol and its nine esters in this group (nos 304–312) resulting from their use as flavouring agents is about 99 mg in Europe and 10 mg in the USA.

Saturated aliphatic acyclic secondary alcohols, ketones and related esters are the main flavouring components of alcoholic beverages and occur naturally in a wide variety of fruits. Quantitative data on natural occurrence have been reported for 22 of the 39 substances in this group. In the USA, the intake of 20 of these 22 substances from natural sources exceeds the intake from their use as flavouring agents; for the two remaining substances, acetone (no. 139) and 3-octanol (no. 291), the intake from their use as flavouring agents exceeds that from natural sources.

Absorption, metabolism and elimination

In general, saturated aliphatic acyclic secondary alcohols and ketones are absorbed through the gastrointestinal tract. The esters in this group of flavouring agents are anticipated to be hydrolysed to their component secondary alcohols and aliphatic, saturated and unsaturated carboxylic acids, which are also readily absorbed.

Three of the esters in this group (3-octyl acetate (no. 313), 1-ethylhexyl tiglate (octen-3-yl 2-methyl-2-butenate; no. 448) and isopropyl tiglate (isopropyl 2-methyl-2-butenate; no. 312)) may not be completely hydrolysed in the gastrointestinal tract, but these esters and other esters in this group of flavouring agents that reach the general circulation intact would be expected to be hydrolysed by tissue esterases to their component alcohols and carboxylic acids. The metabolism of these flavouring agents is primarily by oxidation, reduction and/or conjugation (see section 4.1).

Two of the substances in this group, 3-heptanone (no. 285) and 3-heptanol (no. 286) meet the special structural requirements for possible oxidation to form neurotoxic γ -diketone metabolites.

Application of the Procedure for the Safety Evaluation of Flavouring Agents Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned saturated aliphatic acyclic secondary alcohols, ketones and related esters, the Committee assigned 28 of the 39 substances to structural class I. The remaining 11 substances, which included acyclic aliphatic 2-alkanones (nos 283, 288, 292, 296, 298, 299 and 301), 3-alkanones (nos 285, 290 and 294) and a 4-alkanone (no. 302) with four or more carbons on either side of the keto group, were assigned to structural class II.

Step 2. The available data indicated that 26 of the 28 saturated aliphatic acyclic secondary alcohols and ketones would be predicted to be metabolized to or are innocuous substances. The 11 esters in this group are anticipated to be hydrolysed to their component secondary alcohols (isopropyl alcohol or 3-octanol) and carboxylic acids (aliphatic saturated acids or the unsaturated tiglic acid). These hydrolysis products are either endogenous compounds or can be predicted to be metabolized to innocuous substances. At current levels of per capita intake, 37 of the 39 flavouring agents in this group would not be expected to saturate the metabolic pathways and were considered to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. The remaining two substances, 3-heptanone (no. 285) and 3-heptanol (no. 286), may undergo oxidation to neurotoxic γ -

diketones. The evaluation of these substances therefore proceeded via the right-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 26 of the 28 substances in class I were below the threshold for class I (1800µg), indicating that they pose no safety concern when used at current levels of estimated intake as flavouring agents. Only acetone (no. 139) and isopropyl alcohol (no. 277) had estimated intakes greater than the threshold for class I. The evaluation of these two substances therefore proceeded to step A4.

The total estimated daily per capita intake of isopropyl alcohol (no. 277) and its nine esters from their use as flavouring agents (99mg in Europe and 10mg in the USA) is greater than the threshold for human intake for class I. The intake of three additional esters of isopropyl alcohol (isopropyl benzoate, isopropyl cinnamate and isopropyl phenylacetate), which were not evaluated as part of this group, would contribute approximately an additional 15µg per day in Europe and 5µg per day in the USA to the total intake of isopropyl alcohol. This potential additional intake is relatively minor and would not alter the safety evaluation.

The estimated daily per capita intakes of all 11 substances in class II were below the threshold for class II (540µg), indicating that they pose no safety concern when used at current levels of estimated intake as flavouring agents.

Step A4. Acetone (no. 139) and isopropyl alcohol (no. 277) are both endogenous components of fatty acid and carbohydrate metabolism and have been detected in the blood. Therefore, these substances were determined to be of no safety concern when used at current levels of intake as flavouring agents.

Step B3. The estimated daily per capita intakes of 3-heptanol (no. 286) and 3-heptanone (no. 285) were below the threshold for structural classes I and II, respectively. Accordingly, the evaluation of these substances proceeded to step B4.

Step B4. On the basis of neurotoxic effects seen in a limited study in which 3-heptanone (no. 285) was administered orally to male rats, the NOEL was 1000mg/kg of body weight per day. This NOEL provides a large safety margin (>1 000 000 for 3-heptanone and even higher for 3-heptanol (no. 286)) when compared with the current levels of estimated intake of 3-heptanone and 3-heptanol (which might be metabolized to 3-heptanone). Therefore, these substances were determined to be of no safety concern when used at current levels of intake as flavouring agents.

Table 3 summarizes the stepwise evaluations of the 39 saturated aliphatic acyclic secondary alcohols, ketones and related esters used as flavouring agents.

Consideration of combined intakes

In the unlikely event that all foods containing all 28 saturated aliphatic acyclic secondary alcohols, ketones and related esters of saturated and unsaturated carboxylic acids in structural class I were consumed concomitantly on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would exceed the threshold for class I.

In the unlikely event that all foods containing all 11 saturated aliphatic acyclic secondary ketones in structural class II were consumed concomitantly on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would exceed the threshold for class II.

Of the 39 substances, 37 are expected to be metabolized via well-known biochemical pathways to innocuous metabolic and/or endogenous substances; in the opinion of the Committee, the endogenous levels of these metabolites would not give rise to perturbations outside the physiological range. Accordingly, even a combined theoretical intake would be of no safety concern. For the remaining two substances that could potentially form γ -diketones, the combined intake would also be of no safety concern.

Conclusion

The Committee concluded that the substances in this group would not present safety concerns at the current levels of estimated intake.

No toxicity data were required for application of the Procedure to 37 of the 39 substances in this group. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure. For the remaining two substances, 3-heptanone (no. 285) and 3-heptanol (no. 286), the toxicity data were also consistent with the results of the safety evaluation using the Procedure.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.3 Linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters

The Committee evaluated a group of 42 flavouring agents that included linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters (Table 4) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

The Committee had evaluated one member of the group, oleic acid, previously. At its twenty-ninth meeting (Annex 1, reference 70), the Committee noted that the safety evaluation of fatty acids, including oleic acid, is based on knowledge of their metabolism and excretion and on their occurrence in edible fats and oils that have a long history of use as foods or food components. At the thirty-third meeting, ADIs “not specified” were allocated to the calcium, potassium and sodium salts of oleic acid (Annex 1, reference 83).

Estimated daily per capita intake

The total annual volume of production of the 42 linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, carboxylic acids and related esters is approximately 39 tonnes in Europe and 13 tonnes in the USA. Three substances in the group, *cis*-3-hexen-1-ol (no. 315), linoleic acid (no. 332) and oleic acid (no. 333) account for about 96% of the total annual volume in Europe. Four substances in the group, *cis*-3-hexen-1-ol, oleic acid, 2,6-dimethyl-5-heptenal (no. 349) and a mixture of hexyl 2-methyl-3-pentenoate and hexyl 2-methyl-4-pentenoate (no. 352) account for about 91% of the total annual volume in the USA. On the basis of the reported annual volumes of production, the estimated daily per capita intake of *cis*-3-hexen-1-ol, linoleic acid and oleic acid from their use as flavouring agents is approximately 4300 µg (72 µg/kg of body weight per day), 130 µg (2 µg/kg of body weight per day) and 970 µg (16 µg/kg of body weight per day), respectively, in Europe. Similarly, the estimated daily per capita intake of *cis*-3-hexen-1-ol, oleic acid, 2,6-dimethyl-5-heptenal and the mixture of hexyl 2-methyl-3-pentenoate and hexyl 2-methyl-4-pentenoate from their use as flavouring agents is approximately 1100 µg (18 µg/kg of body weight per day), 440 µg (7 µg/kg of body weight per day), 250 µg (4 µg/kg of body weight per day) and 530 µg (9 µg/kg of body weight per day), respectively, in the USA.

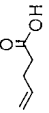
Of the 42 aliphatic unsaturated primary alcohols and unconjugated aldehydes, acids and related esters in this group, 27 are reported to occur naturally in a wide variety of foods, including fruits and vegetables, dairy products, fish and alcoholic beverages. Oleic and linoleic acids (nos 333 and 332) are some of the most common unsaturated fatty acids found in vegetable oils and animal fats, often constituting more than 50% of the total concentration of fatty acids.

Absorption, metabolism and elimination

The metabolism of flavouring agents in this group involves common pathways of intermediary metabolism which are discussed in section 4.1.

Table 4

Summary of the results of safety evaluations of 42 linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters^a

Substance	No.	CAS no. 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 A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
4-Pentenoic acid	314	591-80-0 	No Europe: 5 USA: 3	NR	NR	Converted to the CoA thioester, which undergoes dehydrogenation and then participates in the fatty acid and tricarboxylic acid pathways See note 1	No safety concern
<i>cis</i> -3-Hexen-1-ol	315	928-96-1 	Yes Europe: 4300 USA: 1100	No	Yes The NOEL of 120–150 mg/kg of body weight reported in a 98-day study in rats is > 1000 times the daily per capita intake		

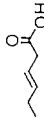
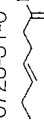
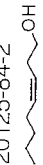
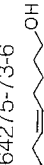
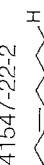
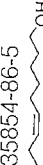
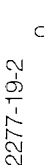
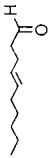
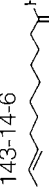
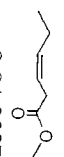
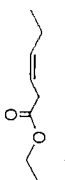
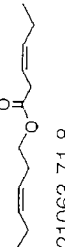
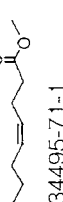
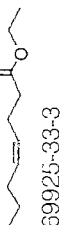
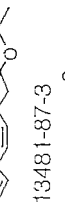
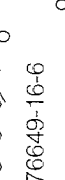
<i>cis</i> -3-Hexenal	316	6789-80-6 	No Europe: 5 USA: 3	NR	NR	See note 2
3-Hexenoic acid	317	4219-24-3 	No Europe: 11 USA: 1	NR	NR	See note 3
4-Hexen-1-ol	318	6126-50-7 	No Europe: 3 USA: 0.04	NR	NR	See note 1
<i>cis</i> -4-Hexenal	319	4634-89-3 	No Europe: 0.03 USA: 0.1	NR	NR	See note 2
4-Heptenal	320	6728-31-0 	No Europe: 2 USA: 0.1	NR	NR	See note 2
<i>cis</i> -3-Octen-1-ol	321	20125-84-2 	No Europe: 6 USA: 0.01	NR	NR	No safety concern
<i>cis</i> -5-Octen-1-ol	322	64275-73-6 	No Europe: 0.5 USA: 0.04	NR	NR	
<i>cis</i> -5-Octenal	323	41547-22-2 	No Europe: 0.001 USA: 29	NR	NR	
<i>cis</i> -6-Nonen-1-ol	324	35854-86-5 	No Europe: 3 USA: 0.1	NR	NR	
<i>cis</i> -6-Nonenal	325	2277-19-2 	No Europe: 2 USA: 0.1	NR	NR	

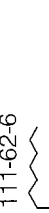
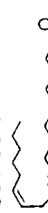
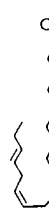
Table 4 (continued)

Substance	No.	CAS no. 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 A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
4-Decenal	326	30390-50-2 	No Europe: 1 USA: 0.01	NR	NR	See note 2	No safety concern
Mixture of 5- and 6-decenoic acid	327	72881-27-7 	No Europe: 4 USA: 0.2	NR	NR	See note 3	
9-Decenoic acid	328	14436-32-9 	No Europe: 0.1 USA: 0.2	NR	NR	See note 3	
9-Undecenal	329	143-14-6 	No Europe: 1 USA: 0.2	NR	NR	See note 2	
10-Undecenal	330	112-45-8 	No Europe: 0.4 USA: 0.04	NR	NR	See note 2	
10-Undecenoic acid	331	112-38-9 	No Europe: 30 USA: 0.8	NR	NR	See note 3	
Linoleic acid	332	60-33-3 	No Europe: 130 USA: 6	NR	NR	See note 4	

Oleic acid ^c	333	112-80-1		No Europe: 970 USA: 440	NR	NR	See note 4
Methyl 3-hexenoate	334	2396-78-3		No Europe: 0.7 USA: 0.01	NR	NR	See note 5
Ethyl 3-hexenoate	335	2396-83-0		No Europe: 4 USA: 1	NR	NR	See note 5
cis-3-Hexenyl cis-3-hexenoate	336	6144-38-0		No Europe: 4 USA: 0.8	NR	NR	See note 5
Methyl cis-4-octenoate	337	21063-71-8		No Europe: 0.4 USA: 0.04	NR	NR	See note 5
Ethyl cis-4-octenoate	338	34495-71-1		No Europe: 1 USA: 0.04	NR	NR	See note 5
Ethyl cis-4,7-octadienoate	339	69925-33-3		No Europe: 2 USA: 0.01	NR	NR	See note 5
Methyl 3-nonenoate	340	13481-87-3		No Europe: 2 USA: 76	NR	NR	See note 5
Ethyl trans-4-decenoate	341	76649-16-6		No Europe: 2 USA: 0.04	NR	NR	See note 5

No safety
concern

Table 4 (continued)

Substance	No.	CAS no. 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 A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Methyl 9-undecenoate	342	5760-50-9 	No Europe: 39 USA: 0.4	NR	NR	See note 5	No safety concern
Ethyl 10-undecenoate	343	692-86-4 	No Europe: 2 USA: 44	NR	NR	See note 5	
Butyl 10-undecenoate	344	109-42-2 	No Europe: 0.04 USA: 5	NR	NR	See note 5	
Ethyl oleate	345	111-62-6 	No Europe: 69 USA: 3	NR	NR	See note 5	
Mixture of methyl linoleate and methyl linolenate	346	301-00-8 	No Europe: ND USA: 23	NR	NR	See note 6	
							

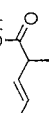
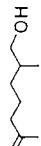
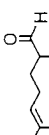
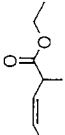
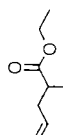
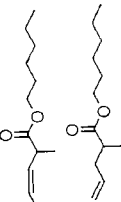
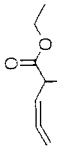
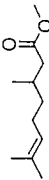
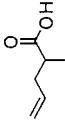
2-Methyl-3-pentenoic acid	347	37674-63-8		No Europe: 1 USA: 2	NR	NR	See note 7	No safety concern
2,6-Dimethyl-6-hepten-1-ol	348	36806-46-9		No Europe: ND USA: 0.1	NR	NR	See note 7	
2,6-Dimethyl-5-heptenal	349	106-72-9		No Europe: 31 USA: 250	NR	NR	See note 7	
Ethyl 2-methyl-3-pentenoate	350	1617-23-8		No Europe: 6 USA: 6	NR	NR	See note 5	
Ethyl 2-methyl-4-pentenoate	351	53399-81-8		No Europe: 0.3 USA: 0.02	NR	NR	See note 5	
Mixture of hexyl 2-methyl-3- and hexyl 2-methyl-4-pentenoate	352	58625-95-9		No Europe: 0.03 USA: 530	NR	NR	See note 6	
Ethyl 2-methyl-3,4-pentadienoate	353	60523-21-9		— Europe: 0.01 USA: 10	NA	NA	Cannot be predicted to be metabolized to innocuous substances	Not evaluated ^d

Table 4 (continued)

Substance	No.	CAS no. and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Methyl 3,7-dimethyl-6-octenoate	354	2270-60-2 	No Europe: 0.2 USA: 0.1	NR	NR	See note 5	No safety concern
2-Methyl-4-pentenoic acid	355	1575-74-2 	No Europe: ND USA: 0.04	NR	NR	See note 7	

CAS: Chemical Abstracts Service; NA: not applicable; ND: no intake data reported; NR: not required for evaluation because consumption was determined to be of no safety concern at step A3 of the Procedure.

^a Step 1: All of the substances in this group are in structural class I.

Step 2: All of the substances in this group are predicted to be metabolized to innocuous products, except 2-methyl-3,4-pentadienoate.

^b The threshold for human intake of class I is 1800 µg per day. All intake values are expressed in µg per day.

^c The ADI "not specified" for the calcium, potassium and sodium salts established at the thirty-third meeting of the Committee (Annex 1, reference 83) was maintained.

^d Evaluation postponed, pending review of a 90-day toxicity study not available at the present meeting.

Notes to Table 4

- Expected to be oxidized to the corresponding aldehyde and carboxylic acid; the latter is completely metabolized in the fatty acid and tricarboxylic acid pathways.
- Expected to be oxidized to the corresponding carboxylic acid, which is completely metabolized in the fatty acid and tricarboxylic acid pathways.
- Metabolized in the fatty acid and tricarboxylic acid pathways.
- Readily metabolized in the fatty acid oxidation pathway.
- Expected to be hydrolysed to its component alcohol and carboxylic acid, which are completely metabolized in the fatty acid and tricarboxylic acid pathways.
- Expected to be hydrolysed to their component alcohols and carboxylic acids, which are completely metabolized in the fatty acid and tricarboxylic acid pathways.
- Undergoes β-oxidation to yield products that are completely metabolized in the tricarboxylic acid cycle.

Application of the Procedure for the Safety Evaluation of Flavouring Agents
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters, the Committee assigned all 42 substances to structural class I.

Step 2. The metabolic fates of the linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, carboxylic acids and related esters in this group of flavouring agents can (with the exception of ethyl 2-methyl-3,4-pentadienoate (no. 353)) be predicted readily because of their close structural relationship to endogenous substrates and the broad substrate specificity of the relevant enzymes. The prediction that the metabolites would be innocuous was supported by available toxicity data on members of this group. The Committee considered that the available data were inadequate to predict the metabolism of ethyl 2-methyl-3,4-pentadienoate because of its terminal diene structure.

At current levels of intake, the remaining 41 substances in this group would not be expected to saturate the metabolic pathways, and all of them are predicted to be metabolized to innocuous products (see section 4.1). The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

Since it could not be predicted to be metabolized to innocuous products, the evaluation of ethyl 2-methyl-3,4-pentadienoate proceeded via the right-hand side of the decision-tree.

Step A3. The current levels of intake of 40 of the 41 substances were below the threshold for class I (1800 µg per day). These 40 substances were considered to be of no safety concern at this step on the basis of their structural class and low levels of estimated intake. For the remaining substance, *cis*-3-hexen-1-ol (no. 315), the estimated daily per capita intake in Europe exceeded the threshold for class I. The evaluation of this substance therefore proceeded to step A4.

Step A4. The substance *cis*-3-hexen-1-ol (no. 315) is not endogenous in humans. Accordingly, its evaluation proceeded to step A5 of the Procedure.

Step A5. A NOEL of 120–150 mg/kg of body weight per day was reported for *cis*-3-hexen-1-ol (no. 315) in a 98-day study in rats. This NOEL provides a safety margin of >1000 when compared with the daily per capita intakes of this substance from its use as a flavouring agent in Europe and the USA. Therefore, this substance would not be expected to be of safety concern.

Step B3. The estimated daily per capita intake of ethyl 2-methyl-3,4-pentadienoate (no. 353) in Europe and the USA did not exceed the threshold for class I. The evaluation of this substance therefore proceeded to step B4.

Step B4. Adequate data to determine a NOEL for ethyl 2-methyl-3,4-pentadienoate (no. 353) were not available. The Committee was aware of a study in rats given ethyl 2-methyl-3,4-pentadienoate in the diet at a concentration equivalent to a daily intake of 1.0mg/kg of body weight for 90 days; however, a full report of the study was not available. The Committee therefore postponed the evaluation of this substance, pending the review of the 90-day toxicity study.

Table 4 summarizes the stepwise evaluations of the linear and branched-chain aliphatic unsaturated alcohols and unconjugated aldehydes, acids and related esters in this group of flavouring agents.

Consideration of combined intakes

In the unlikely event that all foods containing all 41 linear and branched-chain aliphatic unsaturated alcohols and unconjugated aldehydes, acids and related esters in this group were consumed concomitantly on a daily basis, the estimated combined intake would exceed the threshold for class I. However, all 41 substances are expected to be efficiently metabolized and would not saturate the metabolic pathways. On the basis of the evaluation of the collective data, the combined intake was judged by the Committee not to raise safety concerns. The consideration of the combined intakes excludes the intake of ethyl 2-methyl-3,4-pentadienoate (no. 353) because the evaluation of this substance was deferred, pending the review of a 90-day toxicity study not available to the Committee at its present meeting.

Conclusions

The Committee concluded that 41 of the 42 linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters in this group would not present safety concerns when used at current levels of intake as flavouring agents. Data on the toxicity of *cis*-3-hexen-1-ol (no. 315) were required for the application of the Procedure. The Committee noted that these data and the available toxicity data on other substances in the group were consistent with the results of the Procedure. The evaluation of ethyl 2-methyl-3,4-pentadienoate (no. 353) was deferred, pending the evaluation of a 90-day study in rats that was not available to the Committee at its present meeting. The ADI "not specified" for the calcium, potassium and sodium salts of oleic acid established at the thirty-third meeting was maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.4 **Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances**

The Committee evaluated a group of 23 flavouring agents that included selected tertiary alcohols and related esters (Table 5) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Two members of the group, linalool and linalyl acetate, were previously evaluated as part of a group of other terpenoid flavouring substances that included citral, citronellol and geranyl acetate at the eleventh meeting (Annex 1, reference 14). At that meeting, the Committee recommended that at least one member of the group be studied for the effects of long-term exposure. Linalool and linalyl acetate were re-evaluated at the twenty-third meeting (Annex 1, reference 50). A group ADI of 0–0.5 mg/kg of body weight was established, based on the clearly defined metabolism of these substances, their rapid excretion and their low toxicity in short-term studies.

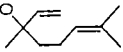
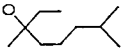
At its forty-ninth meeting (Annex 1, reference 131), the Committee evaluated a group of 26 geranyl, neryl, citronellyl and rhodinyl esters formed from branched-chain terpenoid primary alcohols and aliphatic acyclic linear and branched-chain carboxylic acids using the Procedure for the Safety Evaluation of Flavouring Agents. The Committee concluded that these substances pose no safety concerns, based on knowledge of their metabolism and low levels of intake.

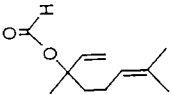
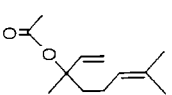
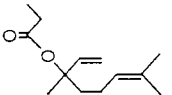
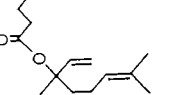
Estimated daily per capita intake

The total annual volume of production of the 23 tertiary alcohols and related esters is approximately 58 tonnes in Europe and 15 tonnes in the USA. Four substances in the group, linalool (no. 356), linalyl acetate (no. 359), α -terpineol (no. 366) and terpinyl acetate (no. 368) account for approximately 96% of the total annual volume in Europe and the USA. On the basis of the reported annual volumes of production, the total estimated daily per capita intake of linalool from use of linalool and eight of its esters as flavouring agents is approximately 4800 μ g (80 μ g/kg of body weight) in Europe and 1300 μ g (22 μ g/kg of body weight) in the USA. Similarly, the total estimated daily per capita intake of α -terpineol from use of α -terpineol and six of its esters as flavouring agents is approximately 3200 μ g (53 μ g/kg of body weight) in Europe and 1400 μ g (23 μ g/kg of body weight) in the USA.

Tertiary alcohols and related esters occur naturally in a wide variety of foods, including fruits, spices and tea. Thirteen of the substances in this group have been reported to occur naturally in foods.

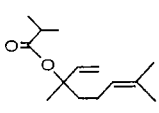
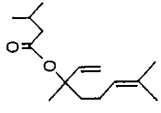
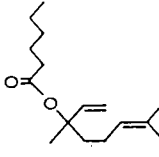
Table 5
Summary of the results of safety evaluations of 23 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances^a

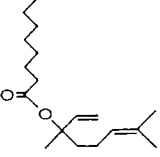
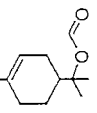
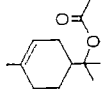
Substance	No.	CAS no. and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step B4/A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
Linalool ^c	356	78-70-6 	Yes Europe: 2600 USA: 1100	No	Yes The NOEL of 50 mg/kg of body weight per day reported in a 90-day study in rats is > 1000 times the daily per capita intake of linalool in Europe and the USA	Oxidation of the allylic methyl group may occur after repeated exposure. See also note 1	No safety concern
Tetrahydrolinalool	357	78-69-3 	No Europe: 55 USA: 0.1	NR	NR	See note 1	

Linalyl formate	358	115-99-1 	No Europe: 8 USA: 13	NR	NR	See note 2
Linalyl acetate ^c	359	115-95-7 	Yes Europe: 2100 USA: 180	No	Yes The NOEL of 24mg/kg of body weight per day reported in a 90-day study in rats is >500 times the daily per capita intake of linalyl acetate in Europe and the USA	See note 2
Linalyl propionate	360	144-39-8 	No Europe: 16 USA: 2	NR	NR	See note 2
Linalyl butyrate	361	78-36-4 	No Europe: 10 USA: 4	NR	NR	See note 2

No safety concern

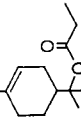
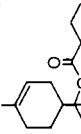
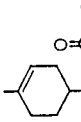
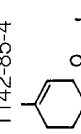
Table 5 (continued)

Substance	No.	CAS no. 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/A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Linalyl isobutyrate	362	78-35-3 	No Europe: 36 USA: 1	NR	NR	See note 2	No safety concern
Linalyl isovalerate	363	1118-27-0 	No Europe: 5 USA: 6	NR	NR	See note 2	
Linalyl hexanoate	364	7779-23-9 	No Europe: 1 USA: 0.4	NR	NR	See note 2	

Linalyl octanoate	365	10024-64-3		No Europe: 0.1 USA: 1	NR	NR	See note 2
α -Terpineol	366	98-55-5		Yes Europe: 3000 USA: 1100	No	Yes The NOEL of 500mg/kg of body weight per day reported in a 20-week study in rats is > 10000 times the daily per capita intake of α -terpineol in Europe and the USA	See note 3
Terpinyl formate	367	2153-26-6		No Europe: 0.1 USA: 2	NR	NR	See note 4
Terpinyl acetate	368	8007-35-0		No Europe: 260 USA: 390	NR	NR	See note 4

No safety concern

Table 5 (continued)

Substance	No.	CAS no. 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/A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Terpinyl propionate	369	80-27-3 	No Europe: 0.03 USA: 1	NR	NR	See note 4	No safety concern
Terpinyl butyrate	370	80-26-2 	No Europe: 6 USA: 6	NR	NR	See note 4	
Terpinyl isobutyrate	371	7774-65-4 	No Europe: 0.7 USA: 0.02	NR	NR	See note 4	
Terpinyl isovalerate	372	1142-85-4 	No Europe: 0.1 USA: 1	NR	NR	See note 4	

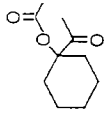
<i>p</i> -Menth-3-en-1-ol	373	586-82-3 	No Europe: 41 USA: 0.4	NR	NR	See note 3
4-Carvomenthenol	439	562-74-3 	No Europe: 170 USA: 51	NR	NR	See note 3
<i>p</i> -Menth-8-en-1-ol	374	138-87-4 	No Europe: 2 USA: 21	NR	NR	See note 3
4-Thujanol	441	546-79-2 	No Europe: 1 USA: 0.04	NR	NR	See note 1
Methyl 1-acetoxycyclohexylketone	442	52789-73-8 	— Europe: ND USA: 4	NA	No	—
						No safety concern
						Not evaluated ^a

Table 5 (continued)

Substance	No.	CAS no. 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/A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class II 2-Ethyl-1,3,3-trimethyl-2-norbornanol	440	18368-91-7 	No Europe: 1 USA: 0.02	NR	NR	See note 1	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data 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 substances in this group are expected to be metabolized to innocuous products, except methyl 1-acetoxycyclohexylketone.

^b The thresholds for human intake for classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.

^c The group ADI of 0–0.5 mg/kg of body weight per day established at the twenty-third meeting (Annex 1, reference 50) for citral, geranyl acetate, citronellol, linalool and linalyl acetate expressed as citral, was maintained.

^d This substance was not evaluated because a NOEL for this or a related substance was not available and the per capita intake exceeds 1.5 µg per day (step B5).

Notes to Table 5

1. Metabolized primarily by conjugation with glucuronic acid and excreted in the urine.
2. Hydrolysed to linalool and the corresponding carboxylic acid. Linalool is metabolized primarily by conjugation with glucuronic acid and excreted in the urine. Oxidation of the allylic methyl group may occur after repeated exposure.
3. Metabolized primarily by conjugation with glucuronic acid and excreted in the urine. Oxidation of the allylic methyl group followed by hydrogenation to yield the corresponding saturated acid may occur after repeated exposure.
4. Hydrolysed to α-terpineol and the corresponding carboxylic acid. Metabolized primarily by conjugation with glucuronic acid and excreted in the urine. Oxidation of the allylic methyl group followed by hydrogenation to yield the corresponding saturated acid may occur after repeated exposure.

Absorption, metabolism and elimination

It is anticipated that the esters in this group would be readily hydrolysed to their component alcohols and carboxylic acids. The products of hydrolysis would be detoxified primarily by conjugation with glucuronic acid and excreted in the urine. Alternatively, the allyl side-chain of unsaturated alcohols may undergo ω -oxidation to yield polar metabolites, which may be conjugated and excreted. Metabolites of acyclic alcohols may be further oxidized to eventually yield carbon dioxide. Hydrolysis of esters and metabolism of the products of hydrolysis are further discussed in section 4.1.

Application of the Procedure for the Safety Evaluation of Flavouring Agents
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances, the Committee assigned all but one of the 23 substances to structural class I. The remaining substance, 2-ethyl-1,3,3-trimethyl-2-norbornanol (no. 440), is a non-terpene bicyclic tertiary alcohol and was therefore assigned to structural class II.

Step 2. Adequate metabolism data were available on both linalool (no. 356) and α -terpineol (no. 366) to allow prediction of the likely pathways of metabolism of all the related compounds in the group. Although the site of oxidative metabolism would differ between compounds, the structures of linalool and α -terpineol would cover all of the functional groups present in the other members of the group of flavouring agents. On the basis of the information available on linalool and α -terpineol, the Committee concluded that, with one exception, the metabolism of compounds in this group could be predicted to give innocuous products.

Methyl 1-acetoxycyclohexylketone (no. 442) contains a sterically hindered ketone group and could not be considered *a priori* to share metabolic or toxicological similarities with other members of the group.

At their current levels of intake, 22 of the substances in this group (21 in class I and one in class II) would not be expected to saturate metabolic pathways and are predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. Methyl 1-acetoxycyclohexylketone (class I) is not predicted to be metabolized to innocuous products; its evaluation therefore proceeded via the right-hand side of the decision-tree.

Step A3. The intakes of 22 substances were evaluated at this step. Of these, 18 of the 21 substances in this group that were assigned to class

I have daily per capita intakes in Europe and the USA that are below the threshold for class I (1800µg). Therefore, these 18 substances were considered to pose no safety concern when used as flavouring agents at their current levels of estimated intake. The daily per capita intakes of the three remaining substances, linalool (no. 356), linalyl acetate (no. 359) and α -terpineol (no. 366), exceed the threshold for class I in Europe. The evaluation of these substances therefore proceeded to step A4.

The total daily per capita intakes of linalool and its esters (nos 358–365) and α -terpineol and its esters (nos 367–372) in Europe from their use as flavouring agents is above the threshold for class I. The total daily per capita intake of linalool and its esters is 4800µg in Europe and 1300µg in the USA. The total daily per capita intake of α -terpineol and its esters is 3200µg in Europe and 1400µg in the USA.

The total daily per capita intake of the class II substance, 2-ethyl-1,3,3-trimethyl-2-norbornanol (no. 440), is below the threshold for class II (540µg), indicating that this substance does not pose a safety concern when used at current levels of estimated intake as a flavouring agent.

Step A4. Linalool (no. 356), linalyl acetate (no. 359) and α -terpineol (no. 366) are not endogenous in humans and they are not predicted to be metabolized to endogenous products. The evaluation of these substances therefore proceeded to step A5.

Step A5. NOELs of 50mg/kg of body weight per day and 24mg/kg of body weight per day have been reported for linalool (no. 356) and linalyl acetate (no. 359), respectively, in 90-day studies in rats. These NOELs provide a margin of safety of >1000 for linalool and >500 for linalyl acetate and for linalool and its esters when compared with the daily per capita intakes of these substances in Europe and the USA. A NOEL of 500mg/kg of body weight per day has been reported for terpinyl acetate in a 20-week study in rats. This NOEL provides a margin of safety of approximately 10000 for α -terpineol and for α -terpineol and its esters when compared with the daily per capita intakes of these substances in Europe and the USA. A carcinogenicity bioassay in rats was conducted using a mixture of the terpenoid esters geranyl acetate and citronellyl acetate, which together with linalool and linalyl acetate, were reviewed at the twenty-third meeting of the Committee (Annex 1, reference 50). The NOEL of 1000mg/kg of body weight per day (710mg/kg of body weight per day for geranyl acetate and 290mg/kg of body weight per day for citronellyl acetate) in this study provides a margin of safety of >10000 for linalool and its esters and for α -terpineol and its esters.

Step B3. The daily per capita intake of methyl 1-acetoxycyclohexylketone (no. 442) in the USA is below the threshold for class I (1800µg). No intake data were reported for Europe. The evaluation of this substance therefore proceeded to step B4.

Step B4. Adequate data to determine a NOEL for methyl 1-acetoxycyclohexylketone (no. 442) or structurally related substances were not available. The evaluation of this substance therefore proceeded to step B5.

Step B5. The conditions of use of methyl 1-acetoxycyclohexylketone (no. 442) result in an intake greater than 1.5µg per day. Accordingly, additional data on this substance were required for its evaluation.

Table 5 summarizes the evaluation of the 23 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances used as flavouring agents.

Consideration of combined intakes

The data were adequate to complete an evaluation of 22 of the 23 tertiary alcohols and related esters in this group. In the unlikely event that all foods containing all 22 of these substances were consumed simultaneously on a daily basis, the estimated combined intake would exceed the threshold for human intake for class I. However, these 22 substances are expected to be efficiently metabolized and would not saturate metabolic pathways. In considering combined intake, the Committee excluded intake of methyl 1-acetoxycyclohexylketone (no. 442) because additional data on toxicity were required to evaluate its safety.

Consumption of foods containing four additional esters of linalool (linalyl anthranilate, linalyl benzoate, linalyl cinnamate and linalyl phenylacetate) that were not evaluated as part of this group, would contribute an extra 10µg or 0.2µg to the total daily per capita intake of linalool (no. 356) in Europe and the USA, respectively. Similarly, intake of foods containing an additional ester of α -terpineol (terpinyl cinnamate) would contribute an extra 0.008µg or 0.5µg to the total daily per capita intake of α -terpineol (no. 366) in Europe and the USA, respectively. The potential consumption of linalool and α -terpineol from these additional esters is minor and would not be expected to lead to saturation of metabolic pathways or alter the outcome of the safety evaluation using the Procedure.

Conclusions

The Committee concluded that 22 of the 23 terpenoid tertiary alcohols and related substances in this group would not present safety

concerns when used at current levels of intake as flavouring agents. Knowledge of the pathways of metabolism of these flavouring agents and toxicity data on three substances (linalool, linalyl acetate and α -terpineol (nos 356, 359 and 366)) were required for application of the Procedure. The Committee noted that these data and the available toxicity data on other structurally related substances were consistent with the results of the safety evaluation using the Procedure. For one substance, methyl 1-acetoxycyclohexylketone (no. 442), the available metabolic data were inadequate to predict that the substance would be metabolized to innocuous products, a relevant NOEL was lacking, and intake exceeded 1.5 μ g per day. According to the Procedure, the Committee concluded that additional data were required for the evaluation of methyl 1-acetoxycyclohexylketone. The group ADI established previously for linalool, linalyl acetate, citral, citronellol and geranyl acetate was maintained.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.5 Carvone and structurally related substances

The Committee evaluated carvone and eight related substances (Table 6) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1). The substances in this group are terpenoid ketones, secondary alcohols and related esters containing a 2-menthyl carbon skeleton.

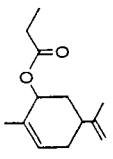
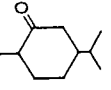
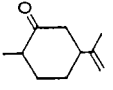
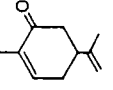
The Committee had previously evaluated carvone on several occasions. A conditional ADI of 0–1.25 mg/kg of body weight for the (+)- and (–)-isomers was established at the eleventh meeting (Annex 1, reference 14). A temporary ADI of 0–1 mg/kg of body weight was established for (+)- and (–)-carvone at the twenty-third meeting (Annex 1, reference 50), which was extended at the twenty-fifth, twenty-seventh, thirtieth and thirty-third meetings (Annex 1, references 56, 62, 73 and 83). At its thirty-seventh meeting, the Committee determined that the (+)- and (–)-enantiomers should be evaluated separately. Owing to a lack of data on (–)-carvone *per se*, the temporary ADI for (–)-carvone was not extended. In its review of (+)-carvone, the Committee considered a long-term toxicity/carcinogenicity study in mice, short-term toxicity studies in mice and rats and *in vitro* tests for mutagenicity. On the basis of a NOEL of 93 mg/kg of body weight per day in a 3-month toxicity study in rats, the Committee established an ADI for (+)-carvone of 0–1 mg/kg of body weight per day.

Table 6

Summary of the results of safety evaluations of carvone and eight structurally related substances^a

Substance	No.	CAS no. and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Endogenous or metabolized to endogenous substances?	Step A5 Adequate NOEL for substance?	Conclusion based on current intake
Structural class I						
<i>p</i> -Menthane-2-ol	376	499-69-4 	No Europe: 0.01 USA: 7	NR	NR	No safety concern
Dihydrocarveol	378	619-01-2 	No Europe: 3 USA: 320	NR	NR	
Dihydrocarvyl acetate	379	20777-49-5 	No Europe: 15 USA: 0.1	NR	NR	
Carveol	381	99-48-9 	No Europe: 15 USA: 140	NR	NR	
Carvyl acetate	382	97-42-7 	No Europe: 6 USA: 36	NR	NR	

Table 6 (continued)

Substance	No.	CAS no. and structure	Step A3 ^a Does intake exceed the threshold for human intake?	Step A4 Endogenous or metabolized to endogenous substances?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Carvyl propionate	383	97-45-0 	No Europe: ND USA: 0.04	NR	NR	No safety concern
Structural class II <i>p</i> -Menthane-2-one	375	499-70-7 	No Europe: 0.01 USA: 1	NR	NR	No safety concern
Dihydrocarvone	377	7764-50-3 	No Europe: 0.02 USA: 180	NR	NR	No safety concern
Carvone ^c	380	2244-16-8 (+)- 6485-40-1 (-)- 	Yes Europe: 2800 USA: 9900	No	Yes	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data 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 substances in this group are metabolized to innocuous substances.

^b The thresholds for human intake for classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.

^c The ADI of 0–1 mg/kg of body weight previously established for (+)-carvone at the thirty-seventh meeting of the Committee (Annex 1, reference 94) was maintained.

Estimated daily per capita intake

The estimated total daily per capita intake of carvone (no. 380) and related substances from their use as flavouring agents is 2.8mg in Europe and 10mg in the USA. In Europe, carvone accounts for approximately 99% of the total annual per capita intake of this group of substances from their use as flavouring agents. In the USA, carvone accounts for approximately 96% of the total annual per capita intake of this group of substances from their use as flavouring agents.

Seven of the substances in this group have been reported to occur naturally in foods, including fruits, spices and berries. (–)-Carvone has been reported to occur in the oils of *Mentha spicata* (spearmint). (+)-Carvone has been reported in *Carum carvi* (caraway) and *Anethum graveolens* (dill). Quantitative data on the natural occurrence and consumption ratios¹ have been reported for five of these substances (nos 377, 379–382), which indicate that they are consumed predominantly in traditional foods (i.e. consumption ratio >1).

Absorption, metabolism and elimination

This group consists of three ketones (nos 375, 377, 380), three secondary alcohols (nos 376, 378, 381) and three esters (nos 379, 382, 383). The metabolism of these substances is discussed in general terms in section 4.1.

Terpenoid esters. The three esters would be expected to be hydrolysed to their corresponding alcohol and carboxylic acid by carboxylesterases, which occur predominantly in hepatocytes. Esters of carveol (no. 381) and dihydrocarveol (no. 378) would be expected to be hydrolysed to yield carveol and dihydrocarveol, respectively, and the corresponding saturated aliphatic carboxylic acids.

Terpenoid alcohols and ketones. The terpenoid alcohols resulting from hydrolysis of esters and their corresponding ketones are metabolized in the same pathways as other alicyclic terpenoid ketones and secondary alcohols. Five detoxification pathways have been identified:

- reduction of the ketone, followed by conjugation of the resulting alcohol with glucuronic acid;

¹ Defined as the ratio of the per capita intake resulting from the natural occurrence of a flavouring agent in food to the per capita intake of the flavouring agent from its intentional addition to food. Thus, a large consumption ratio signifies that the human intake from natural occurrence of the compound in food is much greater than the intake that results from its intentional addition to food. A consumption ratio of zero indicates that the compound does not occur naturally in food as far as is presently known.

- oxidation of the side-chains, yielding polar metabolites, which may be conjugated and excreted;
- conjugation of ketones with glutathione, followed by excretion;
- hydrogenation of the endocyclic double bond of carveol;
- excretion of the unchanged parent compound.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to carvone and the above-mentioned related substances, the Committee assigned six of the nine substances (*p*-menthan-2-ol, dihydrocarveol, dihydrocarvyl acetate, carveol, carvyl acetate and carvyl propionate (nos 376, 378, 379, 381–383)) to structural class I. The remaining three substances (*p*-menthan-2-one, dihydrocarvone and carvone (nos 375, 377 and 380)) were assigned to structural class II as they contain an α,β -unsaturated ketone.

Step 2. Sufficient data were available on carvone to define the major pathways of metabolism, and this compound contained all of the key structural elements and potential sites of metabolism of all other members in the group. Better data on metabolism were available on isophorone (3,4,5-trimethyl-cyclohex-2-en-1-one), which is not a member of this group of flavouring agents, but which shows close structural similarities to carvone and shares the same routes of metabolism (reduction of the carbonyl group, conjugation of the resulting alcohol with glucuronic acid, and side-chain oxidation). It is predicted that carvone and other α,β -unsaturated ketones in the group might undergo conjugation with glutathione, but this would be expected to be a minor route of metabolism because of the low reactivity *in vitro*, a conclusion supported by the toxicological data.

For the three terpenoid esters (nos 379, 382 and 383) in class I, the most likely route of metabolism is hydrolysis to carveol or dihydrocarveol. For the three terpenoid alcohols (nos 376, 378 and 381) which are also in class I, the available data indicated that the most likely route of metabolism is conjugation with glucuronic acid, followed by excretion; however, other routes of metabolism such as side-chain oxidation followed by conjugation and excretion may also be possible. In each case, metabolism yields innocuous metabolites. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

For the three terpenoid ketones (nos 375, 377 and 380) in class II, the available data indicated that the most likely route of metabolism is reduction of the ketone to the corresponding alcohol, followed by conjugation with glutathione and excretion. Other routes of metabolism are also possible, such as side-chain oxidation followed by

conjugation, and direct conjugation of the ketone with glutathione. In all cases, metabolism yields innocuous metabolites. Accordingly, the evaluation of these substances also proceeded via the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of the six terpenoid esters and alcohols in class I are below the threshold of concern for this class (1800 µg per day), and they would not be expected to be of safety concern. The intakes of two of the three terpenoid ketones in class II, *p*-menthan-2-one (no. 375) and dihydrocarvone (no. 377) are below the threshold of concern for this class (540 µg per day), and these would not be expected to be of safety concern. For the remaining substance, carvone (no. 380), the intakes in both Europe and the USA are above the threshold of concern. The evaluation of this substance therefore proceeded to step A4.

Step A4. Carvone (no. 380) is not endogenous in humans. The evaluation of this substance therefore proceeded to step A5.

Step A5. A NOEL of 93 mg/kg of body weight per day was identified for (+)-carvone in a 3-month study in rats at the thirty-seventh meeting of the Committee (Annex 1, reference 94). If it is assumed that all the carvone consumed was (+)-carvone, a margin of safety of >500 exists between this NOEL and the daily per capita intake for the (+)-isomer of carvone. Therefore this substance would not be expected to be of safety concern. The (–)-isomer of carvone would be expected to share a common metabolic pathway with (+)-carvone. A NOEL of 125 mg/kg of body weight per day was identified for carvone (isomer unspecified) at the eleventh meeting (Annex 1, reference 14). The only material that was commercially available at that time was (–)-carvone. This NOEL was considered to apply to (–)-carvone and, if it is assumed that all the carvone intake was (–)-carvone, a margin of safety of >750 exists between this NOEL and the daily per capita intake for (–)-carvone. Accordingly, this substance would not be expected to be of safety concern.

Table 6 summarizes the evaluations of the nine substances in this group.

Consideration of combined intakes

In the unlikely event that all foods containing all six terpenoid alcohols and esters in structural class I were consumed concomitantly on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would be below the threshold for class I.

In the unlikely event that all foods containing all three terpenoid ketones in structural class II were consumed concomitantly on a daily basis, the estimated daily per capita intake of these substances in Europe and the USA would exceed the threshold for class II.

However, all nine substances in this group are expected to be efficiently metabolized to innocuous products and would not give rise to perturbations outside the physiological range.

Conclusions

The results of the evaluations of carvone (no. 380) and related substances (nos 375–379 and 381–383) indicated that these substances would not present safety concerns at the estimated current levels of intake. In using the Procedure, the Committee noted that all of the available data on toxicity were consistent with the results of the safety evaluation. The ADI established previously for (+)-carvone was maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.6 Ionones and structurally related substances

The Committee evaluated a group of 21 flavouring agents that included α - and β -ionone and structurally related substances (Table 7) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Each of these substances has a cyclohexene ring with an allyl or alkyl side-chain containing a ketone or secondary alcohol functional group. With one exception, namely, 1,4-dimethyl-4-acetyl-1-cyclohexene, each contains a 2,6,6-trimethylcyclohexyl carbon skeleton, and an alkyl side-chain of 4–7 carbon atoms located at the C1 position. With the exception of γ -ionone, each of these substances has at least one endocyclic double bond. In the ionones, the carbonyl or hydroxyl group is located in the γ -position in relation to the ring, while in the damascones the carbonyl group is located in the α -position.

The Committee had previously evaluated three members of the group. α -Ionone and β -ionone were both evaluated at the twenty-eighth meeting (Annex 1, reference 66), when a group ADI of 0–0.1 mg/kg of body weight was established for these flavouring agents, individually or in combination. Allyl- α -ionone was evaluated at the twenty-fourth meeting (Annex 1, reference 53), when the Committee concluded that the data were inadequate for setting an ADI.

Table 7

Summary of the results of the safety evaluations of 21 ionones and structurally related substances^a

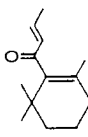
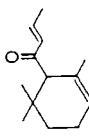
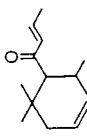
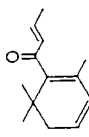
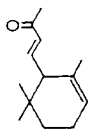
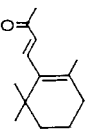
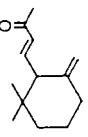
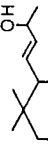
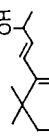
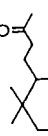
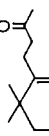
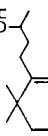
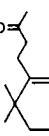
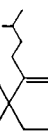
Substance	No.	CAS no. and structure	Step 2 Metabolized to innocuous products?	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Conclusion based on current intake
β -Damascone	384	23726-92-3 	No	No Europe: 43 USA: 10	Yes	No safety concern
α -Damascone	385	43052-87-5 	No	No Europe: 8 USA: 0.4	Yes	
δ -Damascone	386	57378-68-4 	No	No Europe: 0.06 USA: 0.6	Yes	
Damascenone	387	23696-85-7 	No	No Europe: 86 USA: 5	Yes	
α -Ionone ^c	388	127-41-3 	Yes	No Europe: 310 USA: 150	NA	
β -Ionone ^c	389	14901-07-6 	Yes	No Europe: 150 USA: 100	NA	
γ -Ionone	390	79-76-5 	No	No Europe: 0.01 USA: 15	Yes	

Table 7 (continued)

Substance	No.	CAS no. and structure	Step 2 Metabolized to innocuous products?	Step A3/B3 ^a Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Conclusion based on current intake
α -Ionol	391	25312-34-9 	Yes	No Europe: 0.7 USA: 0.06	NA	No safety concern
β -Ionol	392	22029-76-1 	Yes	No Europe: 0.9 USA: 0.1	NA	
Dihydro- α -ionone	393	31499-72-6 	Yes	No Europe: 0.7 USA: 0.02	NA	
Dihydro- β -ionone	394	17283-81-7 	Yes	No Europe: 1 USA: 0.04	NA	
Dihydro- β -ionol	395	3293-47-8 	Yes	No Europe: 0.3 USA: 0.02	NA	
Dehydrodihydroionone	396	20483-36-7 	No	No Europe: 0.1 USA: 0.08	Yes	
Dehydrodihydroionol	397	57069-86-0 	No	No Europe: 8 USA: 0.01	Yes	

Methyl- α -ionone	398	127-42-4 	Yes	No Europe: 100 USA: 7	NA	No safety concern
Methyl- β -ionone	399	127-43-5 	Yes	No Europe: 6 USA: 0.2	NA	
Methyl- δ -ionone	400	7748-98-7 	Yes	No Europe: 0.4 USA: 1	NA	
Allyl- α -ionone	401	79-78-7 	Yes	No Europe: 35 USA: 25	NA	
1,4-Dimethyl-4-acetyl-1-cyclohexene	402	43219-68-7 	—	— Europe: — USA: —	—	Not evaluated ^d
α -Irone	403	79-69-6 	Yes	No Europe: 9 USA: 3	NA	No safety concern
α -iso-Methylionone	404	127-51-5 	Yes	No Europe: 6 USA: 1	NA	

CAS: Chemical Abstracts Service; NA: not applicable.

^a Step 1: All of the substances in this group are in structural class I.

^b The threshold for human intake for class I is 1800 μ g per day. All intake values are expressed in μ g per day.

^c The group ADI of 0–0.1 mg/kg of body weight established at the twenty-eighth meeting of the Committee (Annex 1, reference 66) for α -ionone and β -ionone was maintained.

^d 1,4-Dimethyl-4-acetyl-1-cyclohexene was not considered to have sufficient structural similarities to the ionones to be included in this group. Accordingly, the safety evaluation of this compound was not considered at the present meeting.

Estimated daily per capita intake

The estimated total daily per capita intake of all 21 ionones and related substances from their use as flavouring agents is 0.76mg in Europe and 0.33mg in the USA. In Europe, four substances, damascenone (no. 387), α -ionone (no. 388), β -ionone (no. 389) and methyl- α -ionone (no. 398), account for approximately 85% of the total annual per capita intake of this group of substances from their use as flavouring agents. In the USA, three substances, α -ionone, β -ionone and allyl- α -ionone (no. 401), account for approximately 85% of the total annual per capita intake of this group of substances from their use as flavouring agents.

Eleven of the substances in this group have been reported to occur naturally in foods, including raspberries, carrots, roasted almonds and herbs. Quantitative data on natural occurrence and consumption ratios have been reported for seven substances (nos 388–394), which indicate that they are consumed predominantly in traditional foods (i.e. consumption ratio >1).

Absorption, metabolism and elimination

The available data on metabolism of substances in this group are derived largely from studies on β -ionone and indicate that at least two detoxification pathways are possible (see section 4.1):

- hydroxylation of the cyclohexene ring at the C3 position followed by oxidation of the hydroxyl group to the 3-oxo derivative; and
- reduction of the ketone on the allyl side-chain to the corresponding secondary alcohol.

A combination of these reactions results in the formation of polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acid. β -Ionone can also be excreted unchanged in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

1,4-Dimethyl-4-acetyl-1-cyclohexene (no. 402) was not considered to have sufficient structural similarities to the ionones to be included in this group and therefore the safety of this compound was not evaluated.

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the remaining 20 ionones and related substances, the Committee assigned all of the substances to structural class I.

Step 2. Data were available for β -ionone (no. 389), which showed that it was metabolized by reduction of the carbonyl group and hydroxy-

lation of the alicyclic ring and by conjugation of metabolites containing alcohol groups with glucuronic acid. Only limited data were available on the fate of other compounds in the group of α - and β -ionone and related flavouring agents. Other members of this group had structural characteristics similar to β -ionone, but there were differences in the number and positions of the alicyclic double bonds and the position of the carbonyl group within the side-chain and its relationship to endocyclic and exocyclic double bonds. On the basis of the similarities of the functional groups present in the flavouring agents in this group it was considered that α - and β -ionone (nos 388 and 389), their alcohol analogues, analogues with a single endocyclic double bond, and analogues with saturated side-chains or longer side-chains would be eliminated from the body by common metabolic processes, which would lead to innocuous products.

Members of the group with two endocyclic double bonds, or with the carboxyl group adjacent to the cyclohexene ring, or with an allyl double bond attached to the cyclohexene ring, might show reduced rates of elimination which might affect their toxicological potency. The Procedure takes this possibility into account to some extent since compounds that are sterically hindered are assigned to structural class II, rather than class I. However, the Committee was not able to conclude *a priori* that the products of metabolism of such compounds would be innocuous.

Substances that could be predicted to be metabolized to innocuous products fall into the following two groups:

Group 1: α -Ionone, α -ionol, dihydro- α -ionone, methyl- α -ionone, methyl- δ -ionone, α -irone, 2-iso-methylionone and allyl- α -ionone (nos 388, 391, 393, 398, 400, 403, 404 and 401, respectively). These substances were considered likely to share a common metabolic pathway with α -ionone, although the rate of metabolism for some of these substances may be slower than for α -ionone.

Group 2: β -Ionol, dihydro- β -ionone, dihydro- β -ionol, methyl- β -ionone and methyl- δ -ionone (nos 392, 394, 395, 399 and 400, respectively). These substances were considered likely to share common metabolic pathways with β -ionone, although the rate of metabolism for some of these substances may be slower than for β -ionone.

The evaluation of the substances in groups 1 and 2 proceeded via the left-hand side of the decision-tree.

The substances that could not be predicted to be metabolized to innocuous products are β -damascone, α -damascone, δ -damascone,

damascenone, γ -ionone, dehydrodihydroionone and dehydrodihydroionol (nos 384–387, 390, 396 and 397, respectively). The evaluation of these substances proceeded via the right-hand side of the decision-tree.

Step A3. The daily per capita intakes in Europe and the USA of all 13 substances in groups 1 and 2 are below the threshold for human intake for class I (1800 μ g per day). Therefore, these substances would not be expected to be of safety concern.

Step B3. For β -damascone, α -damascone, δ -damascone, damascenone, γ -ionone, dehydrodihydroionone and dehydrodihydroionol (nos 384–387, 390, 396 and 397, respectively), the daily per capita intakes in Europe and the USA are below the threshold for class I. Accordingly, the evaluation of these substances proceeded to step B4.

Step B4. Information on each of the compounds considered at this step is given below.

The NOEL for β -damascone (no. 384) is 2 mg/kg of body weight per day, based on a 90-day study in rats. The margin of safety between this NOEL and the daily per capita intake under the conditions of intended use is >2000.

α -Damascone, δ -damascone and damascenone (nos 385–387) were considered likely to share common metabolic pathways with β -damascone (no. 384), although the rate of metabolism may be slower. The margins of safety between the NOEL for β -damascone and the daily per capita intakes of α -damascone, δ -damascone and damascenone are >10000, 200000 and >1000, respectively.

γ -Ionone (no. 390) was considered likely to share a common metabolic pathway with α -ionone (no. 388) and β -ionone (no. 389), for which the NOEL was 10 mg/kg of body weight per day, based on a 90-day study in rats. The margin of safety between this NOEL and the daily per capita intake of γ -ionone is 40000. It was also considered likely to share a common metabolic pathway with carvone (no. 380), for which the NOEL was 93 mg/kg of body weight per day, based on a 3-month study in rats. In this case the margin of safety between this NOEL and the daily per capita intake of γ -ionone is 370000.

Dehydrodihydroionone (no. 396) and dehydrodihydroionol (no. 397) were considered to share common metabolic pathways with α -ionone and β -ionone, for which the NOEL was 10 mg/kg of body weight per day. The margins of safety between this NOEL and the daily per capita intakes of dehydrodihydroionone and dehydrodihydroionol are 6×10^6 and 80000, respectively.

Therefore, the seven substances considered at this step would not be expected to be of safety concern.

Table 7 summarizes the evaluations of the 21 α - and β -ionones and related substances used as flavouring agents.

Consideration of combined intakes

All of the 20 ionones and structurally related substances considered in this evaluation would be expected to share common metabolic pathways. In the unlikely event that all foods containing all 20 substances were consumed simultaneously on a daily basis, the estimated daily per capita consumption in Europe and the USA would not exceed the threshold for human intake for substances in class I.

Conclusions

The Committee concluded that use of any of the 20 ionones and related substances as flavouring agents would not present safety concerns at the estimated current levels of intake.

In using the Procedure, the Committee noted that all of the available data on toxicity were consistent with the results of the safety evaluation.

The ADIs previously established for α -ionone and β -ionone were maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.7 Aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones

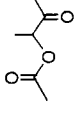
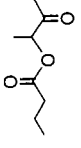
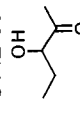
The Committee evaluated a group of 22 flavouring agents that included aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones (Table 8) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1). One member of the group, diacetyl (2,3-butanedione), was previously evaluated at the eleventh meeting of the Committee (Annex 1, reference 14). However, no ADI was allocated at that time because of a lack of data.

Estimated daily per capita intake

In the USA, aliphatic acyclic and alicyclic α -diketones and α -hydroxyketones are generally used as flavouring agents up to average maximum levels of 200 mg/kg. The total annual volume of production of the 22 substances in this group is approximately 44 tonnes in Europe and 56 tonnes in the USA. In both Europe and the USA,

Table 8

Summary of the results of safety evaluations of 22 aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones^a

Substance	No.	CAS no. 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 NOEL for substance or related substance?	Conclusion based on current intake
Acetoin	405	513-86-0 	Yes Europe: 2800 USA: 1800	Yes	NR	No safety concern
2-Acetoxy-3-butanone	406	4906-24-5 	No Europe: 0.03 USA: 23	NR	NR	
Butan-3-one-2-yl-butanolate	407	84642-61-5 	No Europe: 0.02 USA: 0.95	NR	NR	
Diacetyl	408	431-03-8 	Yes Europe: 3300 USA: 8000	Yes	NR	
3-Hydroxy-2-pentanone	409	3142-66-3 	No Europe: ND USA: 0.10	NR	NR	

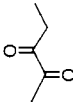
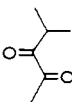
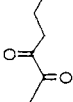
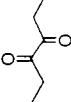
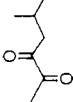
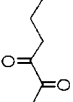
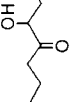
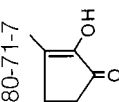
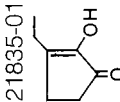
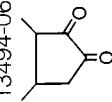
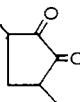
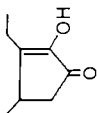
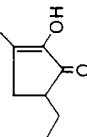
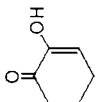
2,3-Pentanedione	410	600-14-6		No Europe: 220 USA: 80	NR	NR
4-Methyl-2,3-pentadione	411	7493-58-5		No Europe: 0.48 USA: 2	NR	NR
2,3-Hexanedione	412	3848-24-6		No Europe: 13 USA: 10	NR	NR
3,4-Hexanedione	413	4437-51-8		No Europe: 33 USA: 0.76	NR	NR
5-Methyl-2,3-hexanedione	414	13706-86-0		No Europe: 2 USA: 6	NR	NR
2,3-Heptanedione	415	96-04-8		No Europe: 2 USA: 5	NR	NR
5-Hydroxy-4-octanone	416	496-77-5		No Europe: 0.02 USA: 0.76	NR	NR
						No safety concern

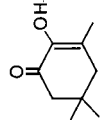
Table 8 (continued)

Substance	No.	CAS no. 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 NOEL for substance?	Conclusion based on current intake
2,3-Undecadione	417	7493-59-6 	No Europe: 0.02 USA: 0.01	NR	NR	No safety concern
Methylcyclopentenolone	418	80-71-7 	Yes Europe: 890 USA: 710	No	Yes The NOEL of 500mg/kg of body weight per day reported in a 6-month toxicity study in rats is >30000 times the daily per capita intake of methylcyclopentenolone	
Ethylcyclopentenolone	419	21835-01-8 	No Europe: 50 USA: 23	NR	NR	
3,4-Dimethyl-1,2-cyclopentanedione	420	13494-06-9 	No Europe: 47 USA: 2	NR	NR	

3,5-Dimethyl-1,2-cyclopentanedione	421	13494-07-0		No Europe: 55 USA: 29	NR	NR
3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one	422	42348-12-9		No Europe: ND USA: 0.17	NR	NR
5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one	423	53263-58-4		No Europe: ND USA: 0.38	NR	NR
2-Hydroxy-2-cyclohexen-1-one	424	10316-66-2		No Europe: 0.08 USA: 0.76	NR	NR
1-Methyl-2,3-cyclohexadione	425	3008-43-3		No Europe: 2 USA: 8	NR	NR

No safety
concern

Table 8 (continued)

Substance	No.	CAS no. 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 NOEL for substance or related substance?	Conclusion based on current intake
2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one	426	4883-60-7 	No Europe: 2 USA: 2	NR	NR	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 (nos 406, 407, 409-417 and 419-426) or step A4 (nos 405 and 408) of the Procedure.

^a Step 1: All of the substances in the group are in structural class II.

^b Step 2: All of the substances in this group are metabolized to innocuous products.

The threshold for human intake for class II is 540 µg per day. All intake values are expressed in µg per day.

more than 95% of the total annual volume is accounted for by three substances: acetoin (3-hydroxy-2-butanone; no. 405; 19 tonnes/year in Europe and 9.2 tonnes/year in the USA), diacetyl (no. 408; 18 tonnes/year in Europe and 42 tonnes/year in the USA) and methylcyclopentenolone (no. 418; 4.7 tonnes/year in Europe and 3.7 tonnes/year in the USA). Two of these substances (acetoin and diacetyl) account for more than 90% of the total annual volume in the USA.

Nineteen of the 22 aliphatic acyclic and alicyclic α -diketones and α -hydroxyketones have been identified as natural components of a variety of foods, including fruits, vegetables, cocoa and coffee. Quantitative data on natural occurrence have been reported for six of these substances. On the basis of the information available, the levels of intake of these substances as natural components of food would appear to be greater than those from their use as flavouring agents, with one exception (diacetyl).

Absorption, metabolism and elimination

In rats and mice, orally administered aliphatic α -diketones are rapidly absorbed from the gastrointestinal tract. It is anticipated that at low levels of intake, humans will metabolize aliphatic acyclic α -diketones principally by α -hydroxylation and subsequent oxidation of the terminal methyl group to yield the corresponding ketocarboxylic acid. The acid may undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which may be completely metabolized in the fatty acid pathway and citric acid cycle. At high levels of intake, aliphatic acyclic α -diketones may be metabolized by reduction to the diol and subsequent conjugation with glucuronic acid. Acyclic α -diketones and α -hydroxyketones without a terminal methyl group and alicyclic α -diketones and α -hydroxyketones are mainly metabolized by reduction to the corresponding diol followed by conjugation with glucuronic acid and excretion (see section 4.1).

Application of the Procedure for the Safety Evaluation of Flavouring Agents
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones, the Committee assigned all 22 substances to structural class II.

Step 2. *In vitro* and *in vivo* data demonstrated two major routes of metabolism for diacetyl (no. 408) and acetoin (no. 405) involving complete oxidation to carbon dioxide and reduction to a diol, but data were not available on the longer-chain linear homologues or cyclic analogues. Alcohol dehydrogenases, aldehyde reductase and

carbonyl reductase are widely distributed enzymes, with broad substrate specificities, and *in vitro* data show that 1,2-cyclohexanedione is a better substrate than diacetyl for aldehyde reductase and carbonyl reductase. In consequence, it was considered that the data on the extensive reduction of acetoin can be extrapolated to other members of the group. The α -diketone group is polar, and it would be expected that all members of the group evaluated would be metabolized by a combination of oxidation (when the carbonyl group is adjacent to a methyl group), reduction of the carbonyl group and excretion of the parent compound and metabolites in urine. The products of these metabolic pathways do not raise toxicological concerns. This conclusion is supported by the toxicological data available on some members of the group.

Step A3. For 19 of the substances, the daily per capita intakes in Europe and the USA are below the threshold for human intake for substances in class II (540 μ g per day), indicating that they pose no safety concern when used at current levels of estimated intake as flavouring agents. Only acetoin (no. 405), diacetyl (no. 408) and methylcyclopentenolone (no. 418) have intakes greater than 540 μ g per day in Europe and the USA.

Step A4. Acetoin (no. 405) and diacetyl (no. 408) are endogenous in humans, but methylcyclopentenolone (no. 418) is not. The evaluation of this substance therefore proceeded to step A5.

Step A5. A NOEL of 500 mg/kg of body weight per day was reported for methylcyclopentenolone (no. 418) in a 6-month toxicity study in rats. A safety margin of >30 000 exists between this NOEL and the estimated daily per capita intake of methylcyclopentenolone. In addition, the results of genotoxicity tests (Ames test and test for unscheduled DNA synthesis) of methylcyclopentenolone were negative. This information indicates that methylcyclopentenolone would not be expected to be of safety concern.

The stepwise evaluations of the 22 aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones used as flavouring agents are summarized in Table 8.

Consideration of combined intakes

In the unlikely event that all foods containing all of the 22 aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones evaluated as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would exceed the threshold for human intake for substances in class II. However, all of the flavouring agents in this

group are expected to be efficiently metabolized and excreted and would not saturate detoxification pathways. On the basis of the evaluation of the collective data, the Committee concluded that combined intake of these substances would not be expected to be of safety concern.

Conclusion

The Committee concluded that the 22 aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones evaluated do not pose a safety concern when used at current levels of intake as flavouring agents. No toxicity data were required for application of the Procedure to 21 of the 22 substances evaluated. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure. For the remaining substance, methylcyclopentenolone (no. 418), the toxicity data indicated that it would not be expected to be of safety concern.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.8 Substances structurally related to menthol

The Committee evaluated 13 substances structurally related to menthol (Table 9) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1). Menthol was evaluated separately (see section 3.2.3). However, it is included here because information on this substance is integral to the evaluation of its structurally related substances.

Menthol was previously evaluated at the eleventh, eighteenth and twentieth meetings of the Committee (Annex 1, references 14, 35 and 41). At its present meeting, the Committee allocated an ADI of 0–4 mg/kg of body weight to menthol (section 3.2.3).

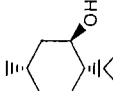
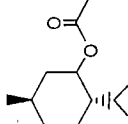
Estimated daily per capita intake

The total annual volume of production of menthol and the 13 structurally related substances from their use as flavouring agents is approximately 140 tonnes in Europe and 79 tonnes in the USA. Menthol (no. 427) and menthone (no. 429) account for 97% of the total annual volume in Europe and 85% of the total annual volume in the USA.

Menthol and some structurally related substances occur naturally in a wide variety of foods, including spearmint oil, cornmint oil, peppermint oil, raspberries, rum, nutmeg and cocoa. Menthol and

Table 9

Summary of the results of safety evaluations of menthol and 13 structurally related substances^a

Substance	No.	CAS structure and no.	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
Structural class I						
Menthol ^c	427	89-78-1 	Yes Europe: 18000 USA: 10000	No	Yes The NOEL of 380 mg/kg of body weight per day reported in a 2-year toxicity/carcinogenicity study in rats is > 1000 times the daily per capita intake of menthol in both Europe and the USA	No safety concern
(+)-neo-Menthol	428	2216-52-6 	No Europe: 3 USA: 27	NR	NR	
Menthyl acetate	431	16409-45-3 	No Europe: 420 USA: 560	NR	NR	

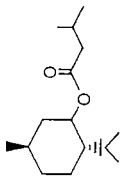
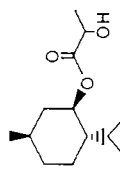
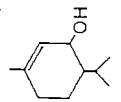
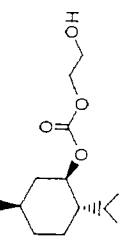
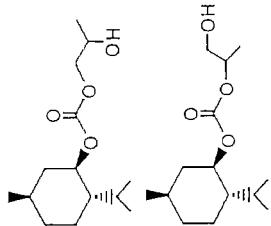
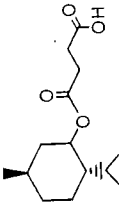
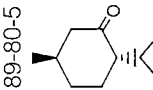
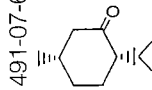
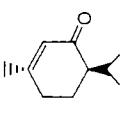
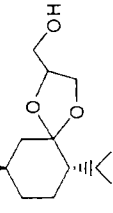
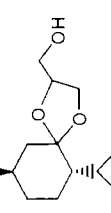
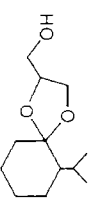
Menthyl isovalerate	432	16409-46-4		No Europe: 9 USA: 27	NR	NR	No safety concern
(-)-Menthyl lactate	433	59259-38-0		No Europe: 26 USA: 0.1	NR	NR	
<i>p</i> -Menth-1-en-3-ol	434	491-04-3		No Europe: 0.02 USA: 0.02	NR	NR	
(-)-Menthol ethylene glycol carbonate	443	156324-78-6		No Europe: ND USA: 760	NR	NR	
Mixture of (-)-menthol 1- and 2-propylene glycol carbonate	444	156329-82-2		No Europe: ND USA: 380	NR	NR	

Table 9 (continued)

Substance	No.	CAS structure and no.	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Conclusion based on current intake
mono-Menthyl succinate	447	77341-67-4 	No Europe: ND USA: 22	NR	NR	No safety concern
Structural class II Menthone	429	89-80-5 	Yes Europe: 1000 USA: 2500	No	Yes The NOEL of 400mg/kg of body weight per day reported in a 28-day toxicity study in rats is about 10000 times the daily per capita intake of menthone in both Europe and the USA	No safety concern
(±)-Isomenthone	430	491-07-6 	No Europe: 200 USA: 0.1	NR	NR	No safety concern

Piperitone	435	6091-50-5		No Europe: 51 USA: 10	NR	NR	No safety concern
(-)-Menthone 1,2-glycerol ketal	445	563187-91-7		No Europe: ND USA: 190	NR	NR	
(±)-Menthone 1,2-glycerol ketal	446	63187-91-7		No Europe: ND USA: 190	NR	NR	
							

CAS: Chemical Abstract Service; ND: no intake data 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 substances in this group are metabolized to innocuous products.

^b The thresholds for human intake for structural classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.

^c An ADI of 0-4 mg/kg of body weight was established for this substance at the present meeting.

menthone are the principal constituents of peppermint oil, accounting for 10–70% and 7–40%, respectively. Eight of the substances in this group have been reported to occur naturally in foods (nos 427–432, 434 and 435); six of the remaining substances are esters of menthol (nos 433, 443, 444 and 447) or ketals of menthone (nos 445 and 446). Quantitative data on natural occurrence and consumption ratios have been reported for five of these substances, which are consumed predominantly in traditional foods (i.e. consumption ratio >1).

Absorption, metabolism and elimination

The esters in this group (nos 431–433 and 447) would be expected to be readily hydrolysed to menthol and their respective carboxylic acids; the latter are endogenous in humans. The carbonate esters of (–)-menthol (nos 443 and 444) can be expected to be hydrolysed to menthol and carbonate and either ethylene glycol or propylene glycol. The ketals (nos 445 and 446) are hydrolysed *in vitro* to yield (–)- or (±)-menthone and simple glycols. The ketones (nos 429, 430 and 435) in this group would be reduced to their corresponding secondary alcohols which, like menthol, are conjugated with glucuronic acid and then excreted in the urine (see section 4.1).

Application of the Procedure for the Safety Evaluation of Flavouring Agents
Step 1. Menthol (no. 427) belongs to structural class I. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned substances structurally related to menthol, the Committee assigned eight of the 13 substances ((+)-neo-menthol, menthyl acetate, menthyl isovalerate, (–)-menthyl lactate, *p*-menth-1-en-3-ol, (–)-menthol ethylene glycol carbonate, the mixture of (–)-menthol 1- and 2-propylene glycol carbonate and mono-menthyl succinate (nos 428, 431–434, 443, 444 and 447, respectively)) to structural class I. The remaining five substances (menthone, (±)-isomenthone, piperitone, (–)-menthone 1,2-glycerol ketal and (±)-menthone 1,2-glycerol ketal (nos 429, 430, 435, 445 and 446, respectively)) were assigned to structural class II.

Step 2. At current levels of intake, the 14 substances would not be expected to saturate the metabolic pathways, and they are all predicted to be metabolized to innocuous products (see section 2.3.1). Consideration of this group of flavouring agents was dependent on data on the metabolic fate of menthol (no. 427) and menthone (no. 429), which are metabolized by a combination of oxidation and conjugation. The extensive conjugation of menthol with glucuronic acid and its rapid elimination in urine and bile, combined with its simple chemical structure, provided assurance that the products of

metabolism are innocuous. The metabolites of piperitone (an α,β -unsaturated ketone) were considered to be innocuous by comparison with menthone and its metabolites (saturated) and carvone (α,β -unsaturated).

Step A3. The daily per capita intakes of all of the substances in this group in structural class I, with the exception of menthol (no. 427), are below the threshold for human intake for class I (1800 μ g) in both Europe and the USA, indicating that they pose no safety concerns when used at the current levels of estimated intake as flavouring agents. The intake of menthol in Europe and the USA is above the threshold for human intake for substances in class I.

The total intake of menthol in Europe and the USA from its use and use of its esters (nos 431–433, 443, 444 and 447) as flavouring agents is above the threshold for human intake for class I. The total daily per capita intake of menthol and its esters is 19000 μ g in Europe and 12000 μ g in the USA.

The daily per capita intakes of all the substances in structural class II, with the exception of menthone (no. 429), are below the threshold for class II (540 μ g per day) in both Europe and the USA. The daily per capita intake of menthone in Europe and the USA is above the threshold for human intake for class II.

The total intake of menthone in Europe and the USA from its use and use of its ketals (nos 445 and 446) as flavouring agents is above the threshold for human exposure for class II. The total daily per capita intake of menthone and its ketals is 1000 μ g in Europe and 2900 μ g in the USA.

Step A4. Menthol (no. 427) and menthone (no. 429) are not endogenous in humans. The evaluation of these substances therefore proceeded to step A5.

Step A5. An ADI of 0–4mg/kg of body weight was allocated to menthol (no. 427) at the present meeting (see section 3.2.3). For menthone (no. 429), a NOEL of 400mg/kg of body weight per day has been reported in a 28-day toxicity study in rats. A safety margin of approximately 10000 exists between this NOEL and the daily per capita intake of menthone (42 μ g/kg of body weight per day in Europe and 17 μ g/kg of body weight per day in the USA) or the total daily per capita intake of menthone and its derivatives (46 and 17 μ g/kg of body weight per day, respectively). This information indicates that neither menthol nor menthone would be expected to be of safety concern.

Table 9 summarizes the evaluation of menthol and the 13 structurally related substances using the Procedure.

Consideration of combined intakes

In the unlikely event that all foods containing menthol (no. 427) and the eight structurally related substances in class I were consumed concomitantly on a daily basis, the estimated total daily per capita intake in Europe and the USA would exceed the threshold for human intake for class I.

In the unlikely event that all foods containing menthone (no. 429) and the four structurally related substances in class II were consumed concomitantly on a daily basis, the estimated total daily per capita intake in Europe and the USA would exceed the threshold for human intake for class II.

All 14 substances are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. On the basis of the evaluation of the collective data, the combined intake was judged by the Committee not to raise safety concerns.

Conclusions

The Committee concluded that the use of menthol and the 13 structurally related substances evaluated as flavouring agents would not pose a safety concern at the current estimated levels of intake. The Committee noted that where toxicity data were available, they were consistent with the results of the safety evaluation using the Procedure.

A monograph summarizing the safety data on the 13 substances structurally related to menthol was prepared.

5. Intake assessments of specific food additives

In response to a request of the Twenty-Ninth Session of the Codex Committee on Food Additives and Contaminants (8), the Expert Committee assessed the intake of five food additives. In this section of the report, the Expert Committee recommends the review of levels of food additives in certain categories of the draft General Standard on Food Additives that is being developed by the Codex Committee. These categories are reproduced in Annex 4 for ease of reference.

5.1 Benzoates

The Committee assessed the intake of benzoates, a class of food additives generally used as preservatives. Maximum limits for benzoates have been proposed in a wide range of foods (solids and liquids) in the draft General Standard for Food Additives. A group ADI of 0–5 mg/kg of body weight, expressed as benzoic acid

equivalents, was allocated to benzyl acetate, benzyl alcohol, benzaldehyde and benzoic acid and its salts at the forty-sixth meeting of the Committee (Annex 1, reference 122).

Information on benzoates was provided by nine Member States (Australia, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The assessments submitted were based on the budget method, “poundage” data, household economic surveys, model diets and individual dietary records (section 2.3). Most Member States provided assessments based on more than one method. Japan submitted assessments based on actual measured concentrations of benzoates in foods. The other assessments were based on benzoate levels assumed to be at the maximum limits specified in the draft General Standard for Food Additives, the maximum limits specified in national food standards or the maximum limits specified by the European Union.

Screening of benzoates using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments were required. The screening of food additives by the Codex Committee using the budget method had identified the benzoates as requiring further assessment. The inclusion of national estimates of the proportion of the food supply that may contain benzoates in the calculations made using the budget method resulted in the same conclusion.

Intake assessments

All the national assessments of intake evaluated by the Committee, except that from Japan, were based on the use of food additives at the maximum limits specified in the draft General Standard for Food Additives, the maximum limits specified by the European Union or the maximum limits specified in national food standards. The intake assessment from Japan was based on an analysis that combined measured levels of additives with data on food consumption to estimate the food additive intake.

Intake estimates based on “poundage” data and household economic surveys. Estimates of daily per capita intake of benzoates based on “poundage” data ranged from 0.7 to 1.4 mg/kg of body weight. These intakes were less than the ADI. The intake estimates based on household economic surveys were also less than the ADI when the maximum limits for benzoic acid specified by the European Union or in national food standards were used. The estimated daily per capita

intakes ranged from 0.4 to 0.6mg/kg of body weight, but it should be noted that the levels that were assumed to be present in foods were lower than the maximum limits in the draft General Standard for Food Additives.

Intake estimates based on model diets. Estimates based on model diets were provided by five Member States. A number of assumptions were included in these assessments. In Japan, the intake estimates were based on levels of residues determined in a market basket survey; these estimates were substantially lower than the maximum limits in the draft General Standard for Food Additives. For example, the average benzoate concentration in non-alcoholic beverages was 190mg/kg as compared with the maximum limit in the draft General Standard for Food Additives of 1000mg/kg in soft drinks and 2000mg/kg in fruit juice. The estimated daily per capita intake of benzoates in Japan was 0.18mg/kg of body weight. In all other national assessments of intake, the maximum limits specified in national standards or the draft General Standard for Food Additives were assumed. The resulting daily per capita intakes ranged from 2.3mg/kg of body weight (for consumers with mean intakes, based on the maximum limits in the USA) to 35mg/kg of body weight (for consumers in the 90th percentile, based on the maximum limits in the draft General Standard for Food Additives). Intake estimates based on model diets were generally greater than the ADI, particularly when it was assumed that the benzoate levels were those proposed in the draft General Standard for Food Additives.

Intake estimates based on individual dietary records. Estimates of the intake of benzoates based on individual dietary records were submitted by four Member States. Only one assessment, the combined assessment provided by Australia and New Zealand, was based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives and individual dietary records. In that assessment, the estimated intakes exceeded the ADI for consumers with mean intakes, as well as for those in the 95th percentile. The mean intake was 20mg/kg of body weight per day (400% of the ADI) and the intake of consumers in the 95th percentile was 36mg/kg of body weight per day (720% of the ADI).

The intakes estimated for benzoates based on individual dietary records and maximum limits specified in national food standards were lower than the ADI for consumers with mean intakes as well as for those with high intakes (in the 95th or 97.5th percentiles) in all four Member States for which assessments were submitted (Australia and New Zealand (combined), France and the United Kingdom).

Conclusions

National intake estimates based on maximum limits in the draft General Standard for Food Additives. National estimates of intake of benzoates for consumers with mean intakes and those in the 95th percentile based on individual dietary records were below the ADI in all Member States when they were calculated using the maximum limits specified in national food standards, but above the ADI when the maximum limits and range of uses specified in the draft General Standard for Food Additives were used. This reflects the fact that the maximum limits specified in the General Standard for Food Additives represent the highest maximum limits submitted by any Member State or international nongovernmental organization.

The available data were insufficient to estimate the number of consumers who may exceed the ADI or to determine the magnitude and duration of intake above the ADI.

Because diets differ among countries, the foods contributing most to benzoate intake would be expected to vary. When foods were categorized according to the draft General Standard for Food Additives, the food category that contributed most to benzoate intake was carbonated water-based flavoured drinks (i.e. soft drinks) (category 14.1.4.1) in Australia and New Zealand (combined), France, the United Kingdom and the USA. Soy sauce was the major contributor to benzoate intake in China and the second most important contributor in Japan.

Typically, estimates of individual dietary intakes rely on short-term (1- to 7-day) surveys of food consumption, which was the case with the benzoates. Generally, estimates of long-term consumption of single foods are lower than estimates from short-term surveys. However, in the case of benzoates, the soft drinks that contribute most to intake are likely to be consumed on a regular basis. Because many consumers are likely to be regular consumers of a certain brand of soft drink, estimates of intake may reflect the levels of benzoates used in specific brands. A consumer who consumes a single bottle (0.5l) of soft drink per day would ingest 8.3mg of benzoates/kg body weight per day (170% of the ADI) if it contains the maximum limit specified in the draft General Standard for Food Additives. Thus, the estimated intake of benzoates would not be likely to be substantially lower if intake data based on a longer-term survey were available. This conclusion assumes that the levels remaining in the foods at the time of consumption are at the maximum limits. If the levels required to meet technological needs were below the maximum limits specified in the draft General Standard for Food Additives, intakes would be correspondingly lower. The data submitted by Japan from a market

basket survey suggest that this might be the case. However, further data would be required to determine the levels that are required to achieve the technological effect. In addition, benzoates are not likely to be used in all foods for which their use is permitted. Information on these aspects could be used to revise both the intake assessment and the maximum limits specified in the draft General Standard for Food Additives.

The Committee concluded that because of the potentially significant intake of benzoates based on the maximum limits specified in the draft General Standard for Food Additives, factors contributing to intakes in excess of the ADI should be further evaluated. Further information on levels in food at the time of consumption is needed.

National intake estimates based on maximum limits specified in national food standards or by the European Union. The best estimates of national mean intakes for consumers of benzoates were below the ADI (ranging from 0.18mg/kg of body weight per day in Japan to 2.3mg/kg of body weight per day in the USA). These estimates were based on analyses that used either model diets or individual dietary records together with maximum limits specified in national food standards or by the European Union. Intake estimates for consumers with high intakes of benzoates based on maximum limits specified in national standards were above the ADI in some cases (14mg/kg of body weight per day in China and 7.3mg/kg of body weight per day in the USA).

Recommendations to the Codex Committee on Food Additives and Contaminants

The Committee noted that the proposed draft General Standard for Food Additives contains several categories in which the proposed levels of use of benzoates submitted by a single country are substantially higher than those in the same or similar foods in other countries. These include the following categories (see Annex 4): ripened cheese (1.6.2), processed cheese (1.6.4), cheese analogues (1.6.5), vegetables in vinegar, oil or brine (4.2.2.3), cooked molluscs, crustaceans and echinoderms (9.2.4.2), semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms (9.3), and liquid egg products (10.2.1). The Codex Committee may wish to review the appropriate levels for these foods.

Levels of benzoates in carbonated drinks (category 14.1.4.1) that ranged from 350 to 1000mg/kg were proposed by several countries and by one nongovernmental organization. Since soft drinks were an important contributor to the estimated intakes of benzoates for all the Member States that submitted data for this assessment (with the

exception of China), the Codex Committee may wish to further consider an appropriate level for this category.

The Committee noted that intake estimates based on maximum limits specified in national standards were below the ADI for benzoates. However, when the intake estimates are based on the maximum limits and the range of uses specified in the draft General Standard for Food Additives, the ADI is exceeded. The differences arise because the range of foods specified in the draft General Standard for Food Additives is wider and the proposed levels of use in specific food categories are generally higher than those specified in national standards.

5.2 Butylated hydroxyanisole (BHA)

The Committee assessed the intake of butylated hydroxyanisole (BHA), for which maximum limits have been proposed in a wide range of solid foods and water-based flavoured non-alcoholic drinks in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. An ADI of 0–0.5 mg/kg of body weight was allocated to BHA at the thirty-third meeting of the Expert Committee (Annex 1, reference 83).

BHA is a food additive that is generally used as an antioxidant in products containing fats or oils and can be used in conjunction with butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate, providing a synergistic combination of antioxidants.

Information was provided by ten Member States (Australia, Brazil, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The intake assessments submitted were based on “poundage” data, household economic surveys or retail sales surveys, model diets, and/or individual dietary records (see section 2.3).

Screening of BHA using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments were required. The screening of food additives by the Codex Committee using the budget method had identified BHA as a food additive requiring detailed assessment. The inclusion of the proportions of the national solid food or beverage supply that may contain BHA in the calculations using the budget method did not change the conclusion that the theoretical maximum levels of use of

BHA in food and beverages were lower than both the national maximum permitted levels in the five Member States that submitted such calculations and the maximum limits specified in the draft General Standard for Food Additives.

Intake assessments

All the national assessments of intake of BHA evaluated by the Committee, except that from Japan, were based on the use of food additives at the maximum limits specified in the draft General Standard for Food Additives, maximum limits specified by the European Union, or maximum limits specified in national food standards.

Intake estimates based on "poundage" data and household economic surveys or retail sales surveys. Intake estimates based on "poundage" data and on household economic surveys or retail sales surveys would generally be expected to be lower than those based on actual consumption of the additive, such as estimates based on model diets or individual dietary records. This was found to be the case for BHA.

Estimates of daily per capita intake based on "poundage" data submitted by five Member States ranged from 0.005mg/kg of body weight in China to 0.48mg/kg of body weight in Spain. Data from household economic surveys or retail sales surveys submitted by three Member States gave estimates of daily per capita intake which ranged from 0.02mg/kg of body weight in France to 0.25mg/kg of body weight in Spain.

Intake estimates based on model diets or individual dietary records. The national mean daily per capita intakes of BHA based on the best estimates of additive consumption (from model diets or individual dietary records) from countries that submitted data ranged from 0.003mg/kg of body weight in Japan (market basket survey) to 0.39mg/kg of body weight in Australia and New Zealand (based on individual dietary records using maximum levels specified in national food standards). The low estimate of intake for Japan was due to the use of analysed BHA levels in the foods "as consumed". All other Member States used maximum permitted levels of food additives derived from national standards.

Intake estimates based on maximum limits specified in the draft General Standard for Food Additives. Intake estimates based on maximum limits and the range of foods specified in the draft General Standard for Food Additives will grossly overestimate the actual intakes in any one country. This is because the maximum limits specified in the draft General Standard for Food Additives are generally compiled by adopting the highest level of use for any one

food category submitted by Member States or nongovernmental organizations. The range of uses specified in the draft General Standard for Food Additives is also usually much wider than in national standards. National estimates of mean daily per capita intake of BHA based on maximum limits and the range of foods specified in the draft General Standard for Food Additives ranged from 0.91 to 0.94 mg/kg of body weight.

Conclusions

National intake estimates based on maximum limits specified in the draft General Standard for Food Additives. National estimates of mean intake of BHA based on maximum limits and the range of foods specified in the draft General Standard for Food Additives were available from only three Member States. The estimates of mean intake exceeded the ADI in these countries (180% of the ADI for Australia and New Zealand (combined), 190% of the ADI for the USA).

National intake estimates based on maximum limits specified in national food standards or by the European Union. All national estimates of mean intake for consumers of BHA were below the ADI (ranging from 1% of the ADI for Japan to 80% of the ADI for Australia and New Zealand (combined) and the USA). These estimates were based either on model diets or on individual dietary records submitted by seven Member States (Australia and New Zealand (combined), China, France, Japan, the United Kingdom and the USA). Intake estimates for consumers with high intakes of BHA based on maximum levels of food additives specified in national standards exceeded the ADI in some cases (ranging from 30% of the ADI for France to 260% of the ADI for Australia and New Zealand (combined)). However, the available data were insufficient to allow an estimate of the number of consumers with a high intake of BHA or the magnitude and duration of intake above the ADI.

All intake estimates, with the exception of that from Japan, assumed that BHA is the only antioxidant in foods where its use is permitted and that all such foods contain the additive at the maximum permitted levels, thus tending to overestimate the actual intake. Actual intakes of BHA will depend on the relative proportions of BHA, BHT, TBHQ and other antioxidants used in foods, on the actual levels of use according to good manufacturing practice and on the proportion of foods in any one category that contain the additive.

Recommendations to the Codex Committee on Food Additives and Contaminants

The Committee identified foods or food groups in the draft General Standard for Food Additives that could potentially contribute to a

high intake of BHA. These include the following categories: fats and oils and fat emulsions (type water-in-oil) (2); dried vegetables (4.2.2.2); cocoa and chocolate products other than 5.1.1, 5.1.2 and 5.1.4 (cocoa mixes (powders and syrups), cocoa-based spreads, including fillings and imitation chocolate and chocolate substitute products) (5.1.3); non-heat-treated processed comminuted meat, poultry and game products (8.3.1); frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms (9.2.1); ready-to-eat soup and broths, including canned, bottled and frozen (12.5.1); and food supplements (13.6). The Codex Committee may wish to review the appropriate levels for these foods.

The Committee noted that intake estimates for BHA based on national maximum permitted levels of use were below the ADI, but exceeded the ADI when they were based on the maximum limits and range of foods specified in the draft General Standard for Food Additives. The differences arise because the range of foods specified in the General Standard for Food Additives is wider and the proposed levels of use in specific food categories are generally higher than those specified in national standards.

5.3 Butylated hydroxytoluene (BHT)

The Committee assessed the intake of butylated hydroxytoluene (BHT), for which maximum limits have been proposed in a wide range of solid foods in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. An ADI of 0–0.3 mg/kg of body weight was allocated to BHT at the forty-fourth meeting of the Expert Committee (Annex 1, reference 116).

BHT is generally used as an antioxidant in products containing fats or oils. BHT can be used in conjunction with BHA, TBHQ and propyl gallate, providing a synergistic combination of antioxidants.

Information was provided by 10 Member States (Australia, Brazil, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The intake assessments submitted were based on “poundage” data, household economic surveys or retail sales surveys, model diets, or individual dietary records (see section 2.3).

Screening of BHT using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments were required. The screening of food additives by the Codex Committee

using the budget method had identified BHT as a food additive requiring further assessment. The inclusion of the proportions of the national food supply that may contain BHT in the calculations made using the budget method did not change the conclusion that the theoretical maximum levels of use of BHT in solid foods were lower than both the national maximum permitted levels and the maximum limits specified in the draft General Standard for Food Additives.

Intake assessments

Intake estimates based on maximum limits specified in the draft General Standard for Food Additives. Intake estimates derived using the maximum limits specified in the draft General Standard for Food Additives will grossly overestimate the actual intakes in any country because the General Standard is generally compiled by adopting the highest level of use submitted by a Member State for any use of the food additive in a given food category. The range of uses specified in the draft General Standard for Food Additives is also much wider than in national standards. National estimates of daily per capita intake of BHT based on the maximum limits specified in the draft General Standard for Food Additives ranged from 0.70 to 0.99 mg/kg of body weight for consumers with mean intakes and from 2.0 to 6.0 mg/kg of body weight per day for consumers with high intakes.

Intake estimates based on maximum limits specified in national food standards. Intakes estimated using different methods based on national maximum permitted levels of use showed results that were relatively consistent. Estimates of daily per capita intake ranged from 0.003 to 0.11 mg/kg of body weight based on “poundage” data, from 0.052 to 0.1 mg/kg of body weight based on household economic surveys or retail sales surveys, from 0.02 to 0.09 mg/kg of body weight (except for the USA) based on model diets (for consumers with average intakes), and from 0.02 to 0.1 mg/kg of body weight based on individual dietary records.

Two exceptions were noted by the Committee. The first was that the estimates of daily per capita intake based on a model diet and the maximum permitted level of use of BHT in the USA were 0.39 and 0.78 mg/kg of body weight for mean and high levels of consumption, respectively. This result can be explained by the high maximum permitted level of use of BHT in the USA. The second exception was a study from Japan, which was based on analytical results for BHT from a market basket survey. The estimate of daily per capita intake (0.000 85 mg/kg of body weight) was lower than all the other intake estimates submitted.

Conclusions

The Committee concluded that BHT intake probably does not exceed the ADI, based on the estimated national intakes of BHT in the 10 Member States for which data were available. However, it may be possible to exceed the ADI if the proposed maximum limits specified in the draft General Standard for Food Additives are assumed.

The Committee recognized that BHT is likely to be used in conjunction with other antioxidants, such as TBHQ and BHA, which are synergistic with BHT. Consequently, the amount of BHT used in practice would be lower and it would be used in fewer foods than assumed in the estimates. All intake estimates, with the exception of that from Japan, assumed that BHT is the only antioxidant in foods where its use is permitted and that all such foods contain the additive at the maximum permitted levels. Actual intakes of BHT would depend on the relative proportions of antioxidants used in foods and on the proportion of foods in any one category that contain the additive.

Recommendations to the Codex Committee on Food Additives and Contaminants

The Expert Committee identified food groups in the draft General Standard for Food Additives that could potentially contribute to a high intake of BHT. These include the following categories: fats and oils and fat emulsions (type water-in-oil) (2); chewing gum (5.3); and processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2). The Codex Committee may wish to review the appropriate levels of BHT for these foods.

The Committee noted that intake estimates based on national maximum permitted levels of use for BHT were below the ADI, but exceeded the ADI when they were based on the maximum limits and range of foods specified in the draft General Standard for Food Additives. The differences arise because the range of foods specified in the General Standard for Food Additives is wider and the proposed levels of use in specific food categories are generally higher than those specified in national standards.

5.4 Sulfites

The Committee assessed the intake of sulfur dioxide and related compounds, including the following: calcium, potassium and sodium hydrogen sulfite; calcium, potassium and sodium metabisulfite; calcium, potassium and sodium sulfite; and sodium thiosulfate. These substances are used as preservatives. Maximum limits for these

substances have been proposed in a wide variety of solid foods and beverages in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. A group ADI of 0–0.7 mg/kg of body weight was allocated to sulfur dioxide and this group of related compounds at the present meeting (see section 3.5).

The sulfites are in some ways unique additives, in that the level of use typically does not reflect the level remaining in a food at the time of ingestion. This is due to losses during the processing and storage of treated foods. For example, dried vegetables, which may contain up to 5000 mg of sulfites/kg according to the draft General Standard for Food Additives, are usually rehydrated and cooked prior to ingestion, resulting in much lower levels of residual sulfite being present in the food at the time of its consumption.

Information on sulfite intake was provided by 10 Member States (Australia, China, Finland, France, India, Japan, New Zealand, Spain, the United Kingdom and the USA). A joint assessment was made by Australia and New Zealand. The intake assessments were based on “poundage” data, household economic surveys or retail sales surveys, model diets, or individual dietary records (see section 2.3).

Screening of sulfites using the budget method

Screening of sulfur dioxide and sulfites by the budget method pointed to the need for detailed assessments of their intake in solid foods and beverages.

Intake assessments

All of the national intake assessments evaluated by the Committee, except those from Japan and the USA, were based on the assumption that sulfites were used at the maximum limits specified in the draft General Standard for Food Additives, maximum levels of use specified by the European Union, or maximum limits specified in national food standards. The intake assessments from Japan and the USA were based on methods that incorporated measured levels of sulfite residues in food.

Intake estimates based on “poundage” data. The estimates of daily per capita intake of sulfites based on “poundage” data ranged from 0.07 to 1.6 mg/kg of body weight. Estimates based on “poundage” data are likely to overstate the actual per capita consumption of sulfites because of unaccounted losses during processing and storage, or household wastage of foods containing sulfites.

Intake estimates based on household economic surveys or food balance sheets. Information from household economic surveys and food

balance sheets gave estimates of daily per capita intake that ranged from 0.3 to 3mg/kg of body weight. Since these estimates are not based on the residual levels of sulfites in foods “as consumed”, they are likely to be overestimates.

Intake estimates based on model diets. Data from model diets yielded estimates of daily per capita intake that ranged from 0.03 to 30mg/kg of body weight. The highest intake estimates, which were based on the maximum limits specified in the draft General Standard for Food Additives, were exceedingly high, but should not be considered realistic estimates of likely intake because of the losses that can be expected to occur during processing and storage of treated foods. Estimates based on national maximum permitted levels of use or measured levels of use, such as those from Japan and the USA, resulted in a much more reasonable measure of actual sulfite intake in a population.

Intakes based on individual dietary records. The data from individual dietary records produced estimates of daily per capita intake ranging from 0.3 to 15mg/kg of body weight per day. The method, which uses national maximum permitted levels of use and the range of permitted uses, also provided the best estimates from Australia and New Zealand (combined), France and the United Kingdom.

Conclusions and recommendations to the Codex Committee on Food Additives and Contaminants

The Committee noted that all intake estimates based on national maximum levels of use for sulfites were below the ADI. However, estimates exceeded the ADI when they were based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives. The differences in intake assessments arise because the range of foods specified in the draft General Standard is wider than that in which the use of sulfites is authorized on a national basis and the proposed levels of use in specific food categories are generally higher than national maximum permitted levels.

The evaluation of the proposed maximum limits for sulfites in the draft General Standard for Food Additives, in conjunction with the data on food intake supplied by national governments, leads to the conclusion that certain foods can be identified as major contributors to overall sulfite intake. Ingestion of a 100-g portion of any food containing sulfite at a concentration of 400mg/kg or above would result in an intake of sulfite equal to or above the ADI. The consumption of certain solid foods and some beverages can commonly lead to intakes above the ADI when the residual level of sulfites in the food approaches the maximum limit for these foods specified in the draft

General Standard for Food Additives. Such foods and beverages are included in the following categories: dried fruit (4.1.2.2); jams, jellies and marmalades (4.1.2.5); fruit preparations, including pulp and fruit toppings (4.1.2.8); dried vegetables (4.2.2.2); vegetable, nut and seed purees and spreads (4.2.2.5); white and semi-white sugar (sucrose or saccharose), fructose, glucose (dextrose), xylose, sugar solutions and syrups, and (partially) inverted sugars, including molasses, treacle and sugar toppings (11.1); concentrates (liquid or solid) for fruit juice (14.1.2.3); wines (14.2.3); and fruit wine (14.2.4).

Some of the high maximum limits specified in the draft General Standard for Food Additives have been incorporated as a consequence of the food groupings in which one type of food in the group requiring a high level of sulfites results in the level of sulfites in the group as a whole being unrealistically high. For example, sucrose, which is included under white and semi-white sugar, requires a maximum limit of only 70mg/kg, but the group maximum limit is taken from the maximum limit for molasses of 500mg/kg. The group maximum limit for jams, jellies and marmalades of 3000mg/kg arises from a request for use in imitation fruit, but where all of the remaining levels requested are at or below 500mg/kg. The maximum limit for concentrates (liquid or solid) for fruit juice of 2000mg/kg comes from a request for use in grape juice concentrate for wine-making, whereas all of the other levels requested for fruit juice concentrates for direct use by consumers are at or below 350mg/kg. Estimates of sulfite intake by consumers of food in this group may not have taken into account either the loss in processing or dilution of the concentrates before consumption. The Codex Committee could consider separating the specific foods that require higher levels of use of sulfites in these categories in order to better differentiate specific products, resulting in lower maximum limits; this would have the potential consequence of lowering the estimated intake of sulfites.

The maximum limit specified in the draft General Standard for Food Additives for fruit preparations, including pulp and fruit toppings, of 3000mg/kg is the result of a request by only one Member State. Products of this type may not be produced using sulfites in other Member States and sulfites may not be needed. The Codex Committee could consider further examination of the need for sulfites in this type of product in order to determine an appropriate level based on good manufacturing practice, if appropriate. The above recommendations are intended to aid the Codex Committee in establishing maximum limits in the General Standard for Food Additives such that the potential for risk to consumers with a high intake of sulfites might be limited.

If intake is estimated using residual sulfite levels measured in foods at the time of consumption, as was done for the estimates from Japan and the USA, the resulting estimates would be insignificantly lower than those presented by the remaining Member States, which used various maximum permitted levels of use. The mean intakes of sulfites estimated from model diets in France, Japan and the USA based on national levels of use, as well as mean intakes from analysis of individual dietary records in the United Kingdom, suggest that the current mean intakes of sulfites worldwide are below the ADI established by the Expert Committee. Although some methods for estimating intake, notably those based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives, showed that consumers with high intakes of sulfites may exceed the ADI, the data submitted were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.

5.5 ***tert*-Butylhydroquinone (TBHQ)**

The Committee assessed the intake of *tert*-butylhydroquinone (TBHQ), for which maximum limits have been proposed in a wide range of food products in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. The highest level of use of TBHQ specified in the draft General Standard for Food Additives is 1000 mg/kg for frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms (category 9.2.1). An ADI of 0–0.7 mg/kg of body weight was allocated to TBHQ at the forty-ninth meeting of the Expert Committee (Annex 1, reference 131).

TBHQ is generally used as an antioxidant in animal-derived food products and in fats and oils. It is often used in conjunction with BHA, BHT and propyl gallate to provide a synergistic antioxidant effect.

Information was provided by six Member States (Australia, Brazil, China, New Zealand, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The intake assessments submitted were based on “poundage” data, household economic surveys or retail sales surveys, model diets, and/or individual dietary records (see section 2.3).

Screening of TBHQ using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments for TBHQ were required. The screening of food additives by the Codex Committee using the budget method had

identified TBHQ as an additive requiring further assessment. The theoretical maximum level of use of TBHQ in food was less than the national maximum permitted level of use in all of the four Member States that submitted such calculations and was also less than the highest level of use specified in the draft General Standard for Food Additives (1000mg/kg).

Intake assessments

All the national assessments of intake of TBHQ evaluated by the Committee were based on the maximum limits specified in the draft General Standard for Food Additives, maximum limits specified by the European Union, or maximum limits specified in national food standards.

Intake estimates based on “poundage” data and household economic surveys or retail sales surveys. Estimates of per capita intake of TBHQ based on “poundage” data and household economic surveys or retail sales surveys would be expected to be lower than estimates based on actual consumption of the additive, obtained using model diets or individual dietary records. This was found to be the case for TBHQ. Estimates of daily per capita intake based on “poundage” data were available from two Member States and ranged from 0.004mg/kg of body weight in China to 0.14mg/kg of body weight in the USA. The estimates of daily per capita intake based on two household economic surveys in Brazil were 0.08 and 0.13mg/kg of body weight.

Intake estimates based on model diets or individual dietary records. National estimates of mean intakes of TBHQ from four Member States based on model diets or individual dietary records ranged from 0.37mg/kg of body weight in the USA to 0.69mg/kg of body weight in China.

Intake estimates based on maximum limits specified in the draft General Standard for Food Additives. Intakes calculated using the maximum limits and the range of foods specified in the draft General Standard for Food Additives will generally be gross overestimates of the actual intakes in any one country. This is because the General Standard for Food Additives is generally compiled by adopting the highest level of use of TBHQ for any food category submitted by Member States and nongovernmental organizations. The range of uses specified in the draft General Standard for Food Additives is also usually much wider than in national standards. The national daily per capita mean intake of TBHQ based on the maximum limits and range of foods specified in the draft General Standard for Food Additives was 1.2mg/kg of body weight in Australia and New Zealand

(combined) and 0.62 mg/kg of body weight in the USA. However, for consumers with high intakes of TBHQ the estimates were 3.5 mg/kg of body weight per day in Australia and New Zealand (combined) and 1.2 mg/kg of body weight per day in the USA.

Conclusions

National intake estimates based on maximum limits specified in the draft General Standard for Food Additives. National estimates of intake based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives were available for only five Member States. The estimates of mean intake were near to or exceeded the ADI for TBHQ (ranging from 90% of the ADI for the USA to 180% of the ADI for Australia and New Zealand (combined)).

National intake estimates based on model diets or individual dietary records. All the best estimates of national mean intake of TBHQ were at or below the ADI (ranging from 50% of the ADI for the USA to 100% of the ADI for China), based on data obtained using model diets or individual dietary records.

National intake estimates based on maximum limits specified by the European Union or in national food standards. Intake estimates for consumers with high intakes of TBHQ based on maximum limits specified in national food standards exceeded the ADI in some cases (e.g. 300% for Australia and New Zealand (combined)). However, the data submitted were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.

All intake estimates assume that TBHQ is the only antioxidant present in foods in which its use is permitted and that all such foods contain the additive at maximum levels, thus tending to overestimate actual intake. The actual intakes of TBHQ will depend on the relative proportions of BHA, BHT, TBHQ and other antioxidants used in foods, on national levels of use according to good manufacturing practice, and on the proportion of foods in any one category that contain the additive.

Recommendations to the Codex Committee on Food Additives and Contaminants

The Expert Committee identified foods or food groups that potentially contribute to the high intake of TBHQ. These included the following categories: fats and oils and fat emulsions (type water-in-oil) (2); processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2); and carbonated drinks (14.1.4.1). On the basis of the assessment, the Codex Committee may wish to review

the appropriate levels of TBHQ for these categories specified in the draft General Standard for Food Additives.

The Committee noted that all national estimates of intake based on national maximum permitted levels of use for TBHQ were below the ADI. However, estimates exceeded the ADI when they were based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives. The differences in intake assessments arise because the range of foods specified in the General Standard for Food Additives is wider than the range of foods in which TBHQ is authorized for use at the national level and the proposed levels of use in specific food categories are generally higher than national maximum permitted levels of use.

6. **Revision of certain specifications**

A total of 40 food additives were examined for specifications only (Annex 2). In general, all specifications monographs were revised in line with current policy on heavy metal contaminants (see section 2.4.6).

The existing specifications for calcium propionate were maintained.

The specifications for canthaxanthin, carnauba wax, cochineal extract, ethyl *p*-hydroxybenzoate, methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, 4-hexylresorcinol, sorbitan monolaurate, potassium sorbate, calcium sorbate, polydextroses, aluminium powder, microcrystalline cellulose and sucrose esters of fatty acids were revised, with minor changes.

The specifications for ferrous gluconate were revised and designated as “tentative”, with a request for information on the need for maintaining the limit test for oxalic acid and maximum limit for mercury, as well as the procedure for mercury analysis, if applicable, and for introducing maximum limits for sulfate and chloride. This information was required by 30 November 1998.

At its forty-ninth meeting (Annex 1, reference 131), the Committee developed revised specifications for “diacetyltartaric and fatty acid esters of glycerol (DATEM)” and “tartaric, acetic and fatty acid esters of glycerol, mixed” but found it difficult to distinguish between the two substances. At that meeting, the Committee requested data that would allow these two substances to be distinguished, and stated that unless such data were provided by 31 March 1998, it would consider combining the specifications for the two substances. Since no such data were received, the Committee, at its present meeting,

decided to combine the specifications for the two substances under the name "diacetyltartaric and fatty acid esters of glycerol".

The existing monograph containing specifications for gum arabic was revised in order to distinguish clearly between gum arabic obtained from *Acacia senegal* and gum arabic obtained from *Acacia seyal*. Gums from other *Acacia* species are excluded from the revised monograph.

The existing specifications for carotenes (algae) and carotenes (vegetable) were revised in order to avoid the use of chloroform in the tests.

The existing specifications for carthamus yellow were revised to include the structural formulae of the main components, and the method of assay was improved.

The existing specifications for carob bean gum and guar gum were revised to include microbiological criteria.

The existing specifications for xanthan gum were revised in order to introduce the modified test methods for microbiological criteria in gums.

The existing specifications for nitrogen were revised and the criteria for hydrogen, carbon dioxide and moisture were deleted.

The existing specifications for talc were revised and the identification test for magnesium was replaced by a test for infrared absorption.

The existing tentative specifications for carthamus red, dichloromethane, propionic acid and petroleum jelly were revised and the "tentative" qualification was deleted.

The existing tentative specifications for shellac were revised and the "tentative" qualification was deleted. Furthermore, the title of the specifications monograph was changed to "shellac, bleached" in order to reflect better the products currently on the market for food use.

The existing tentative specifications for hexane were revised and the "tentative" designation was deleted. In order to reflect that the product defined by the monograph is a mixture of hexanes, the title was changed to "hexanes".

The existing specifications for thaumatin were revised and designated as "tentative". The Committee requested information on a specific identification test by 30 November 1998.

The existing specifications for citric acid were revised and designated as "tentative". The Committee requested information on the need for the test for oxalate and a suitable limit for this impurity by 30 March 1999.

New specifications for ferrous sulfate were prepared and designated as “tentative”. The Committee requested information on the limit and an analytical method for mercury, and the need for a limit on water content. This information was required by 30 March 1999.

No specifications were prepared for sodium sorbate as no information on any commercial production was available.

The existing specifications for acetone, propan-2-ol and isobutyl alcohol (isobutanol) were revised. As these substances are used both as solvents and as flavouring agents, specifications were prepared for incorporation in the table of flavouring agents in the specifications monograph.

7. Future work

1. The two substances “diacetyltartaric and fatty acid esters of glycerol” and “tartaric, acetic and fatty acid esters of glycerol, mixed” were toxicologically evaluated at the seventeenth meeting (Annex 1, reference 32) and were assigned different ADIs. Since the two substances are now covered by one set of specifications (see section 6), the Committee recommended that the material defined in these specifications be evaluated toxicologically.
2. The safety aspects of dietary supplementation with high levels of nutrients should be evaluated at a future meeting, preferably at the same meeting that specifications are being developed for novel forms of such nutrients.
3. Because of the increasing use of gene technology, general guidelines for food additives derived from genetically modified organisms should be developed.
4. The Committee recognized that some specifications established previously may not be adequate in the light of recently improved analytical procedures and the use of new techniques for the production of food additives. The Committee therefore endorsed the recommendation made at the forty-sixth meeting (Annex 1, reference 122) concerning the need for the periodic updating of the *Compendium of food additive specifications* (Annex 1, reference 96) and the *Guide to specifications* (Annex 1, reference 100).

8. 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 Committee recognized the importance of risk analysis for ensuring consistency and a sound scientific basis for food standards. It therefore recommended that FAO and WHO work with the Codex Committee on Food Additives and Contaminants, the Codex Committee on Residues of Veterinary Drugs in Foods and the Codex Committee on Pesticide Residues, together with the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues, to develop appropriate principles and procedures for risk assessment.
3. The Committee recommended that whenever chemical substances undergo toxicological evaluation, assessments of intake should be undertaken so that the risk can be characterized. When substances are referred to the Expert Committee by the Codex Committee on Food Additives and Contaminants for intake assessment, the Expert Committee should determine whether a toxicological evaluation is required.
4. To ensure timely notification of the important issues agreed upon at each meeting, the Committee recommended that draft report items dealing with issues of a general nature be included in the "Summary and conclusions" published shortly after each meeting. When a policy is developed that affects the nature of submissions for the next meeting, the Secretariat should request such information in the "Call for data" for the meeting.
5. The Committee recommended that the opinions of the Codex Committee on Nutrition and Foods for Special Uses and the Codex Committee on Food Additives and Contaminants should be sought on the need to develop specifications for vitamins and minerals for which no internationally recognized food-grade specifications exist and on priorities for the development of such specifications.
6. The Committee recognized that a number of preparations from natural sources have not yet been reviewed, although their main active components have been evaluated as food additives. The Committee recommended that such preparations that are being marketed for use in foods be evaluated.

Acknowledgements

The Committee was saddened to hear of the death of Dr J.P. Modderman, who served on the Committee and contributed greatly to its success for many years. He continued to provide support to the Committee by contributing information until his sudden death in April 1998. He will be greatly missed, both as a valued colleague and as a friend.

The Committee wishes to thank Mrs E. Heseltine, Communication in Science, Lajarthe, Saint-Léon-sur-Vézère, France, for her assistance in the preparation of the report.

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4. **Codex Alimentarius Commission**. General decisions of the Commission. In: *Procedural manual*, 10th ed. Rome, Food and Agriculture Organization of the United Nations, 1997:146–147.
5. *Food consumption and exposure assessment of chemicals. Report of a FAO/WHO Consultation, Geneva, 10–14 February 1997*. Geneva, World Health Organization, 1998 (unpublished document WHO/FSF/FOS/97.5; available on request from Department of Nutrition for Health and Development, World Health Organization, 1211 Geneva 27, Switzerland).
6. **Hansen SC**. Conditions for use of food additives based on a budget method for an Acceptable Daily Intake. *Journal of food protection*, 1979, **42**:429–434.
7. **Codex Alimentarius Commission**. *Advisory lists of mineral salts and vitamin compounds for use in foods for infants and children*. Rome, Food and Agriculture Organization of the United Nations, 1979 (amended 1983, 1991) (unpublished document CAC/GL 10-1979; available from FAO or WHO).
8. **Codex Alimentarius Commission**. *Report of the Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants, The Hague, 17–21 March 1997*. Rome, Food and Agriculture Organization of the United Nations, 1997 (unpublished FAO document ALINORM 97/12A; available from FAO or WHO).

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 Report 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 Report 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 (out of print).
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 Report Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabi-*

- lizers, flour-treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C, 1967; WHO/Food Add/67.29 (out of print).
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 Report Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967 (out of print).
 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 Report Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968 (out of print).
 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 (out of print).
 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 (out of print).
 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 Report Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969 (out of print).
 18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Report Series, No. 45A, 1969; WHO/Food Add/69.34 (out of print).
 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 Report Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970 (out of print).
 20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36 (out of print).
 21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37 (out of print).
 22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents* (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971 (out of print).
 23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39 (out of print).
 24. *Specifications for the identity and purity of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40 (out of print).
 25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41 (out of print).
 26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
 27. *Toxicological evaluation of some enzymes, modified starches, and certain other substances.* FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.

28. *Specifications for the identity and purity of some enzymes and certain other substances*. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972 (out of print).
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 (out of print).
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 Report Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum (out of print).
31. *Evaluation of mercury, lead, cadmium, and the food additives amaranth, diethylpyrocabonate, and octyl gallate*. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of certain 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 (out of print).
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers*. FAO Food and Nutrition Paper, No. 4, 1978.
35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum (out of print).
36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives*. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
37. *Specifications for the identity and purity of some food colours, flavour enhancers, thickening agents, and certain food additives*. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances* (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975 (out of print).
39. *Toxicological evaluation of some food colours, thickening agents, and certain other substances*. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
40. *Specifications for the identity and purity of certain food additives*. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. *Evaluation of certain food additives* (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives*. FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.

45. *Summary of toxicological data of certain food additives*. WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others*. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978 (out of print).
48. *Summary of toxicological data of certain food additives and contaminants*. WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives*. FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives)*. FAO Food and Nutrition Paper, No. 17, 1980.
56. *Evaluation of certain food additives* (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 16, 1981.
58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives)*. FAO Food and Nutrition Paper, No. 19, 1981.
59. *Evaluation of certain food additives and contaminants* (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 17, 1982.
61. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 25, 1982.
62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda (out of print).
63. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 28, 1983.
65. *Guide to specifications — General notices, general methods, identification tests, test solutions, and other reference materials*. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. *Evaluation of certain food additives and contaminants* (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.

67. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 19, 1984.
68. *Specifications for the identity and purity of food colours*. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. *Specifications for the identity and purity of food additives*. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. *Evaluation of certain food additives and contaminants* (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 34, 1986.
72. *Toxicological evaluation of certain food additives and contaminants*. Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 20).
73. *Evaluation of certain food additives and contaminants* (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. *Toxicological evaluation of certain food additives and contaminants*. Cambridge, Cambridge University Press, 1987 (WHO Food Additives Series, No. 21).
75. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 37, 1986.
76. *Principles for the safety assessment of food additives and contaminants in food*. Geneva, World Health Organization, 1987 (WHO Environmental Health Criteria, No. 70) (out of print).¹
77. *Evaluation of certain food additives and contaminants* (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987, and corrigendum.
78. *Toxicological evaluation of certain food additives*. Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 22).
79. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 38, 1988.
80. *Evaluation of certain veterinary drug residues in food* (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.
81. *Toxicological evaluation of certain veterinary drug residues in food*. Cambridge, Cambridge University Press, 1988 (WHO Food Additives Series, No. 23).
82. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41, 1988 (out of print).
83. *Evaluation of certain food additives and contaminants* (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. *Toxicological evaluation of certain food additives and contaminants*. Cambridge, Cambridge University Press, 1989 (WHO Food Additives Series, No. 24).
85. *Evaluation of certain veterinary drug residues in food* (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 25, 1990.
87. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. *Evaluation of certain food additives and contaminants* (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 26, 1990.

¹ The full text is available electronically on the Internet at <http://www.who.int/pes>.

90. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 49, 1990.
91. *Evaluation of certain veterinary drug residues in food* (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 27, 1991.
93. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. *Evaluation of certain food additives and contaminants* (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 28, 1991.
96. *Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990*. Rome, Food and Agriculture Organization of the United Nations, 1992 (2 volumes).
97. *Evaluation of certain veterinary drug residues in food* (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1992.
98. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 29, 1992.
99. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/4, 1991.
100. *Guide to specifications — General notices, general analytical techniques, identification tests, test solutions, and other reference materials*. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.
101. *Evaluation of certain food additives and naturally occurring toxicants* (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.
102. *Toxicological evaluation of certain food additives and naturally occurring toxicants*. WHO Food Additives Series, No. 30, 1993.
103. *Compendium of food additive specifications, addendum 1*. FAO Food and Nutrition Paper, No. 52, Add. 1, 1992.
104. *Evaluation of certain veterinary drug residues in food* (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 31, 1993.
106. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/5, 1993.
107. *Evaluation of certain food additives and contaminants* (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 32, 1993.
109. *Compendium of food additive specifications, addendum 2*. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. *Evaluation of certain veterinary drug residues in food* (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 33, 1994.
112. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/6, 1994.
113. *Evaluation of certain veterinary drug residues in food* (Forty-third report of the

- Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995. and corrigendum.
114. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 34, 1995.
 115. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/7, 1995.
 116. *Evaluation of certain food additives and contaminants* (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
 117. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 35, 1996.
 118. *Compendium of food additive specifications, addendum 3*. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
 119. *Evaluation of certain veterinary drug residues in food* (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
 120. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 36, 1996.
 121. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/8, 1996.
 122. *Evaluation of certain food additives and contaminants* (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
 123. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 37, 1996.
 124. *Compendium of food additive specifications, addendum 4*. FAO Food and Nutrition Paper, No. 52, Add. 4, 1996 (out of print).
 125. *Evaluation of certain veterinary drug residues in food* (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
 126. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 38, 1996.
 127. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/9, 1997.
 128. *Evaluation of certain veterinary drug residues in food* (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
 129. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 39, 1997.
 130. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/10, 1998.
 131. *Evaluation of certain food additives and contaminants* (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
 132. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 40, 1998.
 133. *Compendium of food additive specifications, addendum 5*. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
 134. *Evaluation of certain veterinary drug residues in food* (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999, and corrigendum.
 135. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 41, 1998.
 136. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/11, 1999.

Annex 2

Acceptable Daily Intakes, other toxicological information, and information on specifications

Substance	No.	Specifications ^a	Acceptable Daily Intakes (ADI) in mg/kg of body weight and other toxicological recommendations
Enzyme preparations			
α-Acetolactate dehydrogenase	—	R	Not specified ^b
Maltogenic amylase	—	R	Not specified ^b
Flavouring agents			
<i>trans</i> -Anethole	0217	S	0–2
Furfural	0450	R	No ADI allocated because insufficient data were available
Menthol	0427	R	0–4
Food colours			
Curcumin	—	R, T	0–1 (temporary) ^c
Riboflavin from genetically modified <i>Bacillus subtilis</i>	—	N	0–0.5 (group ADI with synthetic riboflavin and riboflavin-5'-phosphate)
Glazing agents			
Mineral oils (medium- and low-viscosity)		R	
Class I ^d	—		0–1 (temporary) ^c
Class II ^e and Class III ^f	—		0–0.01 (group ADI) (temporary) ^c
Preservatives			
Calcium hydrogen sulfite	—	S, T ^c	0–0.7 ^g
Calcium metabisulfite	—	O	
Calcium sulfite	—	O	
Potassium hydrogen sulfite	—	O	
Potassium metabisulfite	—	R, T ^c	
Potassium sulfite	—	R, T ^c	
Sodium hydrogen sulfite	—	R, T ^c	
Sodium metabisulfite	—	R, T ^c	
Sodium sulfite	—	R, T ^c	
Sodium thiosulfate	—	R, T ^c	
Sulfur dioxide	—	R	
Sweetening agent			
Stevioside	—	O	No ADI allocated ^{c,h}

Substance	No.	Specifications ^a	Acceptable Daily Intakes (ADI) in mg/kg of body weight and other toxicological recommendations
Thickening agents			
Carrageenan	—	R	Not specified (group ADI) (temporary) ^{b,c}
Processed <i>Eucheuma</i> seaweed	—	R	
Sodium carboxymethyl cellulose, enzymatically hydrolysed	—	R	Not specified (group ADI) ^{b,i}
Miscellaneous substances			
γ-Cyclodextrin	—	N	Not specified (temporary) ^{b,c}
Glucono-δ-lactone	—	R	Not specified (group ADI) ^b
Calcium gluconate	—	R	
Magnesium gluconate	—	R, T ^c	
Potassium gluconate	—	R	
Sodium gluconate	—	R	Not specified (group ADI) ^{b,j}
Polyglycitol syrup	—	N	

^a N, New specifications prepared; O, no specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or not requested; T, the existing, new or revised specifications are tentative and comments are invited.

^b 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 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.

^c See Annex 3.

^d Including P70(H) oil.

^e Including N70(H) and N70(A) oils.

^f Including P15(H), N15(H) and N10(A) oils.

^g The Committee reiterated its recommendation made at its thirtieth meeting (WHO Technical Report Series, No. 751, 1987) that, where a suitable alternative method of preservation exists, its use should be encouraged, particularly in those applications (e.g. control of enzymatic browning in fresh salad vegetables) in which the use of sulfites may lead to high levels of acute intake and which have been most commonly associated with life-threatening adverse reactions. Appropriate labelling would help to alert individuals who cannot tolerate sulfites.

^h No ADI was allocated because insufficient data were available and specifications were not prepared.

ⁱ Included in the group ADI with ethyl cellulose, ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, methyl ethyl cellulose and sodium carboxymethyl cellulose.

^j Group ADI for materials conforming to the specifications for polyglycitol syrup and maltitol syrup.

Flavouring agents

The substances listed here were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents. For further details, see section 2.2.1 of the main report.

Substance ^a	No.	Specifications ^b	Conclusion based on current intake
Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters			
Structural class I			
Acetone (propan-2-one)	0139	R	No safety concern
Isopropyl alcohol (2-propanol)	0277	N	
2-Butanone (ethyl methyl ketone)	0278	N	
2-Pentanone	0279	N	
2-Pentanol	0280	N	
3-Hexanone	0281	N	
3-Hexanol	0282	N	
2-Heptanol	0284	N	
3-Heptanol	0286	N	
4-Heptanone	0287	N	
2-Octanol	0289	N	
3-Octanol	0291	N	
2-Nonanol	0293	N	
3-Decanol	0295	N	
2-Undecanol	0297	N	
3-Methyl-2-butanol	0300	N, T	
2,6-Dimethyl-4-heptanol	0303	N	
Isopropyl formate	0304	N, T	
Isopropyl acetate	0305	R	
Isopropyl propionate	0306	N, T	
Isopropyl butyrate (isopropyl butanoate)	0307	N	
Isopropyl hexanoate	0308	N, T	
Isopropyl isobutyrate (isopropyl 2-methylpropanoate)	0309	N	
Isopropyl isovalerate (isopropyl 3-methylbutanoate)	0310	N	
Isopropyl myristate (isopropyl tetradecanoate)	0311	N	
Isopropyl tiglate (isopropyl 2-methyl-2-butenate)	0312	N	
3-Octyl acetate	0313	N	
1-Ethylhexyl tiglate (octen-3-yl 2-methyl-2-butenate)	0448	N	
Structural class II			
2-Heptanone	0283	N	No safety concern
3-Heptanone	0285	N	
2-Octanone	0288	N	
3-Octanone	0290	N	
2-Nonanone	0292	N	
3-Nonanone	0294	N	

Substance ^a	No.	Specifications ^b	Conclusion based on current intake

Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters (continued)

Structural class II (continued)

2-Undecanone	0296	N	No safety concern
2-Tridecanone	0298	N	
2-Pentadecanone	0299	N, T	
4-Methyl-2-pentanone	0301	N	
2,6-Dimethyl-4-heptanone	0302	N	

Linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters

4-Pentenoic acid	0314	N	No safety concern
<i>cis</i> -3-Hexen-1-ol	0315	N	
<i>cis</i> -3-Hexenal	0316	N, T	
3-Hexenoic acid	0317	N, T	
4-Hexen-1-ol	0318	N	
<i>cis</i> -4-Hexenal	0319	N, T	
4-Heptenal	0320	N	
<i>cis</i> -3-Octen-1-ol	0321	N	
<i>cis</i> -5-Octen-1-ol	0322	N	
<i>cis</i> -5-Octenal	0323	N	
<i>cis</i> -6-Nonen-1-ol	0324	N	
<i>cis</i> -6-Nonenal	0325	N	
4-Decenal	0326	N	
Mixture of 5- and 6-decenoic acid	0327	N, T	
9-Decenoic acid	0328	N	
9-Undecenal	0329	N, T	
10-Undecenal	0330	N	
10-Undecenoic acid	0331	N	
Linoleic acid	0332	N	
Oleic acid ^c	0333	N, T	
Methyl 3-hexenoate	0334	N, T	
Ethyl 3-hexenoate	0335	N	
<i>cis</i> -3-Hexenyl <i>cis</i> -3-hexenoate	0336	N	
Methyl <i>cis</i> -4-octenoate	0337	N, T	
Ethyl <i>cis</i> -4-octenoate	0338	N, T	
Ethyl <i>cis</i> -4,7-octadienoate	0339	N, T	
Methyl 3-nonenoate	0340	N, T	
Ethyl <i>trans</i> -4-decenoate	0341	N, T	
Methyl 9-undecenoate	0342	N, T	
Ethyl 10-undecenoate	0343	N	
Butyl 10-undecenoate	0344	N, T	
Ethyl oleate	0345	N	
Mixture of methyl linoleate and methyl linolenate	0346	N	
2-Methyl-3-pentenoic acid	0347	N, T	
2,6-Dimethyl-6-hepten-1-ol	0348	N, T	
2,6-Dimethyl-5-heptenal	0349	N	
Ethyl 2-methyl-3-pentenoate	0350	N, T	

Substance ^a	No.	Specifications ^b	Conclusion based on current intake

Linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters (*continued*)

Ethyl 2-methyl-4-pentenoate	0351	N	No safety concern
Mixture of hexyl 2-methyl-3- and hexyl 2-methyl-4-pentenoate	0352	N, T	
Ethyl 2-methyl-3,4-pentadienoate	0353	N, T	Evaluation deferred ^d
Methyl 3,7-dimethyl-6-octenoate	0354	N	No safety concern
2-Methyl-4-pentenoic acid	0355	N	

Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances

Structural class I

Linalool ^e	0356	R	No safety concern
Tetrahydrolinalool	0357	N	
Linalyl formate	0358	N	
Linalyl acetate ^e	0359	R	
Linalyl propionate	0360	N	
Linalyl butyrate	0361	N	
Linalyl isobutyrate	0362	N	
Linalyl isovalerate	0363	N	
Linalyl hexanoate	0364	N	
Linalyl octanoate	0365	N, T	
α -Terpineol	0366	N	
Terpinyl formate	0367	N	
Terpinyl acetate	0368	N	
Terpinyl propionate	0369	N	
Terpinyl butyrate	0370	N, T	
Terpinyl isobutyrate	0371	N, T	
Terpinyl isovalerate	0372	N, T	
<i>p</i> -Menth-3-en-1-ol	0373	N, T	
4-Carvomenthenol	0439	N	
<i>p</i> -Menth-8-en-1-ol (β -terpineol)	0374	N, T	
4-Thujanol	0441	N, T	Not evaluated ^f
Methyl 1-acetoxycyclohexylketone	0442	N, T	

Structural class II

2-Ethyl-1,3,3-trimethyl-2-norbananol	0440	N, T	No safety concern

Carvone and structurally related substances

Structural class I

<i>p</i> -Menth-2-ol	0376	N, T	No safety concern
Dihydrocarveol	0378	N	
Dihydrocarvyl acetate	0379	N	
Carveol	0381	N	
Carvyl acetate	0382	N	
Carvyl propionate	0383	N, T	

Substance ^a	No.	Specifications ^b	Conclusion based on current intake

Carvone and structurally related substances (continued)

Structural class II

<i>p</i> -Menthan-2-one	0375	N	No safety concern
Dihydrocarvone	0377	N, T	
(+)-Carvone ^g	0380a	R	
(-)-Carvone	0380b	R	

Ionones and structurally related substances

β-Damascone	0384	N	No safety concern
α-Damascone	0385	N	
δ-Damascone	0386	N	
Damascenone	0387	N	
α-Ionone ^h	0388	R	
β-Ionone ^h	0389	R	
γ-Ionone	0390	N, T	
α-Ionol	0391	N	
β-Ionol	0392	N, T	
Dihydro-α-ionone	0393	N	
Dihydro-β-ionone	0394	N, T	
Dihydro-β-ionol	0395	N	
Dehydrodihydroionone	0396	N, T	
Dehydrodihydroionol	0397	N, T	
Methyl-α-ionone	0398	N, T	
Methyl-β-ionone	0399	N	
Methyl-δ-ionone	0400	N, T	Not evaluated ⁱ
Allyl-α-ionone	0401	N	
1,4-Dimethyl-4-acetyl-1-cyclohexene	0402	N, T	
α-Irone	0403	N	
α-Iso-methylionone	0404	N	No safety concern

Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones

Acetoin (3-hydroxy-2-butanone)	0405	N	No safety concern
2-Acetoxy-3-butanone	0406	N, T	
Butan-3-one-2-yl butanoate	0407	N, T	
Diacetyl (2,3-butanedione)	0408	N	
3-Hydroxy-2-pentanone	0409	N, T	
2,3-Pentanedione	0410	N	
4-Methyl-2,3-pentanedione	0411	N, T	
2,3-Hexanedione	0412	N	
3,4-Hexanedione	0413	N	
5-Methyl-2,3-hexanedione	0414	N, T	
2,3-Heptanedione	0415	N	
5-Hydroxy-4-octanone	0416	N, T	
2,3-Undecadione	0417	N, T	
Methylcyclopentenolone	0418	N, T	
Ethylcyclopentenolone	0419	N	
3,4-Dimethyl-1,2-cyclopentanedione	0420	N	
3,5-Dimethyl-1,2-cyclopentanedione	0421	N, T	

Substance ^a	No.	Specifications ^b	Conclusion based on current intake
Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones <i>(continued)</i>			
3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one	0422	N	No safety concern
5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one	0423	N	
2-Hydroxy-2-cyclohexen-1-one	0424	N, T	
1-Methyl-2,3-cyclohexadione	0425	N	
2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one	0426	N	
Menthol and structurally related substances			
Structural class I			
Menthol ⁱ	0427	R	No safety concern
(+)-neo-Menthol	0428	N, T	
Menthyl acetate	0431	N	
Menthyl isovalerate	0432	N	
(-)-Menthyl lactate	0433	N	
<i>p</i> -Menth-1-en-3-ol	0434	N, T	
(-)-Menthol ethylene glycol carbonate	0443	N	
Mixture of (-)-menthol 1- and 2-propylene glycol carbonate	0444	N	
mono-Menthyl succinate	0447	N	
Structural class II			
Menthone	0429	N	No safety concern
($\pm$)-Isomenthone	0430	N	
Piperitone	0435	N	
(-)-Menthone 1,2-glycerone ketal	0445	N	
($\pm$)-Menthone 1,2-glycerol ketal	0446	N	

^a The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 6, 1998). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.

^b N, new specifications prepared; R, existing specifications revised; T, the existing, new or revised specifications are tentative and comments are invited.

^c The ADI "not specified" for the calcium, potassium and sodium salts of oleic acid established at the thirty-third meeting of the Committee (WHO Technical Report Series, No. 776, 1989) was maintained.

^d The evaluation of this substance was deferred, pending review of a 90-day toxicity study that was not available at the meeting.

^e The group ADI of 0–0.5 mg/kg of body weight established at the twenty-third meeting (WHO Technical Report Series, No. 648, 1980) for citral, geranyl acetate, citronellol, linalool and linalyl acetate, expressed as citral, was maintained.

^f Methyl 1-acetoxycyclohexylketone was not evaluated because a NOEL for this or a related substance was not available and the per capita intake exceeds 1.5 μ g per day.

^g The ADI of 0–1 mg/kg of body weight established at the thirty-seventh meeting of the Committee (WHO Technical Report Series, No. 806, 1991, and corrigenda), was maintained.

^h The group ADI of 0–0.1 mg/kg of body weight established at the twenty-eighth meeting of the Committee (WHO Technical Report Series, No. 710, 1984) for α -ionone and β -ionone, was maintained.

ⁱ 1-4-Dimethyl-4-acetyl-1-cyclohexane was not considered to have sufficient structural similarities to the ionones to be included in this group. Accordingly, the safety evaluation of this compound was not considered at the present meeting.

^j An ADI of 0–4 mg/kg of body weight was established for menthol at the present meeting (see section 3.2.3).

Substance ^a (considered for specifications only)	No.	Specifications ^b
Flavouring agents		
<i>Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids^c</i>		
Isobutyl alcohol (2-methyl-1-propanol)	0251	R
Isobutyraldehyde (2-methylpropanal)	0252	N
Isobutyric acid (2-methylpropanoic acid)	0253	N
2-Methylbutyraldehyde (2-methylbutanal)	0254	N
2-Methylbutyric acid (2-methylbutanoic acid)	0255	N
2-Ethylbutyraldehyde (2-ethylbutanal)	0256	N
2-Ethylbutyric acid (2-ethylbutanoic acid)	0257	N
3-Methylbutyraldehyde (3-methylbutanal)	0258	N
Isovaleric acid (3-methylbutanoic acid)	0259	N
2-Methylpentanal	0260	N, T
2-Methylvaleric acid (2-methylpentanoic acid)	0261	N
3-Methylpentanoic acid	0262	N
3-Methyl-1-pentanol	0263	N
4-Methylpentanoic acid	0264	N
2-Methylhexanoic acid	0265	N
5-Methylhexanoic acid	0266	N
2-Ethyl-1-hexanol	0267	N
3,5,5-Trimethyl-1-hexanol	0268	N
3,5,5-Trimethylhexanal	0269	N
2-Methyloctanal	0270	N, T
4-Methyloctanoic acid	0271	N, T
3,7-Dimethyl-1-octanol	0272	N
2,6-Dimethyloctanal	0273	N, T
4-Methylnonanoic acid	0274	N
2-Methylundecanal	0275	N
<i>Aliphatic lactones^c</i>		
4-Hydroxybutyric acid lactone (γ -butyrolactone)	0219	N
γ -Valerolactone	0220	N
4-Hydroxy-3-pentenoic acid lactone	0221	N
5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	0222	N
γ -Hexalactone	0223	N
δ -Hexalactone	0224	N
γ -Heptalactone	0225	N
γ -Octalactone	0226	N
6-Hydroxy-3,7-dimethyloctanoic acid lactone	0237	N
δ -Tetradecalactone	0238	N
ω -Pentadecalactone	0239	N, T
ω -6-Hexadecenlactone	0240	N
ϵ -Decalactone	0241	N, T
ϵ -Dodecalactone	0242	N
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	0243	N
3-Heptyldihydro-5-methyl-2(3H)-furanone	0244	N, T
5-Hydroxy-2,4-decadienoic acid δ -lactone	0245	N
4,4-Dibutyl- γ -butyrolactone	0227	N
δ -Octalactone	0228	N

Substance ^a (considered for specifications only)	No.	Specifications ^b
Flavouring agents (<i>continued</i>)		
Aliphatic lactones^c (<i>continued</i>)		
γ -Nonalactone	0229	R
Hydroxynonanoic acid δ -lactone	0230	N
γ -Decalactone	0231	N
δ -Decalactone	0232	N
γ -Undecalactone	0233	R
5-Hydroxyundecanoic acid δ -lactone	0234	N
γ -Dodecalactone	0235	N
δ -Dodecalactone	0236	N
5-Hydroxy-2-decenoic acid δ -lactone	0246	N
5-Hydroxy-7-decenoic acid δ -lactone	0247	N, T
5-Hydroxy-8-undecenoic acid δ -lactone	0248	N, T
<i>cis</i> -4-Hydroxy-6-dodecenoic acid lactone (1,4-dodec-6-enolactone)	0249	N, T
γ -Methyldecalactone	0250	N
Mixture of 5-Hydroxy-2-decenoic acid δ -lactone, 5-hydroxy-2-dodecenoic acid δ -lactone and 5-hydroxy-2-tetradecenoic acid δ -lactone	0276	O
4-Hydroxy-3-methyloctanoic acid γ -lactone	0437	N
5-Hydroxy-2-dodecenoic acid δ -lactone	0438	N, T
Food additives		
Acetone (extraction solvent)	—	R
Aluminium powder	—	R
Calcium propionate	—	S
Calcium sorbate	—	R
Canthaxanthin	—	R
Carnauba wax	—	R
Carob bean gum	—	R
Carotenes (algae)	—	R
Carotenes (vegetable)	—	R
Carthamus red	—	R
Carthamus yellow	—	R
Citric acid	—	R, T ^d
Cochineal extract	—	R
Diacetyltartaric and fatty acid esters of glycerol (DATEM)	—	R
Dichloromethane	—	R
Ethyl <i>p</i> -hydroxybenzoate	—	R
Ferrous gluconate	—	R, T ^d
Ferrous sulfate	—	N, T ^d
Furfuryl alcohol (flavouring agent)	0451	N
Guar gum	—	R
Gum arabic	—	R
Hexanes	—	R
4-Hexylresorcinol	—	R
Isobutyl alcohol (isobutanol) (extraction solvent)	—	R
Methyl <i>p</i> -hydroxybenzoate	—	R

Substance ^a (considered for specifications only)	No.	Specifications ^b
Food additives (<i>continued</i>)		
Microcrystalline cellulose	—	R
Nitrogen	—	R
Petroleum jelly	—	R
Polydextroses	—	R
Potassium sorbate	—	R
Propan-2-ol	—	R
Propionic acid	—	R
Propyl <i>p</i> -hydroxybenzoate	—	R
Shellac, bleached	—	R
Sodium sorbate	—	O
Sorbitan monolaurate	—	R
Sucrose esters of fatty acids	—	R
Talc	—	R
Tartaric, acetic and fatty acid esters of glycerol, mixed	—	W ^e
Thaumatococcus	—	R, T ^d
Xanthan gum	—	R

^a The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 6, 1998). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.

^b N, new specifications prepared; O, no specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or not required; T, the existing, new or revised specifications are tentative and comments are invited; W, existing specifications withdrawn.

^c These substances were evaluated at the forty-ninth meeting of the Committee (WHO Technical Report Series, No. 884, 1998).

^d See Annex 3.

^e At its forty-ninth meeting (WHO Technical Report Series, No. 884, 1998), the Committee developed revised specifications for "diacetyltartaric and fatty acid esters of glycerol" and "tartaric, acetic and fatty acid esters of glycerol, mixed" but found it difficult to distinguish between the two substances. At that meeting, the Committee requested data that would allow these two substances to be distinguished, and stated that unless such data were provided by 31 March 1998, it would consider combining the specifications for the two substances. Since no data were received, the Committee, at its present meeting, decided to combine the specifications for the two substances under the name "diacetyltartaric and fatty acid esters of glycerol".

Food additives considered for evaluation of national intake assessments

Substance	Conclusions	Recommendations to the Codex Committee on Food Additives and Contaminants
Benzoates	Intake estimates based on levels specified in the draft General Standard for Food Additives^a and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–5 mg/kg of body weight in the five Member States^b that submitted such data. In national data submitted by nine Member States,^b estimates of mean intake of benzoates did not exceed the ADI. The potential exists for consumers with high intakes to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — ripened cheese (1.6.2) — processed cheese (1.6.4) — cheese analogues (1.6.5) — vegetables in vinegar, oil or brine (4.2.2.3) — cooked molluscs, crustaceans and echinoderms (9.2.4.2) — semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms (9.3) — liquid egg products (10.2.1) — carbonated drinks (14.1.4.1)
BHA	Intake estimates based on levels in the draft General Standard for Food Additives^a and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–0.5 mg/kg of body weight for mean intake in the three Member States^b that submitted such data. In national data submitted by seven Member States,^b estimates of mean intake by consumers of BHA did not exceed the ADI. The potential exists for consumers of high levels of BHA to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — fats and oils and fat emulsions (type water-in-oil) (2) — dried vegetables (4.2.2.2) — cocoa and chocolate products other than 5.1.1, 5.1.2 and 5.1.4 (cocoa mixes (powders and syrups), cocoa-based spreads, including fillings, and imitation chocolate and chocolate substitute products) (5.1.3) — non-heat-treated processed comminuted meat, poultry and game products (8.3.1) — frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms (9.2.1) — ready-to-eat soup and broths, including canned, bottled and frozen (12.5.1) — food supplements (13.6)

Food additives considered for evaluation of national intake assessments (*continued*)

Substance	Conclusions	Recommendations to the Codex Committee on Food Additives and Contaminants
BHT	Intake estimates based on levels in the draft General Standard for Food Additives^a and the range of foods in which use is allowed integrated with data on national food consumption exceeded the ADI of 0–0.3 mg/kg of body weight for mean intake in the five Member States^b that submitted such data. In national data submitted by eight Member States,^b estimates of mean and high intake for consumers of BHT exceeded the ADI in only one country.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — fats and oils and fat emulsions (type water-in-oil) (2) — chewing gum (5.3) — processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2)
Sulfites	Intake estimates based on levels in the draft General Standard for Food Additives^a and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–0.7 mg/kg of body weight for mean intake in the three Member States^b that submitted such data. In national data submitted by six Member States,^b estimates of mean intake for consumers of sulfites did not exceed the ADI. The potential exists for consumers of high levels of sulfites to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — dried fruit (4.1.2.2) — jams, jellies and marmalades (4.1.2.5) — fruit preparations, including pulp and fruit toppings (4.1.2.8) — dried vegetables (4.2.2.2) — vegetable, nut and seed purees and spreads (4.2.2.5) — white and semi-white sugar (sucrose or saccharose), fructose, glucose (dextrose), xylose, sugar solutions and syrups, and (partially) inverted sugars, including molasses, treacle and sugar toppings (11.1) — concentrates (liquid or solid) for fruit juice (14.1.2.3) — wines (14.2.3) — fruit wine (14.2.4)

TBHQ	Intake estimates based on levels in the draft General Standard for Food Additives^a and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–0.7 mg/kg of body weight for mean intake for the three Member States^b that submitted such data. In national data submitted by four Member States,^b estimates of mean intake by consumers of TBHQ did not exceed the ADI. The potential exists for consumers of high levels of TBHQ to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — fats and oils and fat emulsions (type water-in-oil) (2) — processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2) — carbonated drinks (14.1.4.1)

BHA: Butylated hydroxyanisole; BHT: butylated hydroxytoluene; TBHQ: *tert*-butyl-hydroquinone.

^a Intake estimates calculated using the additive levels specified in the draft General Standard for Food Additives integrated with national data on food consumption will greatly overestimate actual intakes in any one country because the levels specified in the General Standard are generally compiled by adopting the highest level of use for any one food category submitted by Member States or nongovernmental organizations. The range of food uses specified in the draft General Standard for Food Additives is also usually much wider than in national standards.

^b Eleven Member States made submissions: Australia, Brazil, China, Finland, France, India, Japan, New Zealand, Spain, the United Kingdom and the USA. Australia and New Zealand made a joint submission.

Annex 3

Further toxicological studies and other information required or desired

Flavouring agent

Furfural

Before reviewing this substance again, the Committee would wish to have the following:

1. The results of studies of DNA binding or adduct formation *in vivo* to clarify whether furfural interacts with DNA in the liver of mice.
2. The results of a 90-day study of toxicity in rats to identify a NOEL for hepatotoxicity.

Food colour

Curcumin

The results of a reproductive toxicity study on a substance complying with the specifications for curcumin and information on the need and technological justification for alternative solvents for use in the current manufacturing processes of curcumin are required for evaluation in 2001.

Glazing agents

Mineral oils (medium- and low-viscosity)

Information requested at the forty-fourth meeting of the Committee is required for evaluation in 2002. This includes information about the compositional factors in mineral oils that influence their absorption and toxicity and a study of at least 1 year's duration of one of these materials in F344 rats, including an assessment of immune function at appropriate time periods (with a reversal period of 1 year) and an investigation of the kinetics of accumulation of the material, and particularly whether a plateau is reached. In addition, research on the pharmacokinetics of white mineral oils and their potential effects on immune function known to be in progress should be submitted for review at that time.

Preservatives

Calcium hydrogen sulfite

Information is required on a modified name for the compound that would be more reflective of its physical properties, the extent and functionality of its use as a food additive, and the need for limits for

selenium and arsenic and associated analytical methods. The information is required by 30 November 1998.

Potassium metabisulfite, potassium sulfite, sodium hydrogen sulfite and sodium metabisulfite

Information on iron and selenium in commercial products and on the test method for selenium is required by 30 November 1998. The existing specifications include a limit for selenium, for which the test method is no longer feasible because of a lack of availability of required reagents. The Committee also questioned the need for a limit for selenium because new processes by which sulfur is produced as a by-product of the oil industry have replaced extraction methods from sulfur mines. The Committee noted that the limit for selenium may no longer be necessary and requested information on this point.

Sodium sulfite

Information on the sources of raw materials, on the commercial use of sodium sulfite heptahydrate in food, on iron and selenium levels in commercial products, and on test methods for selenium is required by 30 November 1998.

Sodium thiosulfate

Information on iron and selenium levels in commercial products and on test methods for lead and selenium is required by 30 November 1998.

Sweetening agents

Stevioside

Before reviewing this substance again, the Committee considered that it would be necessary to develop specifications to ensure that the material tested was representative of the commercial product. Further information on the nature of the substance that was tested, data on the metabolism of stevioside in humans and the results of suitable *in vivo* genotoxicity studies with steviol would also be necessary.

Thaumatococin

Information on a specific identification test is required by 30 November 1998.

Thickening agents

Carrageenan and processed Eucheuma seaweed

Information to permit clarification of the significance of the promotion of colon cancer observed in studies in rats is required for evaluation in 2001.

Miscellaneous substances

Citric acid

Information on the need for a test for oxalate and a suitable limit is required by 30 March 1999. Comments are also invited on the new test for sulfate. If no information is forthcoming, the Committee will consider retaining the specifications as they appear in the specifications monograph.

γ-Cyclodextrin

The study of human tolerance known to have been conducted should be reviewed in order to confirm the absence of adverse effects on the gastrointestinal tract at normal levels of intake. These data should be submitted for consideration by the Committee by 1999.

Ferrous gluconate

Information on the need for maintaining the limit test for oxalic acid and the maximum limit for mercury, as well as the procedure for mercury analysis, if applicable, and for introducing maximum limits for sulfate and chloride is required by 30 November 1998.

Ferrous sulfate

Information on the limit and an analytical method for mercury and the need for a limit on water content is required by 30 November 1998.

Magnesium gluconate

Information on the need for maintaining the microbiological criteria included in the specifications, and for introducing maximum limits for sulfate and chloride is required by 30 November 1998.

Annex 4

Food categorization system for the General Standard for Food Additives¹

- 1 Dairy products, excluding products of category 2
 - 1.1 Milk and dairy-based drinks
 - 1.1.1 Milk and buttermilk
 - 1.1.1.1 Milk, including sterilized and UHT goats' milk
 - 1.1.1.2 Buttermilk (plain)
 - 1.1.2 Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, egg-nog)
 - 1.2 Fermented and renneted milk products (plain), excluding drinks
 - 1.2.1 Fermented milks (plain)
 - 1.2.1.1 Fermented milks (plain), not heat-treated after fermentation
 - 1.2.1.2 Fermented milks (plain), heat-treated after fermentation
 - 1.2.2 Renneted milk
 - 1.3 Condensed milk (plain) and analogues
 - 1.3.1 Condensed milk (plain)
 - 1.3.2 Beverage whiteners
 - 1.4 Cream (plain) and similar products
 - 1.4.1 Pasteurized cream
 - 1.4.2 Sterilized, UHT, whipping or whipped and reduced-fat creams
 - 1.4.3 Clotted cream
 - 1.4.4 Cream analogues
 - 1.5 Milk and cream powders (plain)
 - 1.5.1 Milk and cream powders
 - 1.5.2 Milk and cream powder analogues
 - 1.6 Cheese
 - 1.6.1 Unripened cheese
 - 1.6.2 Ripened cheese
 - 1.6.2.1 Total ripened cheese, including rind
 - 1.6.2.2 Rind of ripened cheese
 - 1.6.2.3 Cheese powder (for reconstitution, e.g. for cheese sauces)
 - 1.6.3 Whey cheese
 - 1.6.4 Processed cheese
 - 1.6.5 Cheese analogues
 - 1.7 Dairy-based desserts (e.g. ice cream, ice milk, pudding, fruit or flavoured yoghurt)
 - 1.8 Whey and whey products, excluding whey cheese
- 2 Fats and oils and fat emulsions (type water-in-oil)
 - 2.1 Fats and oils essentially free from water
 - 2.1.1 Butter oil, anhydrous milk fat, ghee
 - 2.1.2 Vegetable oils and fats

¹ Reproduced, with minor editorial corrections, from *Draft Codex General Standard for Food Additives* (Rome, Food and Agriculture Organization of the United Nations, 1999; unpublished document FX/FAC 99/6) by permission of the publisher.

- 2.1.3 Lard, tallow, fish oil and other animal fats
 - 2.2 Fat emulsions mainly of type water-in-oil
 - 2.2.1 Emulsions containing at least 80% fat
 - 2.2.1.1 Butter and concentrated butter
 - 2.2.1.2 Margarine and similar products (e.g. butter-margarine blends)
 - 2.2.2 Emulsions containing less than 80% fat (e.g. minarine)
 - 2.3 Fat emulsions other than 2.2, including mixed and/or flavoured products based on fat emulsions
 - 2.4 Fat-based desserts (excluding dairy-based desserts)
- 3 Edible ices, including sherbet and sorbet
- 4 Fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes) and nuts and seeds
- 4.1 Fruit
 - 4.1.1 Fresh fruit
 - 4.1.1.1 Untreated fruit
 - 4.1.1.2 Surface-treated fruit
 - 4.1.1.3 Peeled or cut fruit
 - 4.1.2 Processed fruit
 - 4.1.2.1 Frozen fruit
 - 4.1.2.2 Dried fruit
 - 4.1.2.3 Fruit in vinegar, oil or brine
 - 4.1.2.4 Canned or bottled (pasteurized) fruit
 - 4.1.2.5 Jams, jellies and marmalades
 - 4.1.2.6 Fruit-based spreads other than 4.1.2.5 (e.g. chutney)
 - 4.1.2.7 Candied fruit
 - 4.1.2.8 Fruit preparations, including pulp and fruit toppings
 - 4.1.2.9 Fruit-based desserts, including fruit-flavoured water-based desserts
 - 4.1.2.10 Fermented fruit products
 - 4.1.2.11 Fruit fillings for pastries
 - 4.1.2.12 Cooked or fried fruit
 - 4.2 Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes) and nuts and seeds
 - 4.2.1 Fresh vegetables
 - 4.2.1.1 Untreated vegetables
 - 4.2.1.2 Surface-treated vegetables
 - 4.2.1.3 Peeled or cut vegetables
 - 4.2.2 Processed vegetables and nuts and seeds
 - 4.2.2.1 Frozen vegetables
 - 4.2.2.2 Dried vegetables
 - 4.2.2.3 Vegetables in vinegar, oil or brine
 - 4.2.2.4 Canned or bottled (pasteurized) vegetables
 - 4.2.2.5 Vegetable, nut and seed purees and spreads (e.g. peanut butter)
 - 4.2.2.6 Vegetable, nut and seed pulps and preparations other than 4.2.2.5
 - 4.2.2.7 Fermented vegetable products
 - 4.2.2.8 Cooked or fried vegetables

- 5 **Confectionery**
 - 5.1 Cocoa products and chocolate products, including imitations and chocolate substitutes
 - 5.1.1 Cocoa mixes (powders and syrups)
 - 5.1.2 Cocoa-based spreads, including fillings
 - 5.1.3 Cocoa and chocolate products other than 5.1.1, 5.1.2 and 5.1.4 (e.g. milk chocolate bars, chocolate flakes, white chocolate)
 - 5.1.4 Imitation chocolate and chocolate substitute products
 - 5.2 Sugar-based confectionery other than 5.1, 5.3 and 5.4, including hard and soft candy and nougats
 - 5.3 Chewing gum
 - 5.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces
- 6 **Cereals and cereal products, including flours and starches from roots and tubers, and pulses and legumes, excluding bakery wares**
 - 6.1 Whole, broken or flaked grain, including rice
 - 6.2 Flours and starches
 - 6.3 Breakfast cereals, including rolled oats
 - 6.4 Pastas and noodles
 - 6.5 Cereal and starch-based desserts (e.g. rice pudding, tapioca pudding)
 - 6.6 Batters (e.g. for fish or poultry)
- 7 **Bakery wares**
 - 7.1 Bread and ordinary bakery wares
 - 7.1.1 Breads and rolls
 - 7.1.2 Crackers, excluding sweet crackers
 - 7.1.3 Other ordinary bakery products (e.g. bagels, pitta, English muffins)
 - 7.1.4 Bread-type products, including bread stuffing and breadcrumbs
 - 7.2 Fine bakery wares
 - 7.2.1 Cakes, cookies and pies (e.g. fruit-filled or custard types)
 - 7.2.2 Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins)
 - 7.2.3 Mixes for fine bakery wares (e.g. cakes, pancakes)
- 8 **Meat and meat products, including poultry and game**
 - 8.1 Fresh meat, poultry and game
 - 8.1.1 Fresh meat, poultry and game, whole pieces or cuts
 - 8.1.2 Fresh meat, poultry and game, comminuted
 - 8.2 Processed meat, poultry and game products in whole pieces or cuts
 - 8.2.1 Non-heat-treated processed meat, poultry and game products in whole pieces or cuts
 - 8.2.1.1 Cured (including salted) non-heat-treated processed meat, poultry and game products in whole pieces or cuts
 - 8.2.1.2 Cured (including salted) and dried non-heat-treated processed meat, poultry and game products in whole pieces or cuts
 - 8.2.1.3 Fermented non-heat-treated processed meat, poultry and game products in whole pieces or cuts

- 8.2.2 Heat-treated processed meat, poultry and game products in whole pieces or cuts
- 8.2.3 Frozen processed meat, poultry and game products in whole pieces or cuts
- 8.3 Processed comminuted meat, poultry and game products
 - 8.3.1 Non-heat-treated processed comminuted meat, poultry and game products
 - 8.3.1.1 Cured (including salted) non-heat-treated processed comminuted meat, poultry and game products
 - 8.3.1.2 Cured (including salted) and dried non-heat-treated processed comminuted meat, poultry and game products
 - 8.3.1.3 Fermented non-heat-treated processed comminuted meat, poultry and game products
 - 8.3.2 Heat-treated processed comminuted meat, poultry and game products
 - 8.3.3 Frozen processed comminuted meat, poultry and game products
- 8.4 Edible casings (e.g. sausage casings)
- 9 **Fish and fish products, including molluscs, crustaceans and echinoderms**
 - 9.1 Fresh fish and fish products, including molluscs, crustaceans and echinoderms
 - 9.1.1 Fresh fish
 - 9.1.2 Fresh molluscs, crustaceans and echinoderms
 - 9.2 Processed fish and fish products, including molluscs, crustaceans and echinoderms
 - 9.2.1 Frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms
 - 9.2.2 Frozen battered fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms
 - 9.2.3 Frozen minced and creamed fish products, including molluscs, crustaceans and echinoderms
 - 9.2.4 Cooked and/or fried fish and fish products, including molluscs, crustaceans and echinoderms
 - 9.2.4.1 Cooked fish
 - 9.2.4.2 Cooked molluscs, crustaceans and echinoderms
 - 9.2.4.3 Fried fish and fish products, including molluscs, crustaceans and echinoderms
 - 9.2.5 Smoked, dried, fermented and/or salted fish and fish products, including molluscs, crustaceans and echinoderms
 - 9.3 Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms
 - 9.3.1 Fish and fish products, including molluscs, crustaceans and echinoderms, marinated and/or in jelly
 - 9.3.2 Fish and fish products, including molluscs, crustaceans and echinoderms, pickled and/or in brine
 - 9.3.3 Salmon substitutes, caviar and other fish roe products
 - 9.3.4 Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms other than 9.3.1–9.3.3
 - 9.4 Fully preserved (including canned or fermented) fish and fish products, including molluscs, crustaceans and echinoderms
- 10 **Eggs and egg products**
 - 10.1 Fresh eggs

- 10.2 Egg products
 - 10.2.1 Liquid egg products
 - 10.2.2 Frozen egg products
 - 10.2.3 Dried and/or heat-coagulated egg products
- 10.3 Preserved eggs, including alkaline, salted and canned eggs
- 10.4 Egg-based desserts (e.g. custard)
- 11 **Sweeteners, including honey**
 - 11.1 White and semi-white sugar (sucrose or saccharose), fructose, glucose (dextrose), xylose, sugar solutions and syrups, and (partially) inverted sugars, including molasses, treacle and sugar toppings
 - 11.2 Other sugars and syrups (e.g. brown sugar, maple syrup)
 - 11.3 Honey
 - 11.4 Table-top sweeteners, including those containing high-intensity sweeteners, other than 11.1–11.3
- 12 **Salts, spices, soups, sauces, salads, protein products, etc.**
 - 12.1 Salt
 - 12.2 Herbs, spices, seasonings (including salt substitutes) and condiments
 - 12.3 Vinegars
 - 12.4 Mustards
 - 12.5 Soups and broths
 - 12.5.1 Ready-to-eat soups and broths, including canned, bottled and frozen
 - 12.5.2 Mixes for soups and broths
 - 12.6 Sauces and similar products
 - 12.6.1 Emulsified sauces (e.g. mayonnaise, salad dressing)
 - 12.6.2 Non-emulsified sauces (e.g. ketchup, cheese sauce, cream sauce, brown gravy)
 - 12.6.3 Mixes for sauces and gravies
 - 12.7 Salads (e.g. macaroni salad, potato salad) and sandwich spreads (excluding cocoa- and nut-based spreads)
 - 12.8 Yeast
 - 12.9 Protein products
- 13 **Foodstuffs intended for particular nutritional uses**
 - 13.1 Infant formulae and follow-on formulae
 - 13.2 Foods for young children (weaning foods)
 - 13.3 Dietetic foods intended for special medical purposes
 - 13.4 Dietetic formulae for slimming purposes and weight reduction
 - 13.5 Dietetic foods other than 13.1–13.4
 - 13.6 Food supplements
- 14 **Beverages, excluding dairy products**
 - 14.1 Non-alcoholic ("soft") beverages
 - 14.1.1 Waters
 - 14.1.1.1 Natural mineral waters and source waters
 - 14.1.1.2 Table waters and soda waters
 - 14.1.2 Fruit and vegetable juices
 - 14.1.2.1 Canned or bottled (pasteurized) fruit juice
 - 14.1.2.2 Canned or bottled (pasteurized) vegetable juice

- 14.1.2.3 Concentrates (liquid or solid) for fruit juice
- 14.1.2.4 Concentrates (liquid or solid) for vegetable juice
- 14.1.3 Fruit and vegetable nectars
 - 14.1.3.1 Canned or bottled (pasteurized) fruit nectar
 - 14.1.3.2 Canned or bottled (pasteurized) vegetable nectar
 - 14.1.3.3 Concentrates (liquid or solid) for fruit nectar
 - 14.1.3.4 Concentrates (liquid or solid) for vegetable nectar
- 14.1.4 Water-based flavoured drinks, including "sport" or "electrolyte" drinks
 - 14.1.4.1 Carbonated drinks
 - 14.1.4.2 Non-carbonated drinks, including punches and ades
 - 14.1.4.3 Concentrates (liquid or solid) for drinks
- 14.1.5 Coffee, coffee substitutes, tea, herbal infusions and other hot cereal beverages, excluding cocoa
- 14.2 Alcoholic beverages, including alcohol-free and low-alcoholic counterparts
 - 14.2.1 Beer and malt beverages
 - 14.2.2 Cider and perry
 - 14.2.3 Wines
 - 14.2.3.1 Still wine
 - 14.2.3.2 Sparkling and semi-sparkling wines
 - 14.2.3.3 Fortified wine and liquor wine
 - 14.2.3.4 Aromatized wine
 - 14.2.4 Fruit wine
 - 14.2.5 Mead
 - 14.2.6 Spirituous beverages
 - 14.2.6.1 Spirituous beverages containing at least 15% alcohol
 - 14.2.4.2 Spirituous beverages containing less than 15% alcohol
- 15 **Ready-to-eat savouries**
 - 15.1 Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)
 - 15.2 Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit)
- 16 **Composite foods (e.g. casseroles, meat pies, mincemeat) — foods that could not be placed in categories 1–15**



Simple esters of linalool: Human health tier II assessment

29 June 2018

- Chemicals in this assessment
- Preface
- Grouping Rationale
- Import, Manufacture and Use
- Restrictions
- Existing Worker Health and Safety Controls
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- References

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Propanoic acid, 2-methyl-, 1-ethenyl-1,5-dimethyl-4-hexenylester	78-35-3
Butanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	78-36-4
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate	115-95-7
1,6-Octadien-3-ol, 3,7-dimethyl-, formate	115-99-1
1,6-Octadien-3-ol, 3,7-dimethyl-, benzoate	126-64-7
1,6-Octadien-3-ol, 3,7-dimethyl-, propanoate	144-39-8
Butanoic acid, 3-methyl-, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	1118-27-0
Benzeneacetic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	7143-69-3
Hexanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	7779-23-9

Chemical Name in the Inventory	CAS Number
Octanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	10024-64-3
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate, (R)-	16509-46-9

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit: www.nicnas.gov.au

Disclaimer

NICNAS has made every effort to assure the quality of information available in this report. However, before relying on it for a specific purpose, users should obtain advice relevant to their particular circumstances. This report has been prepared by NICNAS using a range of sources, including information from databases maintained by third parties, which include data supplied by industry. NICNAS has not verified and cannot guarantee the correctness of all information obtained from those databases. Reproduction or further distribution of this information may be subject to copyright protection. Use of this information without obtaining the permission from the owner(s) of the respective information might violate the rights of the owner. NICNAS does not take any responsibility whatsoever for any copyright or other infringements that may be caused by using this information.

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemicals in this group are esters of linalool (CAS No. 78-70-6), known as linalyl esters. Most of the chemicals in this group have similar uses; they are used in food flavouring or as a fragrance component (see **Use** section). The favoured configuration in genuine lavender oils is the (*R*)-isomer of linalyl acetate (Mosandl, 2004).

Following absorption, linalyl esters are expected to hydrolyse to linalool and the corresponding acids in the gastrointestinal tract and by tissue hydrolases. Among the esters, linalyl acetate (CAS No. 115-95-7) is the main component of lavender and bergamot oils, and is the most widely used (SCCS, 2012). Given the close structural similarities of the chemicals in this group and common hydrolysis product (linalool), they are expected to have similar toxicological effects.

Limited data is available for some of the esters in this group. Therefore, where toxicological data are lacking for specific endpoints in this assessment, data available for linalyl acetate and the metabolite linalool (NICNAS) are considered applicable to these chemicals, particularly for systemic long-term toxicity.

The corresponding carboxylate ions produced by ester hydrolysis in vivo of the chemicals in this group are all expected to have low toxicity.

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified for the majority of the chemicals in this group. However, linalyl acetate (CAS No 115-95-7) has reported domestic use in automotive aftermarket products including car wash soaps, boat wash soaps, polishes and rubbing compounds.

International

The following international uses have been identified through the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; the Organisation for Economic Co-operation and Development Screening information data set International Assessment Report (OECD SIAR); Galleria Chemica; the Substances and Preparations in Nordic countries (SPIN) database; the European Commission Cosmetic Ingredients and Substances (CosIng) database; the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); the US Household Products Database and the International Fragrance Association (IFRA) Transparency List.

All chemicals in this group, except for (*R*)-linalyl acetate (CAS No. 16509-46-9) and linalyl octanoate (CAS No. 10024-64-3), have reported cosmetic use in perfumes or as fragrance ingredients.

Linalyl acetate (CAS No. 115-95-7) has been reported as being used in a number of cosmetic products in the Compilation of Ingredients used in Cosmetics in the United States (CIUCUS, 2011).

The chemicals have reported domestic/commercial uses, including in:

- adhesives and sealants;
- cleaning and maintenance products;
- paints, lacquers and varnishes;
- process regulators;
- welding and soldering agents; and
- odour agents.

The chemicals have reported non-industrial uses including:

- as food additives; and
- in non-agricultural pesticides and preservatives.

Linalyl acetate is a substitute for pettigrain oil (HSDB).

Restrictions

Australian

No known restrictions have been identified.

International

The chemicals are listed on the following (Galleria Chemica):

- as 'Terpenes and terpenoids' in the EU Cosmetic Directive 76/768/EEC Annex III—List of substances which Cosmetic Products must not contain except subject to the restrictions and conditions laid down: Peroxide value less than 10 mmoles/L; and
- as 'Terpene hydrocarbons' in the New Zealand Cosmetic Products Group Standard—Schedule 5: Components cosmetic products must not contain except subject to the restrictions and conditions laid down: Peroxide value less than 10 mmoles/L.

The acceptable daily intake (ADI) for linalyl acetate and linalyl formate is 0.5 mg/kg bw (EFSA, 2012).

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals are not listed on the Hazardous Chemical Information System (HCIS) (Safe Work Australia).

Exposure Standards

Australian

No known restrictions are identified.

International

The following exposure standards are identified for terpenes (Galleria Chemica).

A time weighted average (TWA) of 111–150 mg/m³ (20–25 ppm) in Canada (Alberta), Estonia and Sweden.

Short-term exposure limits (STEL) of 300 mg/m³ (50 ppm) in Estonia and Sweden.

Health Hazard Information

Toxicokinetics

Linalyl esters readily hydrolyse to linalool and the corresponding carboxylic acids following oral exposure. Hydrolysis is catalysed by carboxylesterases or esterases, in particular beta-esterases in the liver. The hydrolysis products then conjugate with glucuronic acid and are excreted in urine. Based on studies for linalyl acetate, it is expected that linalool is available for systemic circulation following oral administration of linalyl esters (OECD, 2002; Bickers et al., 2003a).

Following an inhalation exposure to linalyl acetate at 5 mg/L for 1 hour in mice, serum levels of linalyl acetate and linalool were reported to be 1–2 ng/mL and 4–5 ng/mL, respectively. In another inhalation study, mice exposed to linalyl acetate for 1 hour had serum levels of 1 ng/mL linalyl acetate and 4 ng/mL linalool. When the mice were exposed to 5 mg/mL lavender oil (37 % linalool and 42 % linalyl acetate), the serum levels were 3 ng/mL for linalool and 11 ng/mL for linalyl acetate (Bickers et al., 2003a).

In vitro studies of linalyl acetate have shown hydrolysis to linalool then, to some extent, rapid rearrangement to the ring-closed form alpha-terpineol. Linalyl acetate hydrolyses rapidly (half-life ($t_{1/2}$) < 5 min) at the low pH (acidic) of gastric fluids to form linalool and acetic acid. The hydrolysis rate of this chemical is slow in neutral gastric juice ($t_{1/2}$ = 121 min) and in intestinal fluid with or without pancreatin ($t_{1/2}$ = 153–198 min). Hydrolysis of this chemical in homogenates of rat intestinal mucosa, blood and liver is slower than in acidic gastric juice (Bickers et al., 2003a; HSDB).

In a percutaneous absorption case study, a massage oil containing lavender oil and peanut oil (2:98 ratio) was applied to the skin of a male subject (age 34 years). The lavender oil contained linalool and linalyl acetate at 24.79 % and 29.59 %, respectively. Following application within 5 minutes, linalool and linalyl acetate were found in trace amounts in the blood. Peak plasma concentrations of 121 ng/mL linalyl acetate and 100 ng/mL linalool were reached after 20 minutes. Most of the lavender oil had disappeared from the blood within 90 minutes with a biological half-life of 14.3 minutes. The authors concluded that lavender oil is rapidly absorbed through the skin and excreted within 90 minutes (Bickers et al., 2003a; HSDB).

The dermal permeability constants are reported to be 0.0746 cm/hour for linalyl acetate (CAS No. 115-95-7), 0.711 cm/hour for linalyl benzoate (CAS No. 126-64-7), 0.860 cm/hour for linalyl isovalerate (CAS No. 1118-27-0), and 0.882 cm/hour for linalyl phenylacetate (CAS No. 7143-69-3) (Galleria Chemica).

Linalyl acetate auto-oxidises when exposed to air in a manner similar to linalool, forming allergenic hydroperoxides. A non-sensitising epoxide was identified as a secondary oxidation product for linalyl acetate. The corresponding epoxide was not found in the oxidation products of linalool (Skold et. al., 2008).

Acute Toxicity

Oral

Based on animal test results, the chemicals have low acute oral toxicity following oral exposure.

The reported median lethal dose (LD50) values are (Bickers et al., 2003b; Galleria Chemica):

- linalyl acetate (CAS No. 115-95-7), 10000 mg/kg bw in rats and 13360 mg/kg bw in mice;
- linalyl formate (CAS No. 115-99-1), >5000 mg/kg bw in rats and in rabbits, and 5490 mg/kg bw in male mice;
- linalyl propionate (CAS No. 144-39-8), >5000 mg/kg bw in rats and in rabbits, and 13870 mg/kg bw in male mice;
- linalyl benzoate (CAS No. 126-64-7), >5000 mg/kg bw in rats and 9400 mg/kg bw in male mice;
- linalyl butyrate (CAS No. 78-36-4), >5000 mg/kg bw in rats and in rabbits, and >8900 mg/kg bw in male mice;
- linalyl hexanoate (CAS No. 7779-23-9), 39040 mg/kg bw in mice;
- linalyl octanoate (CAS No. 10024-64-3), 48850 mg/kg bw in mice;

- linalyl isobutyrate (CAS No. 78-35-3), >36500 mg/kg bw in rats and 15100 mg/kg bw in mice;
- linalyl isovalerate (CAS No. 1118-27-0), >5000 mg/kg bw in rats and 25170 mg/kg bw in mice;
- linalyl phenylacetate (CAS No. 7143-69-3), >5000 mg/kg in rats and 15480 mg/kg bw in mice.

Dermal

Based on animal test results, the chemicals have low acute toxicity following dermal exposure.

The reported dermal LD50 values in rabbits are (Bickers et al., 2003b; Galleria Chemica):

- linalyl acetate (CAS No. 115-95-7), >5000 mg/kg bw;
- linalyl formate (CAS No. 115-99-1), >5000 mg/kg bw;
- linalyl propionate (CAS No. 144-39-8), >2000 mg/kg bw;
- linalyl benzoate (CAS No. 126-64-7), >5000 mg/kg bw;
- linalyl butyrate (CAS No. 78-36-4), >2000 mg/kg bw;
- linalyl hexanoate (CAS No. 7779-23-9), >2000 mg/kg bw;
- linalyl isobutyrate (CAS No. 78-35-3), >5000 mg/kg bw;
- linalyl isovalerate (CAS No. 1118-27-0), >2000 mg/kg bw; and
- linalyl phenylacetate (CAS No. 7143-69-3), >5000 mg/kg bw.

No data are available for linalyl octanoate (CAS No. 10024-64-3).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Results for linalyl acetate appeared to be species-specific. Moderate skin irritation effects were reported in a rabbit study but not observed in miniature swine (pig) study. No effects were observed in humans up to a concentration of 32 %. However, it should be noted that concentrations less than 100 % of linalyl acetate were used for human patch test studies. The metabolite linalool is a skin irritant (NICNAS). Therefore, based on the available information, classification is warranted for linalyl acetate (see **Recommendation** section). This classification does not apply to the other chemicals in the group.

In a skin irritation study conducted according to the OECD Test Guideline (TG) 404, 0.5 mL of undiluted linalyl acetate was applied (semi-occlusively) on the skin of rabbits for 4 hours, with observation up to 7 days. Two test runs were conducted, using a total of 7 rabbits (3 in the first test, 4 in the second test). The mean erythema scores (24, 48, 72 hours) for test 1 and test 2 were 1.88 and 1.91, respectively. The mean oedema scores were 1.78 and 1.00, respectively. Slight to marked desquamation was observed in 6/7 animals at the end of the observation period. In another skin irritation study (BASF test), 2 rabbits were applied undiluted linalyl acetate under occlusive patches for 5 minutes, 15 minutes and 20 hours, with observation period up to 8 days. Desquamation was observed and the mean erythema score (24, 48 hours) was ≥ 2 . Based on the above studies, the chemical was determined to be a skin irritant (REACHa).

In several skin irritation studies in rabbits, reactions to linalyl acetate (undiluted) ranged from slight to severe. Severe irritation was observed in a 24-hour open patch test on the dorsal skin of albino Angora rabbits (n = 6). Minimal irritation was observed in a 24-hour closed patch test on New Zealand female rabbits (n = 9). Undiluted linalyl acetate was moderately irritating on male Hartley guinea pigs and not irritating when applied at 0.05 g on miniature swines for 48 hours (OECD, 2002; Bickers et al., 2003a).

Linalyl esters (acetate, benzoate, butyrate, formate, isobutyrate, isovalerate, phenylacetate and propionate) tested at 5 % in diethyl phthalate were slightly irritating in rabbits. Effects generally cleared by 72 hours. Effects for linalyl propionate were reported not to be fully reversible within 72 hours; however, they were not considered severe enough to warrant classification (Bickers et al., 2003a; REACHb).

When tested undiluted, results for linalyl esters (benzoate, butyrate, formate, isobutyrate, isovalerate, phenylacetate and propionate) ranged from very slight to well defined irritation in rabbits (Bickers et al., 2003a).

Linalyl esters were tested in guinea pigs and rats for photoirritation or photoallergy and showed no potential to elicit these effects (Bickers et al., 2003a).

Eye Irritation

Based on the available information, the chemicals are not considered to be eye irritants.

In several eye irritation studies, the results for six undiluted linalyl esters (acetate, benzoate, butyrate, formate, isobutyrate, and propionate) ranged from very slight to slight irritation effects in rabbits. Two linalyl esters (isovalerate and phenylacetate) were reported to cause very slight to well defined irritation in rabbits (Bickers et al., 2003a). When tested at a concentration of 5 % in diethyl phthalate, linalyl esters (acetate, benzoate, butyrate, formate, isobutyrate, isovalerate, phenylacetate and propionate) caused very slight irritation in rabbits (Bickers et al., 2003a).

In an eye irritation study, 0.05 mL of linalyl acetate was administered to one eye of each of 2 Vienna White rabbits, with observation for 8 days. Slight conjunctival redness was observed in both animals, but this was reversible by day 2 (REACHa).

Observation in humans

In human patch-test studies in approximately 380 healthy male and female volunteers, no irritation was observed with linalyl esters tested up to a concentration of 32 % (Bickers et al., 2003a). No signs of irritation were observed up to 120 hours after exposure (OECD, 2002).

In several skin irritation tests on human volunteers, no effects were observed when linalyl esters (benzoate, butyrate, formate, isobutyrate, isovalerate, phenylacetate and propionate) at 8 % in petrolatum were applied (Bickers et al., 2003b).

Sensitisation

Skin Sensitisation

Based on the available data, linalyl acetate in its highly purified form is a weak skin sensitiser. However when exposed to air, the chemical is auto-oxidised into a potent contact allergen. Exposure to linalyl acetate will primarily be as a constituent in natural oils (lavender oil), which also involves co-exposure to linalool, a known skin sensitiser. Given that public exposure to linalyl acetate will be through products exposed to air, linalyl acetate is expected to undergo auto-oxidation. Overall, the above information warrants classification for linalyl acetate as a skin sensitiser (see **Recommendation** section). This classification does not apply to the other esters in this group.

In a skin painting study, female white guinea pigs were topically induced with linalyl acetate (95–99% purity) at concentrations of 10 and 50 %. The animals were challenged at day 28 and day 31 at concentrations of 5 and 10 % linalyl acetate, respectively. No positive responses were reported. However, when repeatedly applied to the clipped flank skin, strong desquamation was observed within the area of application (REACHa).

The skin sensitising effects for auto-oxidised linalyl acetate were investigated in a local lymph node assay (LLNA) (OECD TG 429) with female CBA/Ca mice. The hydroperoxide oxidation products of linalyl acetate were also tested (see **Toxicokinetics** section). The EC₃ values for linalyl acetate as unchanged (97 % purity), distilled, or air-exposed over 10 weeks were reported to be 20 %, 25.4 % and 3.6 %, respectively. For the air-exposed sample, 74 % of the original test substance remained after 10 weeks. The EC₃ value for the identified hydroperoxide derivatives was 3.6 %, similar to that of air-exposed linalyl acetate. The authors concluded that the hydroperoxides were responsible for the sensitising potency of the chemical (Skold et. al., 2008; REACHa).

In guinea pig maximisation tests, linalyl acetate was weak to moderately sensitising at a concentration of 10 %, and non-sensitising when tested at 5 %. The purity of linalyl acetate was not stated. In a Freund's complete adjuvant test (FCAT), female albino Dunkin-Hartley guinea pigs (n = 30) were intradermally induced with linalyl acetate at 10 % in Freund's complete adjuvant and topically at 10 % in acetone. The animals were challenged topically with 5, 10 and 20 % in acetone. Skin reactions were observed in 0/10, 2/10 and 4/10 animals, respectively (Bickers et al., 2003a; REACHa).

No reactions were observed for linalyl isobutyrate and linalyl propionate tested at 8 % in guinea pigs (Bickers et al., 2003a).

The Scientific Committee on Consumer Safety (SCCS) has categorised linalyl acetate as an 'established contact allergen in humans'. This is based on up to 10 positive test reactions reported in humans (SCCS, 2012).

Observation in humans

In a study in Sweden (2008–2011), 1717 subjects were assessed for eczema related to a contact allergy. The patients were patch-tested with oxidised linalyl acetate at 6 % in petrolatum for 48 hours. Around 37 (2.2 %) showed positive reactions to oxidised linalyl acetate. Among these positive reactions, 76 % were weakly positive and 24 % were strongly positive. No irritant reactions were recorded. For patients with positive reactions to oxidised linalyl acetate, 13 (41 %) were positive to oxidised linalool, and 37 % had no other positive reactions to other fragrance markers. The patients were also patch-tested with oxidised linalool and 3.4 % were positive. This study also investigated the auto-oxidation of linalyl acetate and reported that the concentration of hydroperoxides after 42 weeks of air exposure (with stirring) was 37 %. Linalyl acetate hydroperoxides were also found to be relatively stable compared with linalool hydroperoxides which indicated accumulation with time (Hagvall et. al., 2015).

In a number of human maximisation tests, linalyl acetate was tested at concentrations of 10 or 20 % in petrolatum, or at 12 % in an unspecified vehicle. Reactions were observed in a total of 3 out of 131 subjects. No reactions were observed when the same samples were retested, or purified then retested (Bickers et al., 2003a).

No reactions were observed for the rest of the linalyl esters (benzoate, butyrate, formate, isobutyrate, isovalerate, phenylacetate and propionate) tested at concentrations 4–20% in petrolatum in 25 volunteers (Bickers et al., 2003a).

In a multicentre study, a total of 1855 patients with a history of allergy to fragrances were patch-tested with 10 % linalyl acetate (95–99 % purity) in petrolatum for 48 hours. Positive reactions were reported in 4 patients, and 'doubtful positive reactions' (only erythema) were observed in 8 patients (REACHa).

Repeated Dose Toxicity

Oral

There are no reliable repeat dose oral toxicity studies for linalyl acetate. Based on the limited data for the esters and data for linalool, the chemicals in this group are not likely to cause serious damage to health from repeated oral exposure.

In a repeated dose oral toxicity study, a total dose level of 100 mg/kg bw/day of a mixture consisting of linalyl acetate (24.2 mg/kg bw/day), linalyl isobutyrate (27.5 mg/kg bw/day) and geranyl acetate (48.8 mg/kg bw/day) was administered to the diet of rats (n = 10/sex) for 12 weeks. Slight growth retardation was observed in the females only, which were attributed to poor palatability. There were no significant differences between the treated and control groups for haematology, clinical chemistry and urinalysis measurements at weeks 6 and 12. Histopathology revealed no dose-related lesions (Bickers et al., 2003a).

In another repeated dose oral toxicity study, linalyl isobutyrate was administered in the diet to Osborne-Mendel rats (n = 10/sex/dose) at dose levels of 0, 1000, 2500 or 10000 ppm for 17–18 weeks. The estimated daily intakes were 0, 50, 120 or 500 mg/kg bw/day. There were no deaths, and no adverse effects were observed. The no observed adverse effect level (NOAEL) was determined as 500 mg/kg bw/day (Bickers et al., 2003a).

Linalool was not considered to cause serious damage to health from repeated oral exposure. In a 28-day repeated dose oral toxicity study using coriander oil (72.9 % linalool), SD rats (n = 10/sex/dose) were treated by gavage at doses of 160, 400 or 1000 mg/kg bw/day. A NOAEL of 160 mg/kg bw/day was established based on histological effects in the liver and kidney at 400 mg/kg bw/day (NICNAS).

Dermal

There are no repeat dose dermal toxicity studies for linalyl esters. Based on studies on linalool, the chemicals in this group are not likely to cause serious damage to health from repeated dermal exposure.

In a 91-day repeated dose dermal toxicity study (OECD TG 411) in SD rats, a NOAEL of 250 mg/kg bw/day for local effects was established for linalool. No systemic toxicity effects were reported at doses up to 1000 mg/kg bw/day. Depressed motor activity was significant at 4000 mg/kg bw/day (NICNAS).

Inhalation

No data are available.

Genotoxicity

Based on the available in vitro data for the linalyl esters, and the data available for linalool, the chemicals in this group are not considered genotoxic.

In vitro

The following results were reported for linalyl acetate (OECD, 2002; HSDB; REACHa):

- negative in bacterial reverse mutation assay (OECD TG 471) with several strains of *Salmonella* (*S.*) *typhimurium* (TA97, TA98, TA100, TA1535, TA1537, TA1538 and TA102) up to 25000 µg/plate, with or without metabolic activation;
- negative in *Escherichia coli* strain WP2uvrA (trp-) at doses of 125–1000 µg/plate, with or without metabolic activation; but positive at doses of 9 mg/plate and above. The authors concluded that linalyl acetate was cytotoxic at doses >9 mg/plate, and exceeded the regulatory requirement of 5 mg/plate;
- negative in *Bacillus subtilis* H17 (rec+) and M45 (rec-) at 18 µg/plate (Marnett et. al., 2014);
- no induction of chromosome aberrations (OECD TG 473) in human peripheral blood lymphocytes at doses up to 130 µg/mL without metabolic activation, and up to 180 µg/mL with metabolic activation; and
- negative in unscheduled DNA synthesis (UDS) assays in primary rat (Fischer or SD) hepatocytes at doses up to 300 µg/mL, without metabolic activation.

The following result was reported for linalyl propionate (REACHb) and linalyl isobutyrate (RIFM, 2015):

- negative in bacterial reverse mutation assay with several strains of *S. typhimurium* (TA1535, TA1537, TA98, TA100 and TA102) up to 5000 µg/plate, with or without metabolic activation.

The following result was reported for linalyl benzoate (Api et. al., 2016):

- negative in bacterial reverse mutation assay with several strains of *S. typhimurium* (TA1537, TA98 and TA102) up to 5000 µg/plate, with or without metabolic activation; and

- no increase in the frequency of binucleated cells with micronuclei (BNMN) in a micronucleus assay (OECD TG 487) in human peripheral blood lymphocytes at doses up to 640 µg/mL (without metabolic activation), and up to 960 µg/mL (with metabolic activation).

Linalool was generally negative in vitro: negative in bacterial reverse mutation assays (*S. typhimurium* and *E. coli*) and recombination assays with *Bacillus subtilis*; no induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells and Chinese hamster lung V79 fibroblast cells; and negative in mouse lymphoma cell forward mutation assay. Linalool was not clastogenic in an in vivo mouse micronucleus test (NICNAS).

No data are available for the other chemicals in this group.

Carcinogenicity

Limited information available for linalyl acetate does not indicate carcinogenic potential. The metabolite linalool is not considered to be a carcinogen (NICNAS). Therefore, the chemicals in this group are not likely to have carcinogenic effects.

In a carcinogenicity assay, linalyl acetate (purity ≥85 %) was administered (intraperitoneally) in mice (A/He) at 200 or 1000 mg/kg bw, 3 times/week for 8 weeks, with observation up to 24 weeks. Histopathological examinations were conducted on the lungs. The other organs (liver, kidney, spleen, thymus, intestine and salivary and endocrine glands) were subjected to macroscopic investigation. There was no evidence of carcinogenic activity (OECD, 2002). It is stated that the duration for this test was too short to develop into possible carcinogenic effects; however, the positive control (urethane) showed a 100 % response.

In a tumour induction dermal study, distilled linalyl acetate was applied topically alone or with a co-carcinogen benzo[a]pyrene (BaP) (at 1 or 5 µg) on female ICR/Ha Swiss mice, 3 times/week for 67 weeks. No carcinogenicity was observed when the chemical was administered alone, or with 1 µg BaP. Weak co-carcinogenic activity (slight increase in the number of skin papillomas and carcinomas compared with BaP alone) was observed when co-administered with 5 µg BaP (OECD, 2002; Bickers et al., 2003a).

No animal carcinogenicity studies for the metabolite linalool are available with relevant routes of exposure. Linalool was negative for pulmonary tumour response when intraperitoneally (i.p.) injected into A/He mice. No significant effect on the incidence of tumours was observed in rats or mice when linalool was administered orally or dermally in tumour-promotion studies (NICNAS).

Reproductive and Developmental Toxicity

There are no toxicity studies for linalyl esters. The metabolite linalool is not considered a reproductive or developmental toxicant. Therefore, the chemicals in this group are not likely to be reproductive or developmental toxicants.

In a combined study (OECD TG 421), female rats administered coriander oil (containing 72.9 % linalool) displayed urine-stained abdominal fur and neurotoxic effects at the highest dose (1000 mg/kg bw/day). Adverse effects on the offspring were only observed at the highest dose, and did not occur at the absence of significant maternal toxicity. The maternal and developmental NOAELs were both established as 500 mg/kg bw/day (NICNAS). The equivalent dose calculated for linalyl acetate is ~464 mg/kg bw/day (OECD, 2002).

In a developmental toxicity study for linalool, maternal toxicity was only observed at the highest gavage dose (1000 mg/kg bw/day) in female SD rats. Changes in developmental parameters were considered to be secondary to maternal toxicity (NICNAS).

Other Health Effects

Neurotoxicity

Linalyl acetate is one of the primary constituents of lavender oil, which is known as a relaxant.

When the sedative properties of lavender essential oil were studied in mice, the mobility of the animals was observed to decrease significantly and for which this effect was closely dependent on the exposure time. After the animals were injected with caffeine, they exhibited hyperactivity which was reduced to almost normal levels following inhalation of this fragrance (HSDB). In an inhalation study, a decrease in motor activity was observed in Swiss mice exposed to 2.74 mg linalyl acetate/L air for 90 minutes. A 100 % reduction in motor activity was reported in mice aged 6–8 weeks, and up to 81 % reduction in mice aged 6 months old (HSDB).

Linalyl butyrate was reported to strongly decrease the spontaneous mobility of mice at a dose of 100 mg/kg bw (Opdyke, 1979)

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation for linalyl acetate (CAS No. 115-95-7 and CAS No. 16509-46-9) include potential local effects (skin sensitisation in its oxidised form; and at high concentrations, skin irritation).

The other chemicals in this group do not have any critical health effects for risk characterisation.

Public Risk Characterisation

Based on the international uses identified, the chemicals may be used in cosmetics and domestic products in Australia as a fragrance ingredient. The general public could be exposed to the chemicals when using cosmetics or domestic products containing the chemicals. The estimated consumer dermal systemic exposure for linalyl esters in cosmetic products are reported as: 0.03 mg/kg bw/day for linalyl butyrate, formate, hexanoate, isobutyrate, isovalerate and phenylacetate; 0.05 mg/kg bw/day for linalyl propionate; 0.1 mg/kg bw/day for linalyl benzoate and 0.331 mg/kg bw/day for linalyl acetate. For skin sensitisation, the calculated exposure concentrations to linalyl esters used in fine fragrance products are reported as 0.4 % for linalyl isovalerate and at a maximum of 4.6 % for linalyl acetate, based on the use in consumer products at 20 % (Bickers et al., 2003a).

There were no Australian uses identified for the majority of the chemicals except for linalyl acetate (CAS No 115-95-7) which was reported to be used in domestic products including car wash soaps, boat wash soaps, polishes and rubbing compounds. The chemicals were also reported to be used overseas in cosmetics and domestic products, particularly perfumery. Except for linalyl acetate, only low hazards are identified for these chemicals. However, this group of chemicals are expected to be present in domestic and/or cosmetic products at low concentrations due to their known use as fragrance ingredients. Therefore, the general public could be exposed through the skin when using domestic and/or cosmetic products containing these chemicals.

Consumer products containing these chemicals can oxidise over time. While the oxidation products are potentially hazardous (skin sensitisation), these will be present at low concentrations. Products that contain relatively high concentrations but which are used infrequently and have long shelf-lives could contain oxidation products that could pose a sensitisation risk for sensitive individuals. This oxidation potential in consumer products could be reduced by incorporating an anti-oxidant. Stabilised or fresh products are not likely to be a risk to public health.

Overall, the risk to public health from this group of chemicals is not considered to be unreasonable.

Occupational Risk Characterisation

During product formulation, dermal exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to these chemicals at lower concentrations could also occur while using formulated products containing these chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the HCIS (Safe Work Australia) (see **Recommendation** section).

NICNAS Recommendation

Assessment of the chemicals is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Public Health

Products containing the chemicals should be labelled in accordance with state and territory legislation (SUSMP, 2018).

Work Health and Safety

The chemicals are recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards.

Note: The recommended classification applies to linalyl acetate (CAS No. 115-95-7 and CAS No. 16509-46-9) only.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS) ^a	GHS Classification (HCIS) ^b
Irritation / Corrosivity	Not Applicable	Causes skin irritation - Cat. 2 (H315)
Sensitisation	Not Applicable	May cause an allergic skin reaction - Cat. 1B (H317)

^a Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

^b Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

* Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing these chemicals should be used according to the instructions on the label.

Advice for industry

Control measures to minimise the risk from dermal exposure to these chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker's health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals has not been undertaken as part of this assessment.

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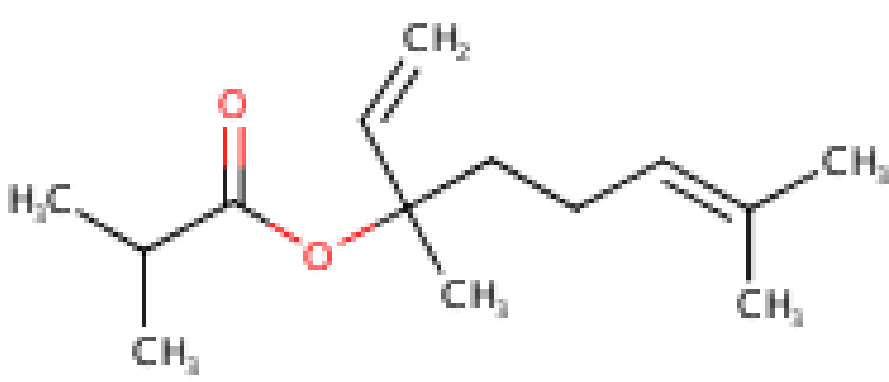
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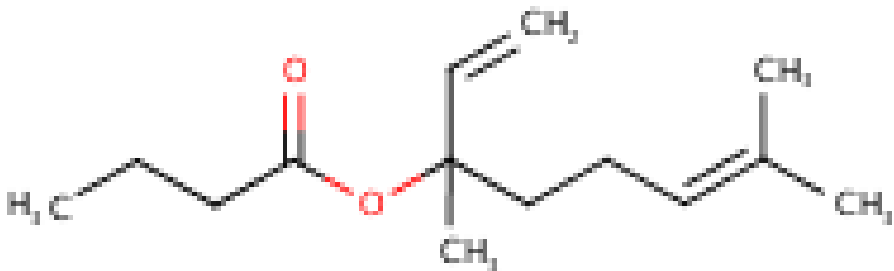
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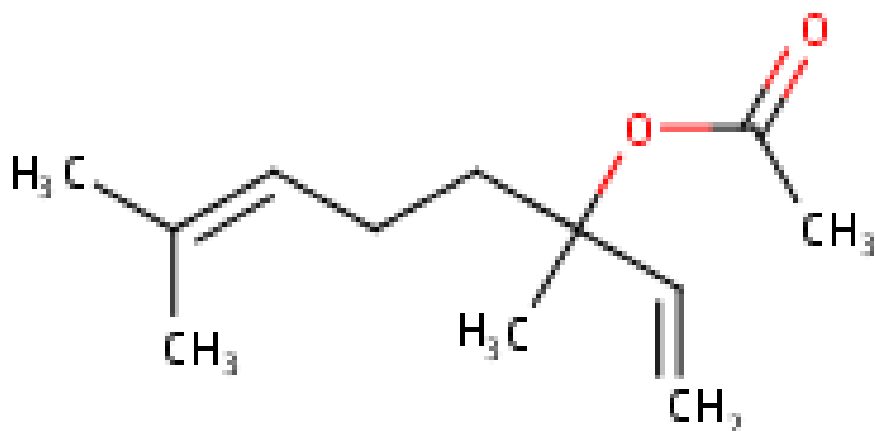
Last Update 29 June 2018

Chemical Identities

Chemical Name in the Inventory and Synonyms	Propanoic acid, 2-methyl-, 1-ethenyl-1,5-dimethyl-4-hexenylester 3,7-dimethyl-1,6-octadienyl isobutyrate linalyl isobutyrate
CAS Number	78-35-3
Structural Formula	
Molecular Formula	C ₁₄ H ₂₄ O ₂
Molecular Weight	224.13

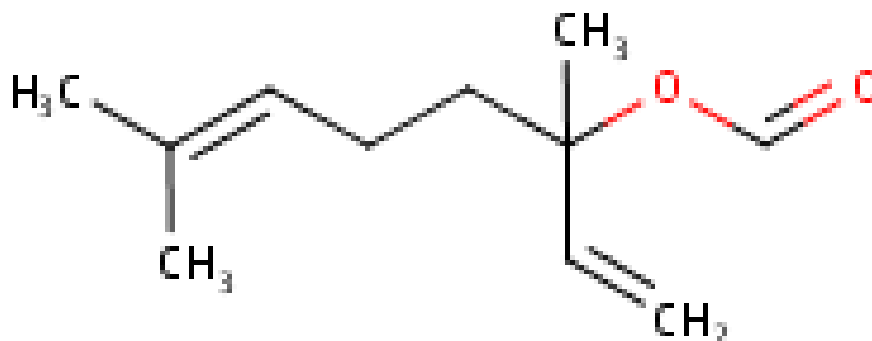
Chemical Name in the Inventory and Synonyms	Butanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester linalyl butyrate 1,6-octadien-3-ol, 3,7-dimethyl-, butyrate
CAS Number	78-36-4
Structural Formula	
Molecular Formula	C14H24O2
Molecular Weight	224.34

Chemical Name in the Inventory and Synonyms	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate 1,5-dimethyl-1-vinylhex-4-enyl acetate linalyl acetate acetic acid linalool ester
CAS Number	115-95-7
Structural Formula	



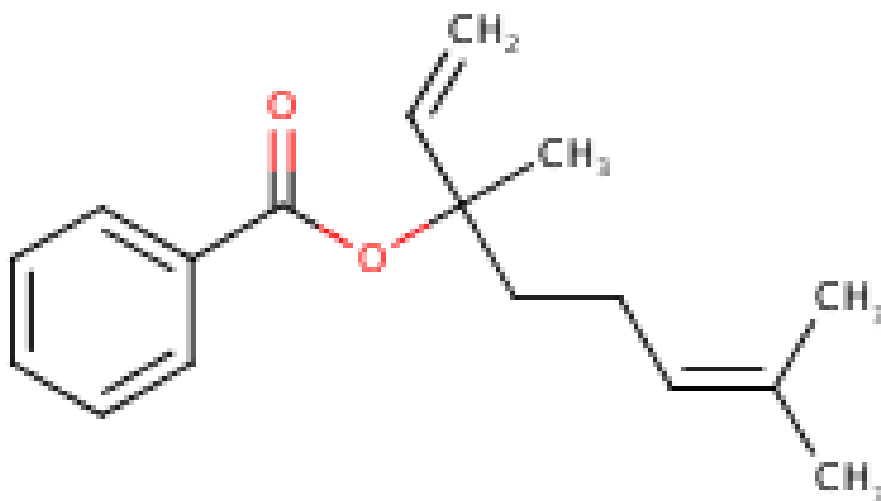
Molecular Formula	C ₁₂ H ₂₀ O ₂
Molecular Weight	196.30

Chemical Name in the Inventory and Synonyms	1,6-Octadien-3-ol, 3,7-dimethyl-, formate linalyl formate formic acid linalyl ester 3,7-dimethyl-1,6-octadien-3-yl formate
CAS Number	115-99-1
Structural Formula	



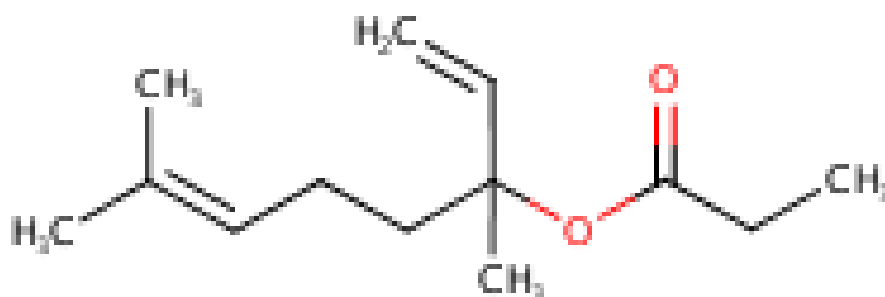
Molecular Formula	C ₁₁ H ₁₈ O ₂
Molecular Weight	182.26

Chemical Name in the Inventory and Synonyms	1,6-Octadien-3-ol, 3,7-dimethyl-, benzoate 3,7-dimethyl-1,6-octadien-3-yl benzoate linalyl benzoate
CAS Number	126-64-7
Structural Formula	



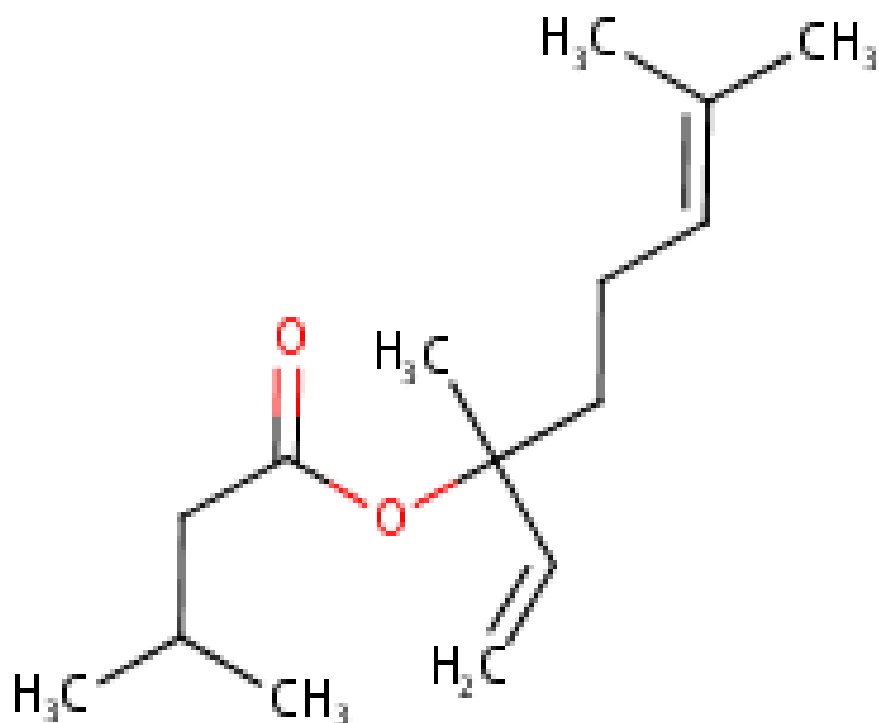
Molecular Formula	C17H22O2
Molecular Weight	258.36

Chemical Name in the Inventory and Synonyms	1,6-Octadien-3-yl, 3,7-dimethyl-, propanoate linalyl propionate 3,7-dimethyl-1,6-octadien-3-yl, propanoate 1,5- dimethyl-1-vinyl hex-4-enyl propionate
CAS Number	144-39-8
Structural Formula	



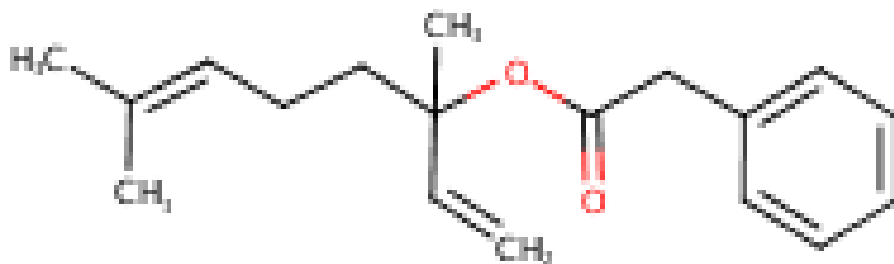
Molecular Formula	C ₁₃ H ₂₂ O ₂
Molecular Weight	210.32

Chemical Name in the Inventory and Synonyms	Butanoic acid, 3-methyl-, 1-ethenyl-1,5-dimethyl-4-hexenyl ester linalyl isovalerate isovaleric acid, 1,5-dimethyl-1-vinyl-4-hexenylester 1,6-octadien-3-ol, 3,7-dimethyl-, isovalerate linalyl isopentanoate
CAS Number	1118-27-0
Structural Formula	



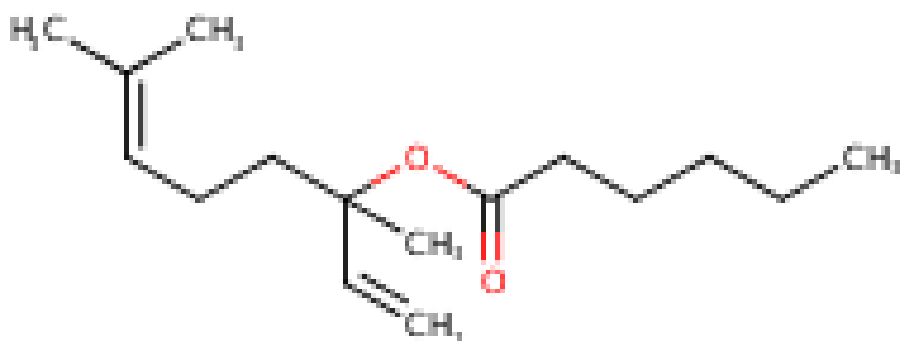
Molecular Formula	C ₁₅ H ₂₆ O ₂
Molecular Weight	238.37

Chemical Name in the Inventory and Synonyms	Benzeneacetic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester linalyl phenylacetate acetic acid, phenyl-1,5-dimethyl-1-vinyl-4-hexenyl ester 1,5-dimethyl-1-vinyl-4-hexenyl phenylacetate linalyl alpha-toluate
CAS Number	7143-69-3
Structural Formula	



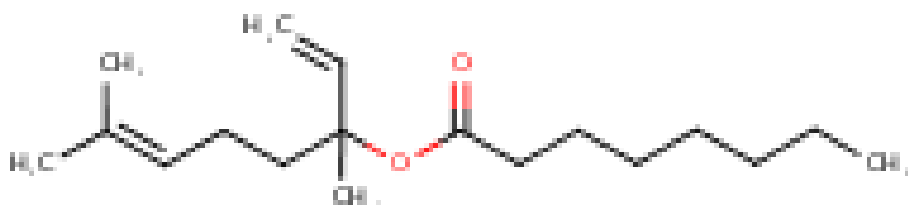
Molecular Formula	C18H24O2
Molecular Weight	272.39

Chemical Name in the Inventory and Synonyms	Hexanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester linalyl caproate hexanoic acid, 1,5-dimethyl-1-vinyl-4-hexenyl ester 1,5-dimethyl-1-vinylhex-4-enyl hexanoate linalyl hexanoate
CAS Number	7779-23-9
Structural Formula	



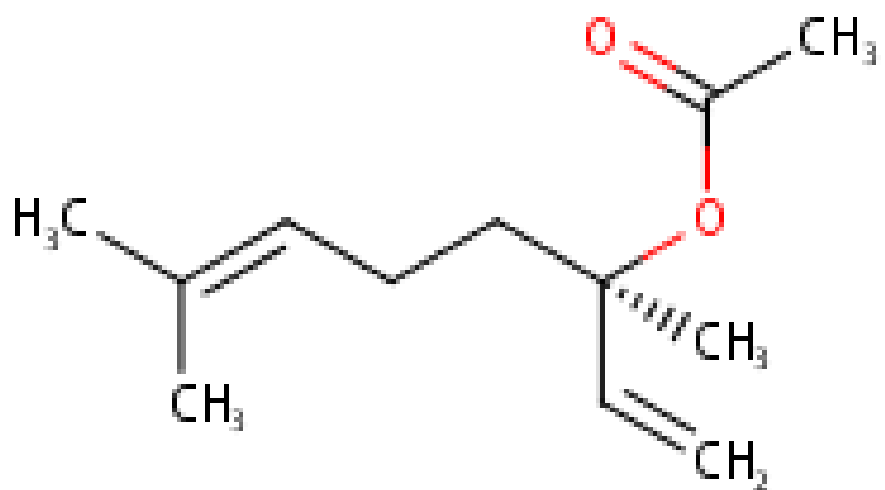
Molecular Formula	C ₁₆ H ₂₈ O ₂
Molecular Weight	252.40

Chemical Name in the Inventory and Synonyms	Octanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester linalyl octanoate linalyl caprylate octanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester 3,7-dimethyl-1,6-octadien-3-yl octanoate
CAS Number	10024-64-3
Structural Formula	



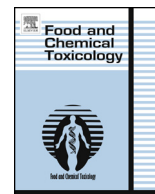
Molecular Formula	C18H32O2
Molecular Weight	280.45

Chemical Name in the Inventory and Synonyms	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate, (R)- linalyl acetate, (-)- (R)-linalyl acetate
CAS Number	16509-46-9
Structural Formula	



Molecular Formula	C ₁₂ H ₂₀ O ₂
Molecular Weight	196.29

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Short Review

RIFM fragrance ingredient safety assessment, Linalyl acetate, CAS Registry Number 115-95-7



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ARTICLE INFO

Article history:

Received 19 November 2014

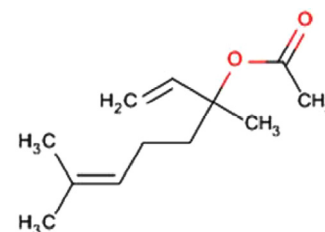
Accepted 13 January 2015

Available online 28 January 2015

Version: 0562014. This version replaces any previous versions.

Name: Linalyl acetate

CAS Registry Number: 115-95-7

**Abbreviation/Definition list:**

Box Model – a RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration

97.5th percentile – The concentration of the fragrance ingredient is obtained from examination of several thousand commercial fine fragrance formulations. The upper 97.5th percentile concentration is calculated from these data and is then used to estimate the dermal systemic exposure in ten types of the most frequently used personal care and cosmetic products. The dermal route is the major route in assessing the safety of fragrance ingredients. Further explanation of how the data were obtained and of how exposures were determined has been previously reported by Cadby et al. (2002) and Ford et al. (2000).

AF – Assessment Factor

DEREK – Derek nexus is an *in silico* tool used to identify structural alerts

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DST – Dermal Sensitization Threshold
ECHA – European Chemicals Agency
EU – Europe/European Union
GLP – Good Laboratory Practice
IFRA – The International Fragrance Association
LOEL – Lowest Observable Effect Level
MOE – Margin of Exposure
MPPD – Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA – North America
NESIL – No Expected Sensitization Induction Level
NOAEC – No Observed Adverse Effect Concentration
NOAEL – No Observed Adverse Effect Level
NOEC – No Observed Effect Concentration
OECD – Organisation for Economic Co-operation and Development
OECD TG – Organisation for Economic Co-operation and Development Testing Guidelines
PBT – Persistent, Bioaccumulative, and Toxic
PEC/PNEC – Predicted Environmental Concentration/Predicted No Effect Concentration
QRA – quantitative risk assessment
REACH – Registration, Evaluation, Authorisation, and Restriction of Chemicals
RIFM – Research Institute for Fragrance Materials
RQ – Risk Quotient
TTC – Threshold of Toxicological Concern
UV/Vis Spectra – Ultra Violet/Visible spectra
VCF – Volatile Compounds in Food
VoU – Volume of Use
vPvB – (very) Persistent, (very) Bioaccumulative
WOE – Weight of Evidence

RIFM's Expert Panel* concludes that this material is safe under the limits described in this safety assessment.

This safety assessment is based on RIFM's Criteria Document (Api et al., 2014) and should be referred to for clarifications.

Each endpoint discussed in this safety assessment reviews the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a two digit month/day/year), both in the RIFM database (consisting of publicly available and proprietary data) and through publicly available information sources (i.e., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative end-point value (e.g., PNEC, NOAEL, LOEL, and NESIL).

* RIFM's Expert Panel is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM guidance relevant to human health and environmental protection.

Summary: The use of this material under current use conditions is supported by the existing information.

This material was evaluated for Genetic, Repeated Dose, Developmental, Reproductive, Respiratory, Phototoxicity, Skin Sensitization potential as well as Environmental assessment. Repeated Dose Toxicity was determined to have the most conservative systemic exposure derived NO[A]EL of 36 mg/kg/day, based on studies conducted on the major metabolite linalool (CAS # 78-70-6), that resulted in an MOE of 462, considering 14.4% absorption from skin contact and 100% from inhalation. An MOE of >100 is deemed acceptable.

Human Health Safety Assessment

Genotoxicity: Not genotoxic (Bickers et al., 2003; Di Sotto et al., 2011)

Repeated Dose Toxicity: NOAEL = 36 mg/kg/day (RIFM, 1980)

Developmental and Reproductive Toxicity: NOAEL = 200 mg/kg/day (ECHA REACH Dossier: Linalyl acetate)

Skin Sensitization: Not sensitizing (Ishihara et al., 1986; RIFM, 1969; RIFM, 1982a; RIFM, 1983a; RIFM, 2002; Skold et al., 2005; Skold et al., 2008)

Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic (RIFM, 1983b; RIFM, 1983c)

Local Respiratory Toxicity: NOAEC = 10 ppm or 63 mg/m³ (0.063 mg/L) (RIFM, 2012)

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 75% and 96.9% (RIFM, 1991a; RIFM, 1994)

Bioaccumulation: Screening Level: 182 L/kg (EPISUITE ver 4.1, 2011)

Ecotoxicity: Critical Ecotoxicity Endpoint: Fish 96 h LC50: 11 mg/L (RIFM, 1977a)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-Level: PEC/PNEC (North America and Europe) > 1 (Salvito et al., 2002)

Critical Ecotoxicity Endpoint: Fish 96 h LC50: 11 mg/L (RIFM, 1977a)

RIFM PNEC is: 11 µg/L

• Revised PEC/PNECs (2011 IFRA VoU): North America and Europe <1

1. Identification

- 1 Chemical Name:** Linalyl acetate
- 2 CAS Registry Number:** 115-95-7
- 3 Synonyms:** Bergamol, 3,7-Dimethyl-1,6-octadien-3-yl acetate, Linalool acetate, Linalyl acetate, 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate, 3,7-Dimethyl-1,6-octadien-3-ol acetate, 酢酸リナリル, 1,5-Dimethyl-1-vinylhex-4-en-1-yl acetate
- 4 Molecular Formula:** C₁₂H₂₀O₂
- 5 Molecular Weight:** 196.29
- 6 RIFM Number:** 138

2. Physical data

- 1 Boiling Point:** 220 °C (IFRA), 228.95 °C (EPI Suite ver 4.1, 2011)
- 2 Flash Point:** 185 °F; CC (IFRA)
- 3 Log K_{ow}:** 4.12 ± 0.40 (Cal, 2006), 2.9 (RIFM, 1996), Log P_{ow} = 4.0 (RIFM, 1991b), 4.3 at 35 °C (RIFM, 2004a), 4.39 (EPI Suite ver 4.1, 2011)
- 4 Melting Point:** less than 20 °C (RIFM, 1991b), <20 °C (RIFM, 1991a), -2.09 °C (EPI Suite ver 4.1, 2011)
- 5 Water Solubility:** 20.12 mg/L (EPI Suite ver 4.1, 2011)

- 6 Specific Gravity:** 0.900 g/ml at 20 °C (RIFM, 1991b), 0.895–0.908 (IFRA), 0.902 D20/4 – 0.898 to 0.903 (RIFM, 1991a), 0.897–0.910 (IFRA), 0.91 g/ml (RIFM, 1994)
- 7 Vapor Pressure:** 0.0864 mm Hg @ 20 °C (EPI Suite ver 4.1, 2011), 0.07 mm Hg 20 °C (IFRA), 0.131 mm Hg @ 25 °C (EPI Suite ver 4.1, 2011)
- 8 UV Spectra:** Absorb in the region of 290–700 nm
- 9 Appearance/Organoleptic:** A clear, colorless liquid having a sweet, floral-fruity odor.

3. Exposure

- 1 Volume of Use (worldwide band):** >1000 metric tons per year (IFRA, 2011)
- 2 Average Maximum Concentration in Hydroalcohols:** 4.60% (IFRA, 2002)
- 3 97.5th Percentile:** 13.00% (IFRA, 2002)
- 4 Dermal Exposure*:** 0.3312 mg/kg/day (IFRA, 2002)
- 5 Oral Exposure:** Not applicable
- 6 Inhalation Exposures**:** 0.03 mg/kg/day (IFRA, 2002)
- 7 Total Systemic Exposure (Dermal + Inhalation):** (0.3312 mg/kg/day × 14.4% absorption) + 0.03 mg/kg/day = 0.078 mg/kg/day

* Calculated using the reported 97.5th percentile concentration based on the levels of the same fragrance ingredient in ten of the most frequently used personal care and cosmetic products (i.e., antiperspirant, bath products, body lotion, eau de toilette, face cream, fragrance cream, hair spray, shampoo, shower gel, and toilet soap) (Cadby et al., 2002; Ford et al., 2000).

** Combined (fine fragrances, hair sprays, antiperspirants/deodorants, candles, aerosol air fresheners, and reed diffusers/heated oil plug-ins) result calculated using RIFM's 2-Box/MPPD *in silico* models, based on the IFRA survey results for the 97.5th percentile use in hydroalcohols for a 60 kg individual.

4. Derivation of systemic absorption

1 Dermal: 14.4%

(RIFM, 2007a (data also available in RIFM, 2007b; and RIFM, 2007c; RIFM, 2008a; RIFM, 2008b, and RIFM, 2001; Lalko et al., 2007; Lalko et al., 2008)) A series of *in vitro* human skin penetration studies were conducted with metabolite linalool (CAS # 78-70-6; see Section 5) under in-use (unoccluded) and occluded conditions in diethyl phthalate (DEP), dipropylene glycol (DPG), ethanol/water, petrolatum, ethanol/DEP or ethanol/DPG vehicles. Twelve active dosed diffusion cells were prepared from seven donors for each application condition (unoccluded, occluded, and an unoccluded control cell). Epidermal membranes were used, and their integrity was assessed by measuring the permeation rate of tritiated water over a period of 1 h. Permeation of 4% linalool from a 5 µl/cm² dose was then measured at 12 time-points over 24 h. Occluded conditions reduced the loss of volatile application vehicles and test compounds but may have also increased skin hydration, factors which caused a significant increase in the permeation of linalool. Under unoccluded experimental conditions, there was a gradual but comprehensive evaporative loss. Total absorbed dose values from an unoccluded application ranged from 1.8% to 3.57% (DPG < ethanol/DPG < ethanol/DEP < DEP < petrolatum < ethanol/water). Total absorbed dose values from an occluded application ranged from 5.73% to 14.4% (DEP < ethanol/DEP < DPG < petrolatum < ethanol/DPG < ethanol/water). Conservatively, 14.4% dermal absorption was selected for this safety assessment.

2 Oral: Data not available – not considered.

3 Inhalation: Assumed 100%

- 4 Total:** Dermal (14.4%) + Inhalation (assume 100%) absorbed = (0.3312 mg/kg/day × 14.4%) + 0.03 mg/kg/day = 0.078 mg/kg/day

5. Computational toxicology evaluation

1 Cramer Classification: Class I, Low

Expert Judgment	Toxtree v. 2.6	OECD QSAR Toolbox v. 3.2
I	I	I

2 Analogs Selected:

- a. Genotoxicity:** None
- b. Repeated Dose Toxicity:** Linalool (CAS # 78-70-6)
- c. Developmental and Reproductive Toxicity:** Linalool (CAS # 78-70-6); dehydrolinalool (CAS # 29171-20-8)
- d. Skin Sensitization:** None
- e. Phototoxicity/Photoallergenicity:** None
- f. Local Respiratory Toxicity:** Linalool (CAS # 78-70-6)
- g. Environmental Toxicity:** None
- 3 Read-across Justification:** See Appendix below

6. Metabolism

Letizia et al., 2003a: Esters are readily hydrolyzed by carboxylesterases or esterases (Satoh, 1987). Linalyl acetate has been demonstrated to be hydrolyzed *in vitro* in rat blood and liver preparations. It is expected to be readily hydrolyzed *in vivo*. Acetate is a normal constituent of the body. The metabolism of linalool is known and is primarily through glucuronic acid conjugation and excretion (Parke et al., 1974).

7. Natural occurrence (discrete chemical) or composition (NCS)

Linalyl acetate is reported to occur in food* and as a component in some natural complex substances (NCS):

Bergamot oil
Cardamom (elettaria cardamomum (L.) Maton)
Citrus fruits
Clary (salvia sclarea L.)
Mentha arvensis oil
Mentha oils
Myrtle (myrtus communis L.)
Myrtle leaf
Salvia species

* VCF Volatile Compounds in Food: database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. [eds]. – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database, contains information on published volatile compounds which have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

8. IFRA standard

None.

9. REACH dossier

Available; accessed on 05/08/13: http://apps.echa.europa.eu/registered/data/dossiers/DISS-9c80e2a4-b39d-6b91-e044-00144f67d249/DISS-9c80e2a4-b39d-6b91-e044-00144f67d249_DISS-9c80e2a4-b39d-6b91-e044-00144f67d249.html.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data and use levels, linalyl acetate does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. The genotoxic potential of linalyl acetate was evaluated extensively, as summarized in the Fragrance Material Review (Letizia et al., 2003a). Ames tests using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100, and *Escherichia coli* strain WP2uvrA, both with and without S9 activation, show that linalyl acetate was not mutagenic (RIFM, 1984; RIFM, 1989a). However, in a non GLP compliant study conducted by Di Sotto et al., 2008, linalyl acetate was found positive for mutagenicity in *E. coli* strain WP2uvr both with and without S9 mixture, indicating that it causes base substitutions at concentrations of 9 mg/plate and above. This study was conducted similar to OECD TG 471 with the exception that only three strains of bacteria were utilized and that the maximum dose exceeded the regulatory requirement of 5 mg/plate. The study also indicated that linalyl acetate was cytotoxic at doses >9 mg/plate. As such, mutagenicity was only observed at levels that approached toxicity and at doses that exceeded regulatory requirements. The guideline states the recommended maximum test concentration for soluble non-cytotoxic substances to be 5 mg/plate unless the substance that is being tested contains substantial amounts of potentially mutagenic impurities. Based on the available data, linalyl acetate does have not mutagenic potential.

With regard to clastogenicity, in one *in vitro* chromosome aberration study following OECD TG 473, linalyl acetate was found to be negative (RIFM, 2000). However, in a subsequent micronucleus assay, which was not compliant to GLP regulations, positive effects were observed (DiSotto et al., 2011). The authors of this second study indicate that the positive effect is likely to be aneugenic rather than clastogenic in nature because while there was an increase in micronuclei there was no effect on nucleoplasmic bridges. It is of note that the doses used in the second assay were lower than those used in the first assay. Moreover, the DiSotto publication has several deficiencies as follows: there is no indication that a preliminary toxicity study was conducted to establish the doses used; there is no indication that there were any treatments with metabolic activation; there is no indication that the slides were independently coded and evaluated; the data suggest that Linalyl acetate is a more potent clastogen than the positive control (ethyl methanesulfonate); sufficient detail with regard to cytochalasin-B treatment is not provided; and lastly, May–Grunwald–Giemsa staining was used which may stain non-DNA material, and can be misinterpreted as micronuclei. Furthermore, positive *in vitro* results for linalyl acetate have little implication for *in vivo* genotoxicity since linalyl acetate would readily be converted to linalool and acetate *in vivo*. Linalool has been identified as not having genotoxic potential (RIFM SA Linalool CAS # 78-70-6); therefore, there is little concern that the positive result observed in the second clastogenicity study would be translated systemically *in vivo*. Additionally, the RIFM Expert Panel has reviewed linalyl acetate and other esters related to linalool and concluded that, based on a weight of evidence, this category of materials does not have significant genotoxic potential (Bickers et al., 2003). Taken together, linalyl acetate does not have potential to be genotoxic.

Additional References: Heck et al., 1989; Oda et al., 1978; RIFM, 1987.

Literature Search and Risk Assessment Completed on: 03/25/13.

10.1.2. Repeated dose toxicity

The margin of exposure for linalyl acetate is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. The repeated dose toxicity data on linalyl acetate includes a 90-day oral (feed) study conducted with 100 mg/kg/day of a mixture containing 24.2 mg/kg/day linalyl acetate, 27.5 mg/kg/day linalyl isobutyrate and 48.8 mg/kg/day geranyl acetate. The only observed effect was slightly depressed food intake and weight gain in females (RIFM, 1958a). These data are not sufficient to determine a NOAEL for repeated dose toxicity due to insufficient details provided. However, the metabolite linalool (CAS # 78-70-6; see Section 5) has several repeated dose toxicity studies. A dermal 90-day subchronic toxicity study conducted in rats determined the NOAEL to be 250 mg/kg/day, based on reduced body weights (RIFM, 1980). To account for bioavailability following dermal application, the data from an *in vitro* dermal absorption study (RIFM, 2007a; see Section 4) with linalool were used to revise the NOAEL of 250 mg/kg/day to reflect the systemic dose. At a dermal penetration of 14.4% of the applied dose, the revised repeated dose toxicity NOAEL from the dermal study is 36 mg/kg/day. Therefore, the MOE is equal to the linalool NOAEL in mg/kg/day divided by the total systemic exposure, 36/0.078 or 462.

Additional References: Letizia et al., 2003a; Bickers et al., 2003; Stoner et al., 1973; Van Duuren et al., 1971; RIFM, 1998a; Jager et al., 1992; Meyer et al., 1959; Meyer, 1965; Cal et al., 2003; RIFM, 1996; Letizia et al., 2003b; Lapczynski et al., 2008a; Lapczynski et al., 2008b; Lapczynski et al., 2008c; Belsito et al., 2008; Belsito et al., 2010; RIFM, 1958b; RIFM, 1979; RIFM, 2012; Randazzo et al., 2013; Hood et al., 1978; Howes et al., 2002; Jirovets et al., 1990; Jirovets et al., 1991; Parke et al., 1974; Green & Tephly, 1996; Meesters et al., 2007; Chadha et al., 1982; Chadha et al., 1984; RIFM, 1998b; Schmitt et al., 2010; Cal, 2006; Cal et al., 2006; Jaaskelainen et al., 2005; Bar et al., 1967; Hagan et al., 1967; Letizia et al., 2003c; RIFM, 1989b; RIFM, 1990; Al-Said et al., 1987; Matsui et al., 1967.

Literature Search and Risk Assessment Completed on: 03/25/13.

10.1.3. Developmental and reproductive toxicity

The margin of exposure for linalyl acetate is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

10.1.3.1. Risk assessment. There are no developmental toxicity data on linalyl acetate. Metabolite linalool (CAS # 78-70-6; see Section 5) has a gavage developmental toxicity study conducted in rats, which concluded a NOAEL of 1000 mg/kg/day for developmental toxicity, the highest dosage tested (Politano et al., 2008). Therefore, the MOE for developmental toxicity is equal to the linalool NOAEL in mg/kg/day divided by the total systemic exposure, 1000/0.078 or 12821.

There are no reproductive toxicity data on linalyl acetate. Read-across material dehydrolinalool (CAS # 29171-20-8; see Section 5) has a gavage reproductive toxicity screening study in rats. The NOAELs were determined to be 750 mg/kg/day for males, the highest dosage tested, and 200 mg/kg/day for the offspring and dams, based on maternal clinical signs and decreased live birth index and viability (ECHA REACH Dossier: Linalyl acetate Read across Subs Key Toxicity to reproduction.003 (accessed 02/19/13)). The gavage developmental toxicity study in rats with the metabolite linalool (CAS # 78-70-6) concluded a NOAEL of 500 mg/kg/day for maternal toxicity, based on reduced maternal body weight gain and feed consumption (Politano et al., 2008). The dermal 90-day subchronic toxicity study with linalool in rats (RIFM, 1980), in addition to the systemic endpoints, included organ weights (testes and ovaries) and histopathology (testes, epididymis, ovaries, pituitary, and thyroid) and no effects were observed. Together, these data indicate there

is no concern for reproductive toxicity. The most conservative NOAEL was selected for this safety assessment. Therefore, the MOE for reproductive toxicity is equal to the dehydrolinalool NOAEL in mg/kg/day divided by the total systemic exposure, 200/0.078 or 2564.

Additional References: Letizia et al., 2003a; Bickers et al., 2003; Stoner et al., 1973; Van Duuren et al., 1971; RIFM, 1998a; Jager et al., 1992; Meyer et al., 1959; Meyer, 1965; Cal et al., 2003; RIFM, 1996; Letizia et al., 2003b; Lapczynski et al., 2008a; Lapczynski et al., 2008b; Lapczynski et al., 2008c; Belsito et al., 2008; Belsito et al., 2010; RIFM, 1958b; RIFM, 1979; RIFM, 2012; Randazzo et al., 2013; Hood et al., 1978; Howes et al., 2002; Jirovets et al., 1990; Jirovets et al., 1991; Parke et al., 1974; Green & Tephly, 1996; Meesters et al., 2007; Chadha et al., 1982; Chadha et al., 1984; RIFM, 1998b; Schmitt et al., 2010; Cal, 2006; Cal et al., 2006; Jaaskelainen et al., 2005; Bar & Griepentrog, 1967; Hagan et al., 1967; Letizia et al., 2003c; RIFM, 1989b; RIFM, 1990; Al-Said et al., 1987; Matsui et al., 1967.

Literature Search and Risk Assessment Completed on: 03/25/13.

10.1.4. Skin sensitization

Based on the available data, linalyl acetate does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Linalyl acetate is not predicted to significantly react, directly, to skin proteins (Roberts et al., 2007; OECD toolbox v3.0). However, linalyl acetate is known to undergo auto-oxidation resulting in degradation products that may be protein reactive (Skold et al., 2008). In guinea pig test methods and the local lymph node assay (LLNA), positive and negative results have been reported (Ishihara et al., 1986; RIFM, 1969; RIFM, 1977b; RIFM, 1982a; RIFM, 1982b; RIFM, 1983a; RIFM, 2002; Skold et al., 2005; Skold et al., 2008). Various qualities of linalyl acetate have been evaluated in the murine local lymph node assay (LLNA) and guinea pig test methods (RIFM, 1969; Ishihara et al., 1986; RIFM, 1977b; RIFM, 1982a; RIFM, 1982b; RIFM, 1983a; RIFM, 2002; Skold et al., 2005; Skold et al., 2008). The lowest EC3 values have been associated with oxidized samples of linalyl acetate, whereas the higher EC3 values have typically been observed when testing higher purity materials. In the LLNA, positive results have been shown to be the result of irritation and sensitizing products due to autooxidation (RIFM, 2010; Skold et al., 2005; Skold et al., 2008). In the human maximization test positive results were reported at concentrations of 10% in petrolatum. These results were also demonstrated to be the result of test sample impurities as retesting of purified samples demonstrated no sensitization potential (RIFM, 1974; RIFM, 1982c). Finally, no reactions indicative of sensitization were observed at higher concentrations (12.5% and 20.0%) in the human maximization test (Greif, 1967; and RIFM, 1975). Based on the available data, linalyl acetate does not present a concern for skin sensitization.

Note: Whereas linalyl acetate is considered to be a non-sensitizer, autooxidation products of this material are known to be contact allergens.

Additional References: None.

Literature Search and Risk Assessment Completed on: 03/25/13.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra along with existing data, Linalyl acetate is not expected to present a concern for phototoxicity.

10.1.5.1. Risk assessment. The UV absorption spectra demonstrate that Linalyl acetate has little to no absorption in the UV range. Though the spectra are not suitable for calculating the molar absorption coefficient for use in benchmark evaluation in the region of 290–700 nm, in Guinea pig and rat studies no reactions indicative of phototoxic responses were observed (RIFM, 1983b; RIFM, 1983c).

Based on the existing data, this material does not present a concern for phototoxicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 03/25/13.

10.1.6. Local respiratory toxicity

The margin of exposure for linalyl acetate is adequate for the respiratory endpoint at the current level of use.

10.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. There are no inhalation data on linalyl acetate. However, in a 2 week acute inhalation study a NOAEC of 10 ppm (63 mg/m³) for metabolite linalool (CAS # 78-70-6; see Section 5) was reported (RIFM, 2012), which was the highest dose tested. The test substance-related effects were limited to nonadverse microscopic findings in the nasal cavity.

This NOAEC expressed in mg/kg lung weight/day is:

- (63 mg/m³) (1m³/1000L) = 0.063 mg/L
- Minute ventilation (MV) of 0.17 L/min for a Sprague-Dawley rat × duration of exposure of 360 minutes per day (min/day) (according to GLP study guidelines) = 61.2 L/d
- (0.063 mg/L) (61.2 L/d) = 3.86 mg/d
- (3.86 mg/d) / (0.0016 kg lung weight of rat*) = 2412.5 mg/kg lw/day

Based on the IFRA survey results for hydroalcohols, the 97.5th percentile was reported to be 13%. Assuming the same amount is used in all product types (fine fragrances, hair sprays, antiperspirants/deodorants, candles, aerosol air fresheners, and reed diffusers/heated oil plug-ins), the combined inhalation exposure would be 1.21 mg/day as calculated based on the IFRA survey results for the 97.5th percentile use in hydroalcohols for a 60 kg individual using RIFM's 2-Box/MPPD in silico models. To compare this estimated exposure with that of Randazzo et al. expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew et al., 2009) to give 1.86 mg/kg lung weight/day resulting in an MOE of 1297 (i.e., [2412.5 mg/kg lw/day]/[1.86 mg/kg lung weight/day]).

Since the MOE is greater than 100, the material exposure, by inhalation, at 13% in a combination of the products noted above, is deemed to be safe under the most conservative consumer exposure scenario.

* Phalen, R.F. Inhalation Studies. Foundations and Techniques, 2nd Ed. 2009. Published by, Informa Healthcare USA, Inc., New York, NY. Chapter 9, Animal Models, in section: "Comparative Physiology and Anatomy", subsection, "Comparative Airway Anatomy."

Additional References: Troy, 1977; Jirovets et al., 1991; Buchbauer et al., 1991; Jirovets et al., 1990; RIFM, 1997; Buchbauer et al., 1993; Perrucci et al., 1996; Perrucci, 1995; Rice et al., 1994; Silver, 1992; Karr et al., 1992; Regnault-Roger et al., 1995; Rice et al., 1994a; Perrucci et al., 1995; Sugawara et al., 1998; Coats et al., 1991; Cometto-Muñiz et al., 1998; Isola et al., 2003a; RIFM, 2003a; Rogers et al., 2003; RIFM, 2003b; Isola et al., 2003b; Isola et al., 2004a; Larsen et al., 1997; Smith et al., 2004; RIFM, 2004b; Isola et al., 2004b; Barocelli et al., 2004; Rogers et al., 2005; Kuroda et al., 2005; Tanida et al., 2006; Yang et al., 2005; Corsi et al., 2007; Sato et al., 2007; Nakamura et al., 2010; Nakamura et al., 2009; Linck et al., 2009; Matsubara et al., 2011.

Literature Search and Risk Assessment Completed on: 03/25/13.

10.2. Environmental endpoint summary

Analogues Identified/Justification: Not Applicable.

10.2.1. Screening-level assessment

A screening level risk assessment of linalyl acetate was performed following the RIFM Environmental Framework (Salvito et al., 2002) which provides for 3 levels of screening for aquatic risk. In Tier 1, only the material's volume of use in a region, its log K_{ow} and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor as discussed in Salvito et al., 2002. At Tier 2, the model ECOSAR (providing chemical class specific ecotoxicity estimates) is used and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Following the RIFM Environmental Framework, linalyl acetate was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening level PEC/PNEC >1).

A screening-level hazard assessment using EPISUITE ver 4.1 did identify linalyl acetate as being possibly persistent but not bioaccumulative based on its structure and physical-chemical properties. This screening level hazard assessment is a weight of evidence review of a material's physical-chemical properties, available data on environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies) and fish bioaccumulation, and review of model outputs (e.g., USEPA's BIOWIN and BCFBAF found in EPISUITE ver 4.1). Specific key data on biodegradation and fate and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.2.2. Risk assessment

Based on current VoU (2011), linalyl acetate presents a risk to the aquatic compartment in the screening level assessment.

10.2.2.1. Biodegradation. A study was conducted following OECD Guideline 301B. 10 mg/L of the test substance was incubated for 28 days. At the end of the study 96.9% biodegradation was observed (RIFM, 1994).

A study was conducted following OECD Guideline 301C. 100 mg/L of the test substance was incubated for 28 days. At the end of the study 75% biodegradation was observed (RIFM, 1991a).

10.2.2.2. Ecotoxicity. A flow through study following OECD Test Guideline 203 using *Cyprinus carpio* was performed. The reported 96 hr LC50 was 11 mg/L (RIFM, 1998c).

10.2.3. Other available data

This material has been registered under REACH. Three additional aquatic toxicity studies are reported. All data are from the ECHA Chemical Information Website accessed 13 March 2013.

An algae growth inhibition study was conducted following OECD Test Guideline 201. The reported 72 hr EC50 was 62 mg/L and the NOEC was 9.6 mg/L.

One *Daphnia* immobilization study is reported following OECD Test Guideline 202. The 48 hr EC50 was reported as 15 mg/L.

An additional fish study using Golden Orfe was also reported following German standard DIN 38412, part L15. The 96 hr LC50 was 68.12 mg/L.

10.2.4. Risk assessment refinement

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in µg/L).

Endpoints used to calculate PNEC are underlined.

Exposure information and PEC calculation (following RIFM Framework: Salvito et al., 2002).

Exposure	Europe	North America
Log K_{ow} used		4.30
Biodegradation Factor Used		1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	>1000	100–1000
Risk Characterization: PEC/PNEC	<1	<1

The RIFM PNEC is 11 µg/L. The revised PEC/PNECs for EU and NA are <1 and, therefore, does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed on: 03/25/13.

11. Literature search*

- **RIFM database:** target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** <http://echa.europa.eu/>
- **NTP:** http://tools.niehs.nih.gov/ntp_tox/index.cfm
- **OECD Toolbox**
- **SciFinder:** <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>
- **PUBMED:** <http://www.ncbi.nlm.nih.gov/pubmed>
- **TOXNET:** <http://toxnet.nlm.nih.gov/>
- **IARC:** (<http://monographs.iarc.fr>)
- **OECD SIDS:** <http://www.chem.unep.ch/irptc/sids/oecd/sids/sidspub.html>
- **EPA Actor:** <http://actor.epa.gov/actor/faces/ACToRHome.jsp;jsessionid=0EF5C212B7906229F477472A9A4D05B7>
- **US EPA HPVIS:** <http://www.epa.gov/hpv/hpvis/index.html>
- **US EPA Robust Summary:** <http://cfpub.epa.gov/hpv-s/>
- **Japanese NITE:** <http://www.safe.nite.go.jp/english/db.html>
- **Japan Existing Chemical Data Base:** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** <https://www.google.com/webhp?tab=ww&ei=KMS0UpiQK-arsQS324GwBg&ved=0CBQQ1S4>

* Information sources outside of RIFM's database are noted as appropriate in the safety assessment.

This is not an exhaustive list.

	LC50 (Fish) (mg/L)	EC50 (Daphnia) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (µg/L)	Chemical Class
RIFM Framework Screening Level (Tier 1)	2.64			1,000,000	0.0026	
ECOSAR Acute Endpoints (Tier 2) Ver 1.11	1.191	1.935	0.572			Esters
ECOSAR Acute Endpoints (Tier 2) Ver 1.11	0.591	2.136	0.471	10,000	0.0471	Vinyl/AllylEsters
ECOSAR Acute Endpoints (Tier 2) Ver 1.11	1.386	0.973	1.745			Neutral Organics
Tier 3: Measured Data (including REACH data)						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	11			1,000	11	
Daphnia		15				
Algae		62	9.6			

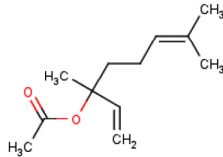
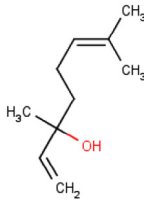
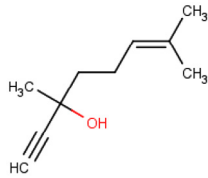
Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

Appendix 1

	Target Material	Read across Material	
Principal Name	Linalyl acetate	Linalool	Dehydrolinalool
CAS No.	115-95-7	78-70-6	29171-20-8
Structure			
3D Structure	http://www.thegoodscentscompany.com/opl/115-95-7.html	http://www.thegoodscentscompany.com/opl/78-70-6.html	http://www.thegoodscentscompany.com/opl/29171-20-8.html
Read-across endpoint		• Skin Absorption • Repeated Dose • Devel/Repro • Respiratory	• Devel/Repro
Molecular Formula	C12H20O2	C10H18O	C10H16O
Molecular Weight	196.29	154.25	152.24
Melting Point (°C, EPISUITE)	−2.09	−11.39	15.40
Boiling Point (°C, EPISUITE)	228.95	204.05	212.37
Vapor Pressure (Pa @ 25 °C, EPISUITE)	17.47	11.09	4.64
Log Kow (KOWWIN v1.68 in EPISUITE)	4.39	3.38	2.75
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPISUITE)	20.12	683.7	1084
J_{max} (mg/cm²/h, SAM)	11.1668059	90.06108298	93.21980338
Henry's Law (Pa·m³/mol, Bond Method, EPISUITE)	176.001525	4.285034	0.449174
Similarity (Tanimoto score)^a		NA ^a	NA ^a
Skin Absorption			
Skin Absorption Percentage (SAM)	80%	80%	
Repeated Dose Toxicity			
Repeated dose (HESS)	Not categorized	Not categorized	
Developmental and Reproductive Toxicity			
ER binding (OECD)	Non binder, non-cyclic structure	Non binder, non-cyclic structure	Non binder, non-cyclic structure
Developmental toxicity model (CAESAR v2.1.6)	NON-Toxicant (low reliability)	NON-Toxicant (low reliability)	NON-Toxicant (low reliability)
Metabolism			
Rat liver S9 metabolism simulator (OECD)	See supplemental data 1	See supplemental data 2	See supplemental data 3

^a Metabolites of the target or analog(s) of metabolites.

Summary

There are insufficient toxicity data on Linalyl acetate (CAS # 115-95-7). Hence, *in silico* evaluation was conducted to determine suitable read-across material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, the above shown read-across materials were identified as proper read across for their respective toxicity endpoints.

Methods

- The identified read-across analogs were confirmed by using expert judgment
- The physicochemical properties of target and analogs were calculated using EPI Suite™ v4.11 developed by US EPA (USEPA, 2012)
- The J_{max} were calculated using RIFM skin absorption model (SAM), the parameters were calculated using consensus model (Shen et al., 2014)

- ER binding and repeat dose categorization were estimated using OECD QSAR Toolbox (v3.1) (OECD, 2012)
- Developmental toxicity was estimated using CAESAR (v2.1.6) (Cassano et al., 2010)
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox (v3.1) (OECD, 2012)

Conclusion/Rationale

- Linalool and Dehydrolinalool (analogues) were used as a read-across for Linalyl acetate (target) based on:
 - The read-across materials are the major metabolite of the target and the analog of the metabolite.
 - The target is the acetate form of the linalool. This difference could be mitigated by the fact the target could readily hydrolyzed into the read-across material and acetic acid (Bickers et al., 2003). Besides, the differences between structures do not essentially change the physicochemical properties nor raise any

additional structural alerts and therefore, the toxicity profiles are expected to be similar.

- The target and analog show the same skin absorption range.
- The target and analog show similar alerts for Repeated Dose (HESS) Categorization and ER Binding. ER Binding is molecular initiating event analogous to protein binding. ER binding is not necessarily predictive of endocrine disruption given the complex pre- and post-receptor events that determine activity.
- As per the OECD Toolbox, the target is predicted to metabolize to the analog (metabolite # 2)

Appendix 2: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.fct.2015.01.010.

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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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ISSN 1831-

Doi:10.2773/

ISBN 978-92-79-

ND-

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

Acknowledgements

Dr. C. Chambers
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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

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Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1. Background

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

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

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

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

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

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

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

Furthermore, two fragrances (natural mixtures) were added

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

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

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

2. Terms of reference

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

3. Introduction

Fragrance ingredients

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

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

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

4. Clinical aspects of contact allergy to fragrance ingredients

4.1. Spectrum of reactions

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

4.1.1. Allergic contact dermatitis

Mechanism

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

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

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

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

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

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

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

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

Overview of clinical features

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

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

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

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

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

Hands

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

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

Axillae

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

Face

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

4.1.2. Irritant reactions (including contact urticaria)

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

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

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

4.1.3. Pigmentary anomalies

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

4.1.4. Photo-reactions

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

4.1.5. General/respiratory

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

4.2. Patch testing

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

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

4.3. Epidemiology of fragrance allergy

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

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

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

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

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

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

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

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

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

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

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

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

4.3.2. Clinical epidemiology

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

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

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

Fragrance Mix I ("Larsen Mix")

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

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

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

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

Note: § Possibly included in (60).

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

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

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

Fragrance Mix II

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

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

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

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

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

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

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

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

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

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

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

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

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

Oil of turpentine

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

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

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

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

4.3.3. Population-based epidemiology

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

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

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

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

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

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

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

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

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

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

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

4.4.1. Clinical relevance

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

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

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

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

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

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

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

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

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

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

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

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

Medical history

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

NACDG 2009 study (65)

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

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

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

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

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

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

Frosch 2002 (a) study (67)

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

Frosch 2002 (b) study (97)

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

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

Frosch 2005 study (35)

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

Danish Contact Dermatitis Group 2005-2008 (69)

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

Cosmetic products

Fragrance formulae from cosmetic products

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

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

IVDK “own perfumes” study (106)

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

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

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

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

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

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

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

4.4.3. Elicitation in diagnostic patch tests without clinical history

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

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

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

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

4.5. Socio-economic impact of contact allergy

4.5.1. Health related quality of life

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

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

4.5.2. Occupational restrictions

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

4.5.3. Costs to health care/health economics

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

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

4.6. Allergen avoidance

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.7. Conclusions

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

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

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

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

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

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

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

Some chemicals might act by all three pathways. One example is geranial (an isomer of citral) which is a hapten itself with a moderate sensitisation potency, but can be activated to more potent sensitisers via air oxidation (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 prehapten and prohapten. This distinction facilitates discussions by emphasizing the differences in activation mechanisms between the two types of compounds requiring activation to become haptens. It is important to note that prehapten activation, in contrast to bioactivation, can be prevented to a certain extent by avoidance of air exposure during the handling and storage of the chemicals. This concerns the most prominent haptens formed by 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 prehapten are presented in Table 5-1 and Table 5-2. In Table 5-1 the results from patch testing with air exposed samples of the prehapten are given. Table 5-2 shows the results from testing with the prehapten themselves without intended air exposure. In addition to the data given in this chapter, animal data (LLNA) on the pure prehapten or after controlled air exposure are given in Table 8-2. Possible pro- and prehapten are identified by SAR analyses in chapter 9.

5.1. Prehapten

Autoxidation is a free radical chain reaction in which hydrogen atom abstraction in combination with addition of oxygen forms peroxy radicals. The reaction shows selectivity for positions where stable radicals can be formed. So far, all fragrance substances that have been investigated with regard to the influence of 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 fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available.

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

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

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

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

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

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

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

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

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

Notes: § Bicentric or multicentre studies.

(ox.) Oxidised.

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

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

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

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

Notes: * Bicentric or multicentre studies.

n.g. Not given.

(ox.) Oxidised.

5.2. Prohaptens

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

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

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

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

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

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

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

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

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

5.3. Conclusions

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

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

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

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

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

6. Retrieval of evidence and classification of fragrance substances

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

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

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

6.1. Retrieval of evidence

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

6.1.1. Search strategy for clinical data

Method of literature search:

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

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

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

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

6.1.2. Collection of experimental (LLNA) data

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

6.2. Grading of evidence

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

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

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

6.2.1. Quality of a clinical study

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

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

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

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

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

6.2.2. Quality of an experimental study

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

6.2.3. Quality of “other” evidence

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

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

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

6.3. Aggregating evidence for a final conclusion

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

6.3.1. Established contact allergen in humans

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

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

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

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

6.3.2. Established contact allergen in animals

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

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

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

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

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

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

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

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

³ It should be noted that the SCCS considers such tests unethical (169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:).

6.4. Conclusions

The present opinion includes (i) a well-defined search strategy for retrieving pertinent evidence; (ii) a definition of criteria used to evaluate available evidence; and, finally (iii) a set of rules to categorise the substances with regard to the relevant toxicological endpoint, i.e. sensitisation in man, based on the evidence.

7. Reported fragrance allergens from the clinical perspective

In this chapter, clinical evidence regarding sensitisation to individual fragrance chemicals and to natural extracts (essential oils) is tabulated. In this report “single chemicals” refers to chemicals of natural or synthetic origin whose chemical identity is fully known. The term “natural extracts” refers to plant or animal derived mixtures of natural chemicals, for example lavender oil, whose composition may be variable and may or may not have been fully or partly established. Full information, including possible synonyms, structural formulas (in the case of single chemicals only), a short summary of available evidence and further information, e.g. on regulatory status, is presented in Annex I.

7.1. Tabular summary of evaluated individual fragrance chemicals

Regarding nomenclature, INCI names are used wherever possible. If an INCI name is not available, the perfuming name as listed by CosIng is used. Detailed information on the publications identified and considered for this report can be found in Annex I. Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I, these substances have not been further evaluated and are thus not included in this chapter.

In this section, a tabular overview on the classification of substances considered is presented in four tables listing:

1. Established contact allergens in humans (→ *sufficient human evidence present*).
2. Substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans” (→ *limited human evidence present*).
3. Substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.
4. Substances eligible for inclusion (see beginning of chapter 6) for which no human data are available.

A critical point in understanding this scheme is that there is publication bias in reporting allergens. This is due to the fact that once a substance has been reported and accepted as a contact allergen in humans, further reports are less likely to be published unless they are part of an epidemiological survey or when there is a novel source of exposure. Moreover, the number of patients displaying positive test reactions obviously not only depends on the underlying prevalence of sensitisation, but also on how often a substance is patch tested. This implies that inclusion of an allergen or allergen mixture in the baseline patch test series (as for Fragrance Mix I and II, *Myroxylon pereirae* and HICC, and partly also other substances/mixtures) will yield the maximum possible number of cases. In contrast, patch testing in “special” series, e.g. as a break-down of single constituents of the respective mix in case of a positive reaction to the latter, or with application only in the case of strongly suspected fragrance intolerance, will mostly result in higher relative numbers than testing the same compound consecutively, but also in lower absolute numbers.

In Table 7-1, the single substances are listed with a semi-quantification of their impact which were categorised as established contact allergens in humans according to the criteria given in chapter 6.3.

Established contact allergens in humans, according to the criteria outlined in chapter 6.3.1, were categorised according to the number of patients reacting positively and to the number of patients tested, based on the publications considered (see annex I for references). The following categories were used:

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+	Up to 10 positive test reactions reported
++	11 to 100
+++	101 to 1000
++++	> 1000

If a test allergen has been tested in less than 1,000 patients, “r.t.” (rarely tested) is added in the following tables. For this categorisation, absolute numbers of cases of sensitisation, and not the relative frequency of positive patch tests, were used, because relative frequencies depend heavily on the selection of patients for patch testing. Thereby, an important allergen tested routinely, in the baseline series, may yield 1 to 2% positive reactions (usually in several thousand patients), while an allergen tested in a selective fashion (in much fewer patients) may yield an even higher relative frequency. Moreover, case reports/series cannot be interpreted in terms of relative frequencies. The calculation of absolute numbers was based on all available literature, as detailed in the annex I to this opinion, i.e., regarding the 26 substances already listed in Annex III to the Cosmetics Directive includes data already evaluated in the previous opinion.

Table 7-1: Established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
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.)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ROSE KETONE-4		
alpha-DAMASCONE (TMCHB) [#]	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE [#]	23726-92-3	+
delta-DAMASCONE [#]	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL	97-53-0	+++
FARNESOL	4602-84-0	+++
GERANIOL	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE (HICC)	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL	107-75-5	+++
ISOEUGENOL	97-54-1	+++
alpha-ISOMETHYL IONONE	127-51-5	++
(DL)-LIMONENE	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN [#]	92-48-8	++ (photo-allergy)
METHYL 2-OCTYNOATE	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	++
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

Those substances which were categorised as +++ or more, i.e. those with the most reported cases, were also the top ranking substances in large series of patients tested with the 26 labelled fragrance ingredients ((44, 74) and additionally (170)). Geraniol is an exception, as it was all negative in the Danish study (170), but was still among the top ten in the Dutch and German studies (44, 74), with prevalences of 0.5%-0.6% positives. Geraniol has, in addition, caused many cases of contact allergy in other areas of Europe (49).

The use of absolute numbers allows the pooling of studies with different selection criteria. Limonene and linalool were not tested in their oxidized forms in the three studies (44, 74, 170) and would not have been identified, if only these publications had been the basis of assessment.

It should be noted that oxidised fragrance terpenes with defined content of the major haptens formed after autooxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available (see also chapter 5).

Table 7-2 lists those substances which gave rise to a few reported cases of contact sensitisation only, or where results have been reported from just one clinical department. Thus, the level of evidence concerning human data must be regarded as *limited*, according to the definitions given in chapter 6.3.

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Table 7-2: Fragrance substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
AMBRETTOLIDE	7779-50-2	3.4% positive reactions in 178 patients	(171)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (172))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(139)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(173)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(171)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(174)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(175).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(171)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(173)
ETHYL VANILLIN	121-32-4	1 occupational case	(176)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(97)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(173)
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, possibly “excited back syndrome” 0 / 95 in another study with <= 1/10 of above test conc.	(139) (70)
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(171)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(177)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(173)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(178)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(179)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(97) (70)
METHYLIONANTHEME	55599-63-8	1 case	(180)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(97)
METHYL OCTINE CARBONATE	111-80-8	1 case	(181)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(133)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(182)
NEROL	106-25-2	6.0% positive	(173)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(183)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly "excited back syndrome"	(139)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(139) (70)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(178) (184)
PHENYLPROPANOL	122-97-4	2 / 218	(173)
PHYTOL	150-86-7	1 case in human max. test	(185)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(186)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(187)

For a number of substances negative patch tests results were obtained, usually in rather small patient samples (max. 313 patients). For some of these substances exposure is substantial, according to data submitted from IFRA. It should be noted that a negative result does not rule out a notable sensitisation prevalence, as the study size has to be larger than, e.g. n=298 to yield a 95% CI which excludes a prevalence of 1% and larger than n=597 to exclude a prevalence of 0.5%.

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Table 7-3: Fragrance substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Results / Comment	Ref.
6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	21145-77-7	0 / 313 consecutive patients in 2 centres	(70)
AMYL CYCLOPENTANONE	4819-67-4	0 / 178	(171)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(70)
2-TERT-BUTYL CYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(70)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(70)
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(171)
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(70)
HEXYL SALICYLATE	6259-76-3	0 / 218 "top 100" substance and classified as R43	(173)
HIBISCOLIDE	6707-60-4	0 / 178	(171)
alpha-IONONE	127-41-3	0 / 205	(70)
beta-IONONE	79-77-6	0 / 205 "top 100" substance	(70)
ISOBORNYL ACETATE	125-12-2	0 / 107 "top 100" substance	(70)
METHYL ANTHRANILATE	134-20-3	0 / 91 "top 100" substance	(188)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 "top 100" substance	(70)
OXALIDE	1725-01-5	0 / 178	(171)
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 "top 100" substance	(70)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(139)
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(171)

For yet another subset of substances, no human data were publicly available. However, exposure to these substances is important as they are used in high volumes (this being the sole criterion for inclusion in this list) and, therefore their hazard with regard to contact sensitisation should be examined.

Table 7-4: Fragrance substances lacking human data and used in high volumes according to industry information.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
ANISALDEHYDE	123-11-5
BENZYL ACETONE	2550-26-7
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0
CITRONELLYL NITRILE	51566-62-2
CYCLAMEN ALDEHYDE	103-95-7
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0
DECANAL	112-31-2
DIHYDROMYRCENOL	18479-58-8
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	68039-49-6
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6
DIPHENYL ETHER	101-84-8
ETHYL 2-METHYLBUTYRATE	7452-79-1
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6
ETHYLENE BRASSYLATE	105-95-3
EUCALYPTOL	470-82-6
GERANYL ACETATE	105-87-3
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0
HEXYL ACETATE	142-92-7
IONONE isomeric mixture	8013-90-9
ISOAMYL ACETATE	123-92-2
ISOBERGAMATE [#]	68683-20-5
Longifolene	475-20-7
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0
METHYLBENZYL ACETATE	93-92-5
METHYL DECENOL	81782-77-6
METHYL beta-NAPHTHYL ETHER	93-04-9
METHYLUDECANAL	110-41-8
OXACYCLOHEXADECENONE	34902-57-3
PENTADECALACTONE	106-02-5
PHENETHYL ACETATE	103-45-7
PHENOXYETHYL ISOBUTYRATE	103-60-6
PHENYLISOHEXANOL	55066-48-3
Tetrahydrolinalool	78-69-3
TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	63500-71-0

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
TRICHLOROMETHYL PHENYL CARBINYL ACETATE	90-17-5
TRICYCLODECENYL PROPIONATE	17511-60-3
TRIMETHYLHEXYL ACETATE	58430-94-7
gamma-UNDECALACTONE	104-67-6
VERDYL ACETATE	2500-83-6/ 5413-60-5

7.2. Tabular summary of evaluated natural extracts/essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic the demand for perfumes based on natural materials is considerable (189).

The three main methods used to concentrate plant fragrance substances (190); distillation, mechanical separation ("pressing"), and solvent extraction, yield very different extracts. Essential oils are obtained by water steam, water, ethanol, or water/ethanol distillation. Essence oils are essential oils that separate from the aqueous phase in the distillation receiver during the distillative concentration of fruit, usually citrus, juices. Citrus peel oils, apart from distilled lime oil, are prepared in a special way by pressing the peel to release mostly volatile substances from the pericarp in small oil glands, mostly highly volatile terpene hydrocarbons. However, they also contain small amounts of non-volatile compounds such as dyes, waxes and furocoumarins. The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concretes, an extract of fresh plant material with nonpolar solvents, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolutes, which are prepared by taking up concretes in ethanol; compounds that precipitate on cooling are removed by filtration, yielding a wax-free residue called absolute. Resinoids, used for their fixative properties, are prepared by extracting plant exudates with alcohols or nonpolar solvents. The products are usually highly viscous and thus sometimes diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are concentrates prepared from spices by solvent extraction (189).

An ISO norm exists regarding the nomenclature of aromatic natural raw materials (ISO/DIS 9235 Aromatic raw materials - vocabulary; International Standardisation Organisation, Geneva, Switzerland). This nomenclature has been considered in Annex I, whereas in the present opinion, nomenclature is according to the CosIng database. Concerning extraction processes for many essential oils, ISO standards exist; for detailed information see Annex I to this opinion.

Regarding clinical data in terms of contact allergy to essential oils and natural extracts, the main focus is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g. masseurs, physiotherapists (191, 192), aromatherapists (193-197), beauticians performing massages (198). For further details, e.g. PT results with various essential oils, see Annex I.

In this section, a tabular overview on the classification of substances considered is presented in three tables listing:

1. Extracts identified as *established contact allergens* in humans(→ *sufficient human evidence present*).

2. Extracts with positive human data, which are, however, not sufficient to categorise as *established contact allergen* in humans (→ *limited human evidence present*).
3. Extracts with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

In Table 7-5, essential oils with sufficient human evidence to categorise these as *established contact allergens* in humans are presented.

Table 7-5: Natural extracts classified as established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion, including variants of botanical nomenclature.

INCI name (or, if none exists, ^{\$} perfuming name according to CosIng ⁴) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment: see text
CANANGA ODORATA and <i>Ylang-ylang oil</i>	83863-30-3; 8006-81-3	+++
CEDRUS ATLANTICA BARK OIL	92201-55-3; 8000-27-9	++
CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
CITRUS AURANTIUM AMARA FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
CITRUS BERGAMIA PEEL OIL EXPRESSED ^{\$}	89957-91-5	+ (r.t.)
CITRUS LIMONUM PEEL OIL EXPRESSED [#]	84929-31-7	++
CITRUS SINENSIS (syn.: <i>AURANTIUM DULCIS</i>) PEEL OIL EXPRESSED ^{\$}	97766-30-8; 8028-48-6	++
CYMOPOGON CITRATUS / SCHOENANTHUS OILS	89998-14-1; 8007-02-1; 89998-16-3	++
EUCALYPTUS SPP. LEAF OIL ^{\$}	92502-70-0; 8000-48-4	++
EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL	8000-34-8	+++
EVERNIA FURFURACEA EXTRACT ⁵ (Tree moss)	90028-67-4	+++
EVERNIA PRUNASTRI EXTRACT (Oak moss) [#]	90028-68-5	+++
JASMINUM GRANDIFLORUM / OFFICINALE	84776-64-7; 90045-94-6; 8022-96-6	+++
JUNIPERUS VIRGINIANA	8000-27-9; 85085-41-2	++
LAURUS NOBILIS	8002-41-3; 8007-48-5; 84603-73-6	++
LAVANDULA HYBRIDA	91722-69-9	+ (r.t.)
LAVANDULA OFFICINALIS ^{\$}	84776-65-8	++
MENTHA PIPERITA	8006-90-4; 84082-70-2	++
MENTHA SPICATA	84696-51-5	++
MYROXYLON PEREIRAE (Balsam of Peru) [#]	8007-00-9	++++

⁴ <http://ec.europa.eu/consumers/cosmetics/cosing/>

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INCI name (or, if none exists, ^{\$} perfuming name according to CosIng ⁴) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment: see text
NARCISSUS SPP.	<i>diverse</i>	++
PELARGONIUM GRAVEOLENS	90082-51-2; 8000-46-2	++
PINUS MUGO/ PUMILA [#]	90082-72-7 / 97676-05-6	++
POGOSTEMON CABLIN	8014-09-3; 84238-39-1	++
ROSE FLOWER OIL (ROSA SPP.)	<i>Diverse</i>	++
SANTALUM ALBUM	84787-70-2; 8006-87-9	+++
TURPENTINE (oil) [#]	8006-64-2; 9005-90-7; 8052-14-0	++++
VERBENA absolute [#]	8024-12-2	++

Notes: r.t. Rarely tested.

Table 7-6 lists a number of essential oils, mostly tested in just one clinical department, and thus, or for other reasons, not satisfying the criteria for being categorised as *established contact allergen* in humans (i.e. *limited human evidence present*).

Table 7-6: Natural extracts with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment	Ref.
ACORUS CALAMUS ROOT OIL	84775-39-3	n=7 pos. reactions to “calamus”	(199)
CEDRUS DEODARA WOOD OIL	91771-47-0	Rudzki 1976/1986 found 3 / 3 positive reactions	(199, 200).
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Several cases in 2 series from 1 centre	(199, 200)
CITRUS TANGERINA ...	223748-44-5	1 case	(201)
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Several cases in 2 series from 1 centre	(199, 200)
ILLICIUM VERUM FRUIT OIL	84650-59-9	Cases of active sensitisation; 34% consecutive patients pos. to 1%	(202)

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INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment	Ref.
LAVANDULA SPICA	97722-12-8	Several cases in 2 series from 1 centre	(199, 200)
LITSEA CUBEBA	90063-59-5	Several cases in 2 series from 1 centre	(199, 200)
PELARGONIUM ROSEUM	90082-55-6	2.1% pos. of 1483 patients	(203)
ROSMARINUS OFFICINALIS	84604-14-8	3 cases in 2 series from 1 centre	(199, 200)
SALVIA spp.	Diverse	Several cases in 2 series from 1 centre	(199, 200)
TAGETES PATULA	91722-29-1	1 case (aromatherapist)	(193)
THYMUS spp.	84929-51-1	4 / 84 pos	(199)
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	1 / 200 and 9 / 86 pos.	(199, 200)

The final table is an indicative list of natural extracts which lack published human data, but which are of interest: (i) as high-volume exposure; (ii) due to published positive animal experiments; or (iii) because they contain well-known (established) contact allergens.

Table 7-7: Indicative list illustrating natural extracts containing established human allergens or having R43-label or positive LLNA, lacking published human data.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment
CITRUS PARADISI PEEL OIL	8016-20-4	high volume substance, classified as R43
CYMBOPOGON MARTINI HERB EXTRACT	84649-81-0	Pos. LLNA study by RIFM: EC3 value 9.6% (204).
MENTHA ARVENSIS	68917-18-0	high volume, classified as R43
OCIMUM BASILICUM	84775-71-3	Pos. LLNA study by RIFM: EC3 value < 2.5% (204).
PIMENTA RACEMOSA	85085-61-6	Contains, among other substances, the established contact allergen eugenol (42-56%)
SANTALUM SPICATA	8024-35-9	Contains, among other substances, the established contact allergens santalols (75%) and farnesol (10%)

7.3. Conclusions

- According to the criteria described in chapter 6.3 a total of 54 individual chemicals and 28 natural extracts (essential oils) can be categorised as *established contact allergens* in humans, including all currently regulated substances.
- Of the 54 individual chemicals which are established contact allergens in humans, 12 are considered to be of special concern due to the high number of reported cases, (> 100, i.e. category +++ or ++++ in Table 7-1). These are further considered in chapter 5 (limonene and linalool) and the remainder in chapter 11. In particular one ingredient stands out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1,500 reported cases since the 1999 opinion (see also chapter 4.3.1, chapter 11.3 and Annex I).
- For an additional 33 individual chemicals (Table 7-2) and 14 natural extracts (Table 7-6), positive patch test results have been reported. However, they do not qualify for the above category, i.e. only *limited human evidence* is present.
- For a number of fragrance substances (n=18, Table 7-3) patch testing did not yield positive results. However, numbers of patients tested are generally too small to rule out the existence of clinical contact sensitisation with sufficient confidence. No clinical evidence has been identified for 39 individual chemicals that have been reported to be frequently used (Table 7-4).
- For the substances (and, if possible, also for the main constituents of the natural mixtures) with limited or no human evidence, additional animal data and/or SAR considerations are taken into account. Aggregated data for these substances are presented in chapter 13.

8. Animal data

8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test⁶ and the local lymph node assay (LLNA)⁷. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH⁸. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling⁹, substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol “Xi” and the risk phrase “R43: May cause sensitisation by skin contact”; or, according to the recent regulation on classification, labelling and packaging (CLP¹⁰) “H317: May cause an allergic skin reaction”.

As yet, there is no officially validated *in vitro* test method for skin sensitisation. Therefore, for cosmetic ingredients the LLNA, the GPMT and the Buehler test have also been used in risk assessment for regulatory purposes.

Positive results from the OECD guideline animal tests mentioned above which are sufficient to classify a substance as a skin 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 $SI \geq 3$). For positive LLNAs, an EC3 value is calculated which gives the estimated concentration of a chemical necessary to give a 3-fold increase in proliferative activity compared to vehicle-treated controls.

Further categorisation of substances classified with R43 into three groups according to allergen potency (extreme, strong and moderate) has been proposed by a European Commission expert group on skin sensitisation (161, 205), and proposed also in the ECHA guidance document on application of the CLP criteria (162). Such categorisation is based on EC3 values in the LLNA, on intradermal induction concentration in the GPMT, and topical induction concentration in the Buehler test. The potency categories and their default concentration values based on EC3 values in the LLNA as defined in (161): extreme sensitiser (EC3 value ≤ 0.2); strong sensitiser (EC3 $> 0.2 - \leq 2$); 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).

⁶ OECD Guideline for testing of chemicals. Guideline 406: Skin Sensitisation. OECD, Adopted 12 May 1981, updated 17th July 1992.

⁷ OECD Guideline for testing of chemicals. Guideline 429: Skin Sensitisation: Local Lymph Node Assay. OECD, Adopted 22 July 2010.

⁸ Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

⁹ Directives 67/548/EEC and 1999/45/EC.

¹⁰ Regulation No. 1272/2008.

8.1.1. LLNA data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, IFRA submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report.

Table 8-1 displays the EC3 values for fragrance substances in the report submitted by industry (164). EC3 values for some additional fragrance substances in two published reviews (165, 166) have also been included in Table 8-1. Table 8-2 presents LLNA results for oxidised substances. Full data are given in Annex II. Table 8-3 summarises the distribution of fragrance substances, by potency category, according to EC3 values.

Additional EC3 values may be available in the scientific literature. Many more animal experiments may have been performed, but have not been published.

Table 8-1: Summary of local lymph node assay (LLNA) data on 66 fragrance substances, based on a report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009 (164)) and in published reviews by Gerberick et al. 2005 (165) and Kern et al. 2010 (166), respectively. EC3 values (% and M) are given. The order of substances is by decreasing sensitisation potency as assessed by LLNA EC3 values (lowest EC3 value indicating highest potency).

Substance	CAS no.	EC3 value		Reference
		%	M	
Hexyl salicylate	6259-76-3	0.18	0.008	(164, 166)
Cinnamal	104-55-2	0.2	0.015	(164)
Methyl 2-octynoate	111-12-6	<0.5	<0.032	(164, 166)
Isoeugenol	97-54-1	0.54	0.033	(164)
Citral	5392-40-5	1.2	0.079	(164)
2-Hexylidene cyclopentanone	17373-89-6	2.4	0.14	(164)
Methyl octine carbonate	111-80-8	2.5	0.15	(164)
Peru balsam absolute	8007-00-9	2.5	n/a	(164)
trans-2-Hexenal	6728-26-3	2.6	0.26	(164)
Benzyl Salicylate	118-58-1	2.9	0.23	(164, 166)
Butylphenyl methylpropional (BMHCA)	80-54-6	2.9	0.14	(164)
Phenylacetaldehyde	122-78-1	3	0.25	(164, 165)
Allyl phenoxyacetate	7493-74-5	3.1	0.16	(164)
Benzylideneacetone	122-57-6	3.7	0.25	(165)
3-Propylidenephthalide	17369-59-4	3.7	0.21	(164, 165)
<i>Evernia prunastri</i> extract oak moss	90028-68-5	3.9	n/a	(164)
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	4	n/a	(164)
Farnesol	4602-84-0	4.1	0.18	(164)
p-t-Butyl-dihydrocinnamaldehyde	18127-01-0	4.3	0.23	(164)
α-Methyl cinnamic aldehyde	101-39-3	4.5	0.31	(164, 165)

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Substance	CAS no.	EC3 value		Reference
		%	M	
Eugenol	97-53-0	5.3	0.32	(164)
Hexyl cinnamal	101-86-0	5.3	0.25	(164)
Dihydrocoumarin	119-84-6	5.6	0.38	(165)
Geraniol	106-24-1	5.6	0.36	(164)
Carvone	6485-40-1	5.7	0.38	(164)
Diethyl maleate	141-05-9	5.8	0.34	(165)
2-Methoxy-4-methylphenol	93-51-6	5.8	0.42	(164, 165)
Anise alcohol	105-13-5	5.9	0.43	(164, 166)
Jasmine absolute (<i>Grandiflorum</i>)	8022-96-6	5.9	N/a	(164)
Dibenzyl ether	103-50-4	6.3	0.32	(164)
<i>Cananga odorata</i> leaf/flower oil ylang ylang "extra"	8006-81-3	6.8	N/a	(164)
Isocyclocitral	1335-66-6	7.3	0.48	(164)
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	7.5	0.50	(165)
Amyl cinnamal	122-40-7	7.6	0.38	(164)
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	8.1	0.54	(164, 165)
p-Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	9.5	0.46	(164)
d-Limonene*	5989-27-5	<10	<0.73	(164)
Methylundecanal	110-41-8	10	0.54	(165)
Acetylcedrene	32388-55-9	13.9	0.57	(166)
Methylenedioxyphenyl methylpropanal	1205-17-0	16.4	0.85	(164, 166)
Benzyl benzoate	120-51-4	17	0.80	(165)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	17.1	0.81	(164, 165)
Benzyl cinnamate	103-41-3	18.4	0.77	(164, 166)
Hydroxycitronellal	107-75-5	19.3	1.12	(164)
Cinnamyl alcohol	104-54-1	21	1.57	(165)
α -iso-Methylionone	127-51-5	21.8	1.06	(164, 166)
Cyklamen aldehyde	103-95-7	22	1.64	(165)
4-Methoxy- α -methyl benzenpropanal	5462-06-6	23.6	1.32	(164)
Amyl cinnamyl alcohol	101-85-9	~25	~1.22	(164, 166)
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	25.1	1.07	(164)
Ethyl acrylate	140-88-5	28	2.8	(165)
Linalool*	78-70-6	30	1.94	(165)
Trimethylbenzenepropanol Majantol	103694-68-4	30	~1.68	(164)
Jasminum Sambac Flower CERA/Extract/Water	91770-14-8	35.4	N/a	(164)

Substance	CAS no.	EC3 value		Reference
		%	M	
Citronellol	106-22-9	43.5	2.78	(164, 166)
No EC3 value was established; higher concentrations should also have been tested				
6-Methyl-3,5-heptadien-2-one	1604-28-0	>5	>0.40	(164)
<i>Camellia sinensis</i> leaf tea leaf absolute	84650-60-2	>5	N/a	(164)
Cinnamyl nitrile	1885-38-7	>10	>0.77	(164)
Menthadiene-7-methyl formate	68683-20-5	>10	>0.51	(164)
<i>Evernia furfuracea</i> extract tree moss absolute	90028-67-4	>20	N/a	(164)
Isocyclogeraniol	68527-77-5	>25	>1.62	(164)
1-Octen-3-yl acetate	2442-10-6	>30	>1.76	(164)
Benzyl alcohol	100-51-6	>50	>4.62	(164)
Coumarin	91-64-5	>50	>3.42	(164)
Vanillin	121-33-5	>50	>3.3	(164)
No EC3 value calculated				
Benzaldehyde	100-52-7	-		(165)

Notes: * Material with low levels of oxidation according to (164)

n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

8.1.2. LLNA data on oxidised fragrance substances

For fragrance substances that can autoxidise upon air exposure, it is also important to investigate the sensitisation potency after air exposure. The oxidised compounds are clinically relevant as they represent what the consumers could come in contact with from perfumes and fragranced products. In Table 8-2 the LLNA data for some of the most commonly used fragrance substances, pure and after autoxidation, are presented. The EC3 values obtained for the pure substances are 5-10 times higher compared to those obtained for the same substances after air exposure. The experimental air exposure simulated air exposure that can take place during normal handling and storage. In the production process, some perfumes are “matured” aerobically, stirring included. During this process, some fragrance substances may be oxidised. It should be noted that, although only a few substances capable of oxidation have so far been investigated, structural alerts indicating possible autoxidation are common among the fragrance substances listed in this document (see chapter 9). It is important to further investigate this issue for increased understanding of the associated risk.

Table 8-2: Local lymph node assay (LLNA) data on four fragrance substances and one essential oil before and after air exposure, comparing the sensitisation potency of the pure (not oxidised) substance with the potency of the oxidised.

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
D-Limonene (ox. 10 w)	5989-27-5	1, 5, 25	3.0	(207)
D-Limonene (pure)	5989-27-5	25, 50, 100	30	

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
Linalool (ox. 10 w)	78-70-6	5, 10, 25	9.4	(127)
Linalool (ox. 45 w)	78-70-6	2.5, 10, 25	4.8	
Linalool (pure)	78-70-6	25, 50, 100	46.2	
Linalyl acetate (ox. 10 w)	115-95-7	0.5, 10, 40	3.6	(128)
Linalyl acetate (pure)	115-95-7	10, 30, 100	25	
Geraniol (ox. 10 w)	106-24-1	1, 3, 6, 10, 20	4.4	(119)
Geraniol (ox. 45 w)	106-24-1	0.5, 1, 3, 6, 10	5.8	
Geraniol (pure)	106-24-1	5, 10, 15, 20, 30	22.4	
Lavender oil (ox. 10 w)		1, 5, 10, 20, 50	11	(140)
Lavender oil (ox. 45 w)		1, 5, 10, 20, 50	4.4	
Lavender oil (not ox.)		5, 25, 100	36	

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.

Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.

Ox. x w: Oxidised by air exposure during x weeks.

* Acetone:olive oil.

8.2. Methodological considerations

EC3 mean values

In the submitted poster (163) and the report by IFRA (164), the LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) are presented. The SCCS considers it is misleading to present EC3 values as mean values from tests performed with different vehicles. It is generally agreed that the lowest EC3 value should be used if there is more than one study fulfilling the OECD guideline requirements (161, 205), and these have been introduced into Table 8-1. The EC3 values in the reviews by Gerberick et al. and Kern et al. (165, 166) were based on single representative experiments with a vehicle described in the OECD guideline 429 (see above), and preferably with acetone:olive oil. EC3 mean values, as in the submission by IFRA, were not presented in these two reviews.

Vehicle

The most frequently used *vehicle* in the submission by IFRA (164) was ethanol:diethyl phthalate (1:3), followed by acetone:olive oil (4:1). In some experiments, antioxidants were mixed with ethanol:diethyl phthalate. The vehicle was not reported in some of the references, and no rationale for using vehicles other than those recommended was given in the report (164). According to the OECD guideline 429 (see above), the recommended vehicles are acetone:olive oil (4:1), N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulphoxide, but others may be used if sufficient scientific rationale is provided. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus as an *additional control*, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.

Number of doses and animals

According to the OECD guideline 429 (see above), a minimum of three concentrations should be tested. The number of consecutive doses used in the reported data, was generally five, sometimes three and in few experiments two. The SCCS considers that too few concentrations were tested in four studies in which only two concentrations were used. Lower concentrations than those tested should have been used in experiments with five fragrance substances, in which the EC3 value could not be determined. Higher concentrations than those tested should also have been used in experiments with 12 substances, in which the EC3 value could not be determined.

The *number of animals* per dose group was generally four plus a non-exposed control group, sometimes five, and in few experiments six; the minimum according to the OECD guideline being four.

Units for concentrations

In the submission by IFRA (164) the EC3 values are given in weight per area unit ($\mu\text{g}/\text{cm}^2$). The SCCS considers that the EC3 values (%) are the values of primary interest in communicating risk assessment, as EU legislation, OECD guideline 429 and scientific literature refer to EC3 values (%). However, the SCCS recommends that molar (M) EC3 values should be considered, as they give the concentration based on the molecular weight of substances. They have thus been calculated and introduced into Table 8-1.

EC3 values (%) overestimate the intrinsic molecular sensitisation potency for low molecular weight compounds while compounds with a high molecular weight are underestimated. Regarding the differences in molecular weight between the studied fragrance substances, a variation is seen if the ranking list of the sensitisation potency is based on EC3 (%) or EC3 (M) since some substances have a molecular weight twice as high as others.

From comparisons in Table 8-1, we notice that, e.g. hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has an EC3 value of 17.1 %, or 0.81 M when the calculation includes its molecular weight, while for trans-2-hexenal the corresponding values are 2.6% and 0.26 M. The example shows that comparing the sensitisation potency between these two substances using the EC3 values in % exaggerates the sensitisation potency of trans-2-hexenal compared to that of HICC. When using the EC3 values in molar concentrations the difference is not so pronounced.

8.3. Summary of animal data by LLNA

The distribution of sensitising potency of fragrance substances compared to other substances, (e.g. biocides, dyes, plastic materials) taken from three references (164-166) as assessed by EC3 values in the LLNA, is shown in Figure 8-1 and Table 8-3.

For 10 substances, no EC3-value could be established. These should have been tested at higher concentrations – some of these would most probably have generated an EC3 value. However, we reported here “No EC3 value established”. 5 substances should have been tested also at lower concentration and in these cases the EC3 value could have been lowered, meaning a more severe potency category could have been achieved. In all, approx 150 experiments were reported in (164), listed in Annex II.

The median EC3 value of evaluable fragrance substances (5.9%) is similar to other substances tested (5.5%). However, very few fragrance substances have low EC3 values (≤ 2).

Substances with an EC3 value ≤ 2 may be categorised as strong or extreme sensitisers. Such potent sensitisers are comparatively rare among fragrance substances assessed in the LLNA. Nevertheless, fragrances are important allergens in humans, which points to repeated skin exposure to less potent sensitisers as a factor strongly determining sensitisation risk.

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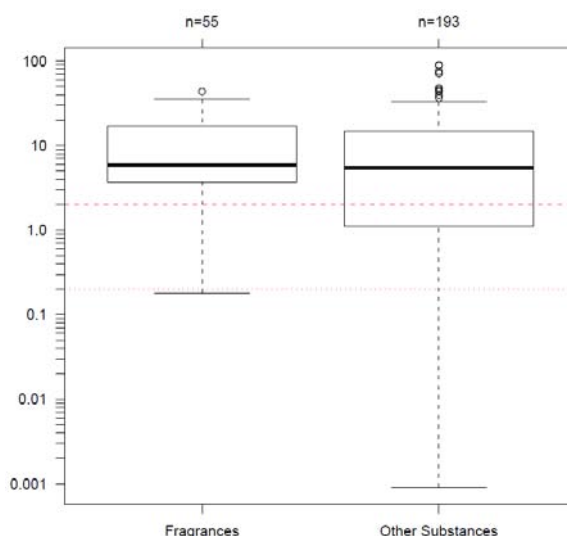


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in the box the median, and the top of the box the 3rd quartile (75% percentile). Outliers, i.e. below the 25% and above the 75% percentiles, are shown as whiskers. Beyond the 1.5-fold interquartile range, single values are shown as circles instead of whiskers. The difference in distribution is not significant (Wilcoxon test: $p=0.061$).

Note: EC3 values for the five oxidised fragrances additionally examined (Table 8-2) range from 3.0 to 4.8 (median 4.4) and are lower by a factor of around 7 than EC3 values of the respective non-oxidised material.

Table 8-3: Summary of EC3 values for fragrance substances in Table 8-1 and for other substances, all taken from the three references (164-166). The EC3 value intervals for potency categorisation (161, 205) were used for comparison of fragrances substances vs other substances.

EC3 value interval	Fragrance substances		Other substances	
	n	%	n	%
≤ 0.2	2	3%	28	11%
$> 0.2 - \leq 2$	3	4%	38	15%
> 2	50	71%	127	49%
No EC3 value established *	10	14%	0	0%
No EC3 value calculated (NC)	5	7%	69	26%
All substances	70		262	

Note: * Substances should have been tested also at higher concentrations.

8.4. Conclusions

- In the event that human data are lacking, the LLNA provides important information on skin sensitising potential and potency.
- Animal data on fragrance substances submitted by IFRA (164) and assessed in this opinion were generated exclusively by LLNA. Other guideline methods are, however, also available.
- The vast majority of the submitted (164) and additional (165, 166) fragrance substances tested by the LLNA are skin sensitisers.
- Several studies in the IFRA report (164) were of insufficient quality, not following the OECD guideline.

- Fragrance substances that can be predicted to autoxidise upon air exposure should also be tested after air exposure, as oxidation may significantly increase their sensitising potency.
- It can be concluded that the skin sensitising potency, as assessed by the LLNA, is only one of several factors that are of importance for sensitisation to fragrance substances. This is illustrated by the fact that only a small fraction of sensitising fragrance substances can be categorised as an extreme allergen based on LLNA test results. Therefore, doses from repeated deposition onto skin must be considered a driving force of sensitisation risk.

9. Structure activity relationships (SAR): grouping of substances based on expert judgement

Whether or not a particular chemical will be a sensitiser, and how potent it will be if it is a sensitiser, depends on its ability, either directly or after activation, to react with appropriate proteins in the skin. This fundamental concept was initially demonstrated by Landsteiner and Jacobs in 1936 (208) and subsequently validated by numerous studies with various types of chemicals (some key references: (209-213)). The ability to predict sensitisation potency, or lack of it, depends on being able to predict reactivity to skin proteins. This is the basis of SAR analysis for skin sensitisation. The prediction can often be made based on the chemical structure, recognising structural features (referred to as **structural alerts**) that are associated with reactivity.

The relationships between molecular structure and reactivity that form the basis for structural alerts are based on well established principles of mechanistic organic chemistry (214). Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and α,β -unsaturated carbonyl groups, C=C-CO- (alerting to the possibility of sensitisation via Michael addition of protein thiol groups). Major mechanistic reactivity domains have been discussed in detail by Aptula and Roberts (215). Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) is more complex compared to that of compounds that act as direct haptens without any activation. The autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers linalool and geraniol results in different major haptens/allergens. Moreover, the complexity of the prediction increases further for those compounds that can act both as pre- and prohaptens. In such cases, the impact on the sensitisation potency depends on the degree of abiotic activation (e.g. autoxidation) in relation to the metabolic activation.

These structural alerts can be applied by computerized expert systems, i.e. *in silico* or by estimations made by organic chemists (*in cerebro*) using their experience. When an organic chemist looks at a chemical structure, they recognise parts of the structure that they can associate with reactivity, the type of reactivity (i.e. assign the reaction mechanistic domain), and other features of the molecular structure that will affect the reactivity positively or negatively. Human experts should be aware of the complexities, and how structural modification can alter the reactivity associated with structural alerts, etc. Importantly, they can also recognise where there are unfamiliar structural features whose effects they cannot confidently predict. In such cases they can call for experimental chemistry work (*in chemico*) to be done to ascertain the presence or nature of, and degree of reactivity. *In chemico* methods include organic chemistry experimentation to identify chemical reaction products from oxidation and/or reaction with model nucleophiles, identification of mechanisms of reaction. In so called *in chemico* reactivity methods, the ability of a specific chemical to react with selected peptides is determined so as to predict the sensitisation potential of the chemical under investigation (216, 217). To make *in chemico* reactivity methods able to predict the activity of prohaptens, the addition of horseradish peroxidase and hydrogen peroxide oxidation system has been tested to model the enzymatic oxidation in the skin (218, 219).

Although computerized expert systems are derived from input by human experts, they are less well able to capture the subtleties of structure reactivity relationships, and they sometimes fail to detect aspects of chemistry that are obvious to organic chemists. Human experts should be aware of the complexities, as well as how structural modification can alter the reactivity associated with structural alerts, etc.

The SAR evaluation made in the section below is based on *in cerebro* alerts applied by organic chemists.

Depending on the type of reactivity (the **reaction mechanistic domain**), it is sometimes possible to make a quantitative prediction of potency in the LLNA, which can be used to predict potency in humans relative to related known human sensitisers. These predictions use quantitative mechanistic models (**QMMs**) based on reactivity expressed quantitatively

by model parameters, and sometimes in combination with hydrophobicity. For example, potency of aliphatic aldehydes and ketones (the Schiff base domain) in the LLNA is modelled by a combination of reactivity and hydrophobicity (220), whereas the LLNA potency of DNCB analogues (the S_NAr domain) is well modelled by reactivity alone (221).

QMMs aiming not only to predict the potential to be a sensitiser but also to predict the potency, promise to be a useful tool in non-animal based risk assessment for skin sensitisation. However, in the field of fragrance substances there are major gaps in our present ability to apply QSAR/QMM. This is largely because many of the fragrance substances of interest have the potential to act via abiotic or metabolic activation (pre- and/or prohaptens, i.e. they themselves are only weak or non-sensitisers, but have the potential to be activated to form more potent sensitisers. Resulting sensitisation potency will depend on the extent of activation and the nature of the resulting products. It is possible to apply SAR analysis to identify these plausible possibilities, but QSAR modelling for these cases is not yet developed. However, much progress has been made in identifying structural alerts for the various activation mechanisms that have been recognised. This is reviewed by Karlberg et al. (122).

Chemicals with no structural alerts for direct reactivity, or for known activation mechanisms, and no unfamiliar structural features that might be associated with as yet unidentified activation mechanisms, can be predicted to be non-sensitising. Chemicals that do have alerts for reactivity (direct or via activation) are not necessarily sensitisers – they may be insufficiently reactive and/or insufficiently hydrophobic.

Substances meeting the inclusion criteria (see chapter 6), for which, however, no categorisation as established contact allergen in humans or established contact allergen in animals was possible, have been assessed for structural alerts. The results are presented in four tables based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitiser; structural alerts:
Compounds containing structural alerts comprising direct reactive compounds and for compounds that after specific abiotic or metabolic activation (prohaptens and prehaptens) can be predicted to be sensitisers by structural comparison to known allergens.
- Possible sensitiser; structural alerts:
Compounds containing structural alerts that by comparison to known allergens with similar structures were expected to be less reactive and hence less likely to be sensitising. Also compounds with structural alerts indicating a possible abiotic or metabolic activation (possible prehaptens or prohaptens) but with no structural data available for comparison, were included in this group. Consequently, a possible sensitiser may turn out to be a non sensitiser when tested in vivo.
- Predicted non-sensitiser (NS); no obvious structural alerts
- Not predictable due to insufficient/conflicting data

Table 9-1: Predicted sensitisers.

Substance (INCI) name	CAS number	Structural alerts
p-tert.-Butyldihydrocinnamaldehyde ^s	18127-01-0	Schiff base
Citronellal	106-23-0	Schiff base and possible prehapten
Citronellyl nitrile	51566-62-2	Possible prehapten
Decanal	112-31-2	Schiff base
3,7-Dimethyl-1,6-nonadien-3-ol	10339-55-6	Prehapten
Geranyl acetate	105-87-3	Prehapten and prohapten

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Isoamyl salicylate	87-20-7	Acyltransfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehaptent
Nerol	106-25-2	Prehaptent and prohaptent
Nerolidol (isomer not specified)	7212-44-4	Possible prehaptent
Oxacyclohexadecanone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehaptent

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

Substance (INCI) name	CAS number	Structural alerts
Ambrettolide	7779-50-2	Possible prehaptent
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohaptent via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehaptent
Cuminaldehyde	122-03-2	Schiff base and possible prehaptent
alpha-Cyclohexylidene benzeneacetone	10461-98-0	Possible Michael acceptor
Cyclopentadecanone	502-72-7	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
trans-beta-Damascone	23726-91-2	Possible Michael acceptor
trans-trans-delta-Damascone	71048-82-3	Possible Michael acceptor and possible prehaptent
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehaptent
Dihydromyrcenol	18479-58-8	Possible prehaptent
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehaptent and possible prohaptent
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehaptent
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehaptent
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehaptent
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehaptent
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohaptent
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl	54830-99-8	Possible prehaptent

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Substance (INCI) name	CAS number	Structural alerts
acetate		
Hexahydro-methanoindenyl propionate	68912-13-0	Possible prehapten
Ionone isomeric mixture	8013-90-9	Possible Michael acceptor and possible prehapten
alpha-Ionone	127-41-3	Possible Michael acceptor and possible prehapten
beta-Ionone	79-77-6	Possible Michael acceptor
Isobergamate	68683-20-5	Possible prehapten
Isolongifoleneketone	33407-62-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Longifolene [§]	475-20-7	Possible prehapten
Methoxycitronellal	3613-30-7	Schiff base
Methyl decenol	81782-77-6	Possible prehapten
Methyl ionone (mixture of isomers)	1335-46-2	Possible Michael acceptor and possible prehapten
Methylionantheme	55599-63-8	Possible Michael acceptor and possible prehapten
5-Methyl-alpha-ionone	79-69-6	Possible Michael acceptor and possible prehapten
Myrtenol	515-00-4	Possible prehapten
Nopyl acetate	128-51-8	Possible prehapten
Phytol	150-86-7	Possible prehapten and/or prohaptent
Rhodinol	6812-78-8	Possible prehapten
Terpineol acetate (isomer mixture)	8007-35-0	Possible prehapten
alpha-Terpinyl acetate	80-26-2	Possible prehapten
Tricyclodecenyl propionate	17511-60-3	Possible prehapten
Verdyl acetate	2500-83-6/ 5413-60-5	Possible prehapten

Note: [§] Classified as R43.

Table 9-3: Predicted non-sensitisers with no obvious structural alerts.

Substance (INCI) name	CAS number	Structural alerts
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	
Benzyl acetone	2550-26-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
2-tert.-Butylcyclohexyl acetate	88-41-5	
4-tert.-Butylcyclohexyl acetate	32210-23-4	
Cyclohexyl acetate	622-45-7	
Diphenyl ether	101-84-8	

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Substance (INCI) name	CAS number	Structural alerts
Ethyl 2-methylbutyrate	7452-79-1	
Ethylene dodecanioate	54982-83-1	
Ethylene brassylate	105-95-3	
Eucalyptol	470-82-6	
Hexyl acetate	142-92-7	
Hibiscolide	6707-60-4	
Hydroxycitronellol	107-74-4	However, dehydration followed by autoxidation could give sensitising impurities
Isoamyl acetate	123-92-2	
Isobornyl acetate	125-12-2	
Methoxytrimethylheptanol	41890-92-0	
Methyl p-anisate	121-98-2	
Methyl anthranilate	134-20-3	
Methylbenzyl acetate	93-92-5	
Methyl dihydrojasmonate	24851-98-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
Oxalide	1725-01-5	
Pentadecalactone	106-02-5	
Phenethyl acetate	103-45-7	
Phenethyl alcohol	60-12-8	
Phenoxyethyl isobutyrate	103-60-6	
Phenylisohexanol	55066-48-3	
Phenylpropanol	122-97-4	
Tetrahydrolinalool	78-69-3	
Tetrahydro-methyl-methylpropyl)-pyran-4-ol	63500-71-0	
Trimethylhexyl acetate	58430-94-7	
Trimethyl-propylcyclohexanepropanol (tmch)	70788-30-6	
gamma-Undecalactone	104-67-6	

Table 9-4: Not predictable.

Substance (INCI) name	CAS number	Structural alerts
Anisaldehyde	123-11-5	Due to insufficient /conflicting data; structural similarities to benzaldehyde suggest certain activity in man
Trichloromethyl phenyl carbonyl acetate	90-17-5	Due to insufficient /conflicting data
Methyl beta-naphthyl ether	93-04-9	Due to insufficient /conflicting data

9.1. General results

From this work with the included SAR predictions, the following observations can be made.

- For substances for which sufficient experimental/clinical evidence is missing, SAR analyses have been performed to predict a probable or possible risk of allergenic (sensitising) effect. These predictions are based on chemical reactivity and the recognition of structural features in a substance that are in common with the structural features that have been shown to cause sensitisation from other substances. In cases where the SAR analysis indicates a sensitisation potential, the substance should be investigated further to confirm or reject the conclusion drawn from the SAR analysis.
- Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) becomes more complex compared to that of compounds that act as direct haptens without any activation.
- The complexity of the prediction increases further for those compounds that can act both as prehaptens and prohaptens.
- Prediction of the sensitisation potential of compounds that can act as prehaptens is further complicated by the fact that the autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers of linalool and geraniol results in different major haptens/allergens.

9.2. Conclusions

The SAR evaluation made in this section is based on *in cerebro* alerts applied by organic chemists.

- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods.
- SAR, as performed here, is only one consideration in the overall weight of evidence.

10. Exposure

Exposure to fragrance chemicals and other potential allergens is most commonly by direct skin contact. Exposures to fragrance chemicals occur from:

- Personal cosmetic use;
- Detergents and other household products;
- Medicaments;
- Occupation, i.e. personal hygiene, manufacturing ingredient(s), product in work process, plant materials;
- Secondary exposure from another individual (e.g. spouse, child);
- Toys;
- Oral intake;
- Airborne exposure.

Factors that are important for both the induction and elicitation of contact allergy are:

- Dose per unit area;
- Vehicle effects including penetration enhancers;
- Presence of skin irritants;
- Presence of other allergens (combination effects);
- Duration of skin exposure;
- Frequency of applications;
- Anatomical sites of exposure;
- Condition of the skin (barrier function impairment, pre-existing inflammation);
- Occlusion (e.g. in flexures, under clothing and personal protective equipment).

Fragrance mix ingredients are commonly present in cosmetic formulations (71, 222-224). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (225). The clinical significance of exposure to natural extracts is difficult to determine as there is often “hidden and variable” exposure to important and potent allergens in natural products.

10.1. Concentrations and quantities used

Consumers are exposed in daily life to fragrance chemicals from a large variety of products, such as cosmetics, toys, detergents and other cleaning products, etc. The fragrance exposure may be via dermal and/or inhalation route. With respect to “Terms of Reference” to the SCCS, only dermal exposure from cosmetics is addressed in this opinion. As cosmetics are the perfumed products most commonly used in daily life, potential fragrance allergens identified by the use of cosmetics also represent the exposures of these chemicals from other product categories. In recent years, it has become a trend to add fragrance chemicals to many other types of consumer products, such as children’s toys, toilet paper and nappies, which may contribute significantly to the fragrance exposure of the consumer by the dermal route.

Factors for the fragrance exposure assessment by the dermal route require knowledge on:

- Product types (categorisation of scented products) used by the consumer.
- Market survey (impression of the qualitative and quantitative contents of different allergens in consumer products).

- Hydrolysis, metabolism or oxidation of a fragrance material, which may generate a potential skin allergen.
- Chemicals in the product matrix, which may significantly enhance or reduce dermal absorption of a fragrance material.

Fragrance materials, both defined chemical substances and natural mixtures of chemicals (essential oils), are used in all types of cosmetic products: perfumes, eau de cologne, eau de perfume (EDP), and eau de toilette (EDT), aftershave lotion, deodorants, skin care products, skin cleansers, make-up cosmetics, hair care products, and oral care products, etc. However, some unscented cosmetic products have also reached the market in the last decade. Products containing the highest concentration of fragrance chemicals are perfumes, followed by eau de cologne, eau de perfume (EDP) and eau de toilette (EDT). Concentrations of fragrance chemicals in deodorant products are lower than those in EDT/EDP products, but still significant. Aftershave products also contain relatively high amounts of fragrance chemicals. Other cosmetic products contain relatively low amounts, 0.1-1% of fragrance compound, compared to up to 30% fragrance compound in EDT/EDP (226). The fragrance compound are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (226). For the exposure assessment, levels of fragrance chemicals in cosmetics containing significant amounts of fragrance materials (i.e. EDP/EDT/aftershave/deodorant) should be selected. It may not be possible to detect/measure the amounts of all fragrance chemicals when present in highly diluted form in a cosmetic product such as skin care products, make-up cosmetics etc. On the other hand, if a fragrance is evaluated safe for use when present in significant amounts in a product, it will also be safe for use in other products. Also the analysis of trend of the use of individual fragrance materials should be based on monitoring their contents in fine perfumes and deodorants.

Ninety of the 100 fragrance materials used in annual volumes > 175 tons in perfume formulations are fragrances and the remaining ten are used for other functions such as solvents or antioxidants (IFRA, personal communication 2010).

Among the 26 fragrances currently requiring individual labelling, amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone, and linalool are used in volumes greater than 175 ton. α -Amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri*), tree moss (*Evernia furfuracea*) are used in volumes less than 175 ton.

According to the information from the fragrance industry, 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products.

Since the implementation of the regulation of labelling of 26 fragrance substances in cosmetic products, qualitative information on fragrance exposure from cosmetics is provided in some market surveys performed on cosmetics (Table 10-1, (227)) and (Table 10-2, (228)) and on consumer products including cosmetics (Table 10-3, (229); Table 10-4, (115); and Figure 10-1, (105)). Thus, the implementation of the regulation of fragrance allergens in detergents (Directive 648/2004/EC), similar to that for cosmetics, has also added to the knowledge of fragrance exposure to the consumer. These market surveys revealed that fragrance ingredients which are potent allergens and frequently cause allergies in consumers are used as ingredients in consumer products including cosmetics. The results of these surveys further revealed that limonene and linalool were the most commonly used fragrance chemicals in cosmetics, while anisyl alcohol, cinnamal, α -amylcinnamyl alcohol, oak moss and tree moss were the least used fragrance ingredients in cosmetics and other consumer products. In general, the most potent allergens were also the most infrequently used ingredients. Prior to the regulation of the 26 allergens, analysis of 21 selected fragrance chemicals in deodorants also revealed additional 66 potential allergens in these products on the basis of structure activity relationship (230).

Table 10-1: Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (227).

Fragrance substance		% Products labelled to contain the fragrance substance
INCI name	CAS number	
Amyl cinnamal	122-40-7	8.2
alpha-Amylcinnamyl alcohol	101-85-9	2.9
Anise alcohol	105-13-5	0
Benzyl alcohol	100-51-6	9.6
Benzyl benzoate	120-51-4	9.1
Benzyl cinnamate	103-41-3	2.9
Benzyl salicylate	118-58-1	9.6
Butyl phenyl methyl propional	80-54-6	7.7
Cinnamal	104-55-2	1
Cinnamyl alcohol	104-54-1	6.7
Citral	5392-40-5	8.2
Citronellol	106-22-9	10.5
Coumarin	91-64-5	4.8
Eugenol	97-53-0	7.2
Farnesol	4602-84-0	2.9
Geraniol	106-24-1	12
Hexyl cinnamal	101-86-0	10.1
Hydroxycitronellal	107-75-5	6.3
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	31906-04-4	5.8
Isoeugenol	97-54-1	0.5
Alpha-isomethyl ionone	127-51-5	5.8
<i>d</i> -Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
<i>Evernia prunastri</i> /oak moss	90028-68-5	0
<i>Evernia furfuracea</i> /tree moss	90028-67-4	0

Opinion on fragrance allergens in cosmetic products

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

Opinion on fragrance allergens in cosmetic products

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
Hexyl cinnamal [□]	101-86-0	33.0	48	1-4434	71	2-1684
Hydroxycitronellal [■]	107-75-5	27.3	70	1-1746	50	1-1023
HICC [□]	31906-04-4	33.0	74	1-4431	53	1-1874
Isoeugenol [■]	97-54-1	9.1	35	1-138	29	1-458
Alpha-isomethyl ionone	127-51-5	46.6	65	6-2588	61	1-2765
D-Limonene [°]	5989-27-5	53.4	70	1022-11386	n.a.	n.a.
Linalool [°]	78-70-6	53.4	96	8-3447	97	9-1927
Methyl-2-octynoat [°]	111-12-6	1.1	-	-	n.a.	n.a.
<i>Evernia prunastri</i> [■] /oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
<i>Evernia furfuracea</i> [■] /tree moss	90028-67-4	2.3	n.a.	n.a.	n.a.	n.a.

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.

- Fragrance not detected in any product.

n.a. Not analysed.

* Benzyl alcohol could not be determined in 49% of the products due to interference.

The most common fragrance allergens are contained in the two mixtures, which are used for diagnosing fragrance allergy, called Fragrance Mix I (■) and Fragrance Mix II (°), besides the oxidation product of terpens (°), and tree moss extract are common allergens. Methyl-2-octynoate is an extreme, but rare allergen.

Opinion on fragrance allergens in cosmetic products

Table 10-3: Frequency of occurrence in consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (229).

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyr TM	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evermia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evermia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0.3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

INCI, International Nomenclature of Cosmetic Ingredients; PCP, personal care products; MP, men's products; HP, household products; WP, women's perfumes; Cos, cosmetics; Deo, deodorants; Dent, dental products; BPMP, butyl phenyl methyl propional; LyrTM, 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
Citronellol	21.1
Benzyl salicylate	18.6
Coumarin	17.0
Eugenol	15.7
Benzyl alcohol	15.3
Benzyl benzoate	14.7
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	12.8

Opinion on fragrance allergens in cosmetic products

Fragrance substance INCI name	% Product containing the chemical
Citral	11.6
Hydroxycitronellal	10.8
Amyl Cinnamal	7.9
Anise alcohol	7.0
Cinnamyl alcohol	6.4
Farnesol	3.9
Isoeugenol	3.1
Cinnamal	2.5
Benzyl cinnamate	2.3
Amylcinnamyl alcohol	1.9
Methyl-2-octynoate	1.0
<i>Evernia prunastri</i> -oak moss	0.8
<i>Evernia furfuracea</i> -tree moss	0.4

Note: * Consumer Products: Cosmetics and household products with labelling of the 26 fragrance allergens. The content of these fragrances was confirmed by chemical analysis.

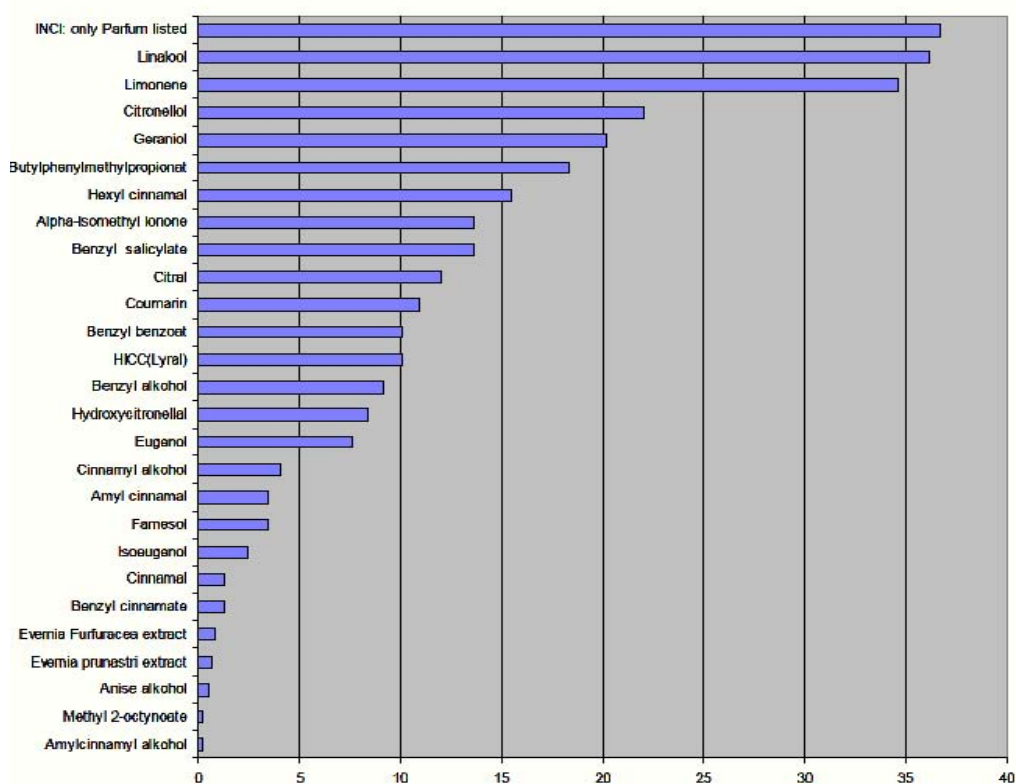


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

Contents of fragrance substances determined in cosmetic products have been described in several studies, both before and after the regulation of the 26 fragrance allergens. The studies prior to the regulation of the 26 fragrance allergens included many, but not all of these 26 allergens. On the other hand, these studies included some other possible fragrance allergens. The quantitative analysis of fragrance substances has been performed in prestige perfumes (5, 157, 232-234), deodorants (228, 231), children's cosmetics and cosmetic toys (115, 227, 235), products marketed as natural cosmetics (225) and in cosmetics used by patients with contact allergy to fragranced products (35, 71). Quantitative analyses have revealed that the consumer is exposed to most, but not all of the 26 fragrance allergens from the use of cosmetics. However, when fragrance exposure from other consumer products, for example detergents and other household products is also taken into consideration (Table 10-3, Table 10-4, Figure 10-1), (105, 115, 229, 236), exposure to all of the 26 allergens is foreseeable in daily life. Although from the data available, the exposure to α -amylcinnamyl alcohol, cinnamal, methyl-2-octynoate, *Evernia prunastri* (oak moss) and tree moss may appear to be low, these are very strong allergens.

The changes in the use of fragrance chemicals in cosmetic formulations, during last 12 years, i.e. before and after the regulation of the 26 fragrance allergens, is reflected in the studies concerning contents of fragrances substances in popular perfumes (5, 232). As described in Table 10-5, the content of FM I allergens in prestige perfumes was significantly reduced from 1996 to 2003. Whether this is also the case for the perfumes sold as natural cosmetics (Table 10-6) has not yet been investigated.

Table 10-5: Concentration of Fragrance Mix I ingredients in five prestige perfumes before and after the regulation of the 26 fragrance allergens.

Fragrance INCI name	Concentration in the perfumes before regulation (5)			Concentration in the perfumes after regulation (232)		
	In no. of perfumes	Range % (w/w)	Mean % (w/w)	In no. of perfumes	Range % (w/w)	Mean % (w/w)
Geraniol*	5	0.072- 0.432	0.340	5	0.090- 0.236	0.156
Cinnamal	2	0.002- 0.002	0.002	0	-	-
Hydroxy- citronellal	5	0.222- 0.979	0.615	5	0.015- 0.478	0.169
Cinnamyl alcohol	4	0.068- 0.232	0.147	0	-	-
Eugenol	5	0.032- 0.738	0.337	2	0.001, 0.001	0.001
Isoeugenol	3	0.026- 0.249	0.119	2	0.001, 0.004	0.003
Amyl cinnamal	1	0.019	0.019	0	-	-

Note: * Due to interference by linalyl acetate, concentration of geraniol+linalyl acetate is reported.

Table 10-6: Concentrations of Fragrance Mix I ingredients, hexyl cinnamal and coumarin in 22 perfumes marketed as natural cosmetics investigated in 1996.

Fragrance	In no. of perfumes	Concentration % (w/w)
Geraniol	14	1.191*
Cinnamal	3	0.089, 0.109, 2.101
Hydroxycitronellal	5	0.135-6.044
Cinnamyl alcohol	8	0.035-2.289
Eugenol	2	0.027, 0.139
Isoeugenol	8	0.194-3.039
Amyl cinnamal	9	0.105-7.706
Coumarin	11	0.046-6.043

Note: * Quantification was performed in one sample only, due to interference by a very large amount of linalyl acetate in other samples.

The trend in the use of most of the fragrance allergens in deodorants before and after their regulation is reflected by the two studies performed by Rastogi et al. (228, 231). The results of these studies cannot be directly compared, because the study from 1998 included randomly selected deodorants, while selection of the deodorants for the 2007 study was based on the labelling of the presence of known strong fragrance allergens in these products. The number of products analysed in the 1998 study were three times more than those analysed in 2007, but not all of the 26 fragrance allergens were analysed in the 1997 study. However, an indication of the change in the use of the fragrance allergens during 1998-2007 may be obtained by reviewing the results of these two studies. Among the 17 common fragrance substances studied in the two studies, the frequency of use of 16 of these substances in deodorants was reduced in 2007 compared to that in 1998 (Table 10-2). The frequency of use of butyl phenyl methyl propional in deodorants appeared to be unchanged. The contents of benzyl alcohol, benzyl salicylate, cinnamal, cinnamyl alcohol, eugenol, geraniol, isoeugenol and linalool were found to be lower in the deodorants analysed in 2007 compared to those in 1998. Citronellol, coumarin and alpha-isomethylionone contents in the deodorants were similar in both studies, but concentrations of benzyl benzoate, butyl phenyl methyl propional, hexyl cinnamal, hydroxyisohexyl-3-cyclohexene carboxyaldehyde and linalool were much higher in deodorants in 2007 compared to those in 1998. This analysis of trend of use of fragrance allergens in cosmetic products indicates that the regulated fragrance allergens are used less frequently, but exposures from some of the regulated fragrance allergens may be much higher compared to those before regulation.

Table 10-7: Atranol and chloroatranol content in eau de toilette/eau de perfume, investigated in 2004 and in 2007.

	2007 Study	2004 Study
No. of samples	22	17
Atranol present in no. of samples	15 (68%)	12 (70%)
Atranol content	ppb (ng/ml)	ppb (ng/ml)
Range	n.d.-880	n.d.-791
Mean±SD	157±249	97±224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Atranol content	ppb (ng/ml)	Ppb (ng/ml)
Range	0.9-208	1-175
Mean±SD	63±73	36±51
Median	22	10

Notes: n.d. Not detected.

* $P < 0.05$ (chi-square test).

SD: Standard deviation.

Atranol (CAS no. 526-37-4) and chloroatranol (CAS no. 57074-21-2), constituents of oak moss and tree moss have been shown to be very potent fragrance allergens (237, 238). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (239). Two other commonly used fragrance chemicals, isoeugenol (240) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (71), have also been shown to be important contact allergens. The contents of atranol, chloroatranol, isoeugenol and hydroxyisohexyl-3-cyclohexene carboxyaldehyde in fine fragrances was determined for the exposure assessment of these fragrances (233). The results revealed that isoeugenol was present in 56%, HICC in 72%, atranol in 59%, and chloroatranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below 0.02% which is the maximum concentration recommended by the fragrance industry. HICC reached a maximum concentration of 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the EC Scientific Committee (241). The concentrations of atranol and chloroatranol in the products investigated in 2007 were comparable to those found in similar products in 2004 (Table 10-7, (233, 234). A significant decrease in the frequency of the presence of chloroatranol in the products was found in 2007 (Table 10-7).

10.2. Global exposure (household and occupational exposures)

Fragrances are used in cosmetics that the consumer applies to themselves, as described in the previous section. In addition, exposure to fragrance substances is possible by a number of other exposure routes briefly outlined in this section.

Topical pharmaceutical products

In a study from Belgium, 370 of the 3,280 topical products marketed in Belgium have been found to contain one or more of 66 fragrance substances (242). This publication also contains a description of causative fragrance allergens in 127 patients reacting to 48 specific topical products. In a broader sense, exposure of the patient by extracts used in aromatherapy falls in this category as well.

Childrens products and toys

Children's products may contain fragrance allergens and high levels may be present (235). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (243).

Clothing

Washed fabrics have been reported to contain fragrances (244). Odour-neutralising agents are sometimes used for shoe insoles. In one case, an insole containing cinnamon, has been reported to lead to plantar vesicular contact dermatitis due to contact sensitisation to FM I and, in the breakdown, to cinnamal and cinnamyl alcohol (245).

Cleaning agents and other household products

Contact dermatitis from geraniol in washing-up liquid has been reported (246). Terpenes are used as solvents and cleansing agents (e.g. limonene) (247) and have been reported as cause of hand dermatitis (248, 249). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (250). In a review of 301 cosmetic and detergent consumer products in Sweden, in half of the cosmetics and one-third of the detergents, one or more of the 26 fragrances requiring labelling were identified (251). In the UK, a review of 300 consumer products showed that linalool and limonene were present in 63% of products. Dental products contained on average 1.1 fragrance substances that are presently required to be labelled and women's perfumes contained 12 of these fragrance substances (Table 4-1 and Table 4-3) (229).

Candles

The dermal hand transfer of three fragrance materials (cinnamic aldehyde, d-limonene and eugenol) from scented candles was determined in ten subjects (i.e. 20 hands) after grasping scented candles for five consecutive 20 second exposures/grasps. The total mean residues of cinnamal and eugenol transferred per grasp from the candles to the hands were 0.255 µg/cm(2) and 0.279 µg/cm(2), respectively (252).

Food

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (253)) or lichen planus-like lesions (e.g. due to cinnamal (254)) or contact gingivitis (e.g. due to eugenol (255)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (256) may cause allergic contact dermatitis by handling this food.

Occupational exposure

In a number of occupations, contact allergy to fragrances is more common than in others, including geriatric nurses, masseurs and physiotherapists, metal furnace operators and potters/glass makers, according to a multifactorial analysis (90). Moreover, hairdressers, beauty therapists and aroma therapists are examples of occupations where there is occupational exposure to fragrance-containing cosmetic and other products. Cleaners are exposed to fragrance-containing household products (e.g. detergents). Cooks and bakers are exposed to flavour chemicals and spices. Healthcare workers are also at risk of acquiring fragrance contact allergy. "Odour maskers" may contain important fragrance allergens (89, 90, 257-259). Occupational exposure and

occupational ACD to fragrances have been described in perfume bottlers (260). Industrial use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (259).

A number of fragrance chemicals are also used as biocides (see Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, published 11.12.2007 EN Official Journal of the European Union L 325/3 –L325/65), see Table 10-8 below.

Table 10-8: Parts of Annex I to (EC) No 1451/2007 (see above): "Active substances identified as existing", if use is 'perfuming' or 'masking' according to CosIng.

Biocide	EINECS	CAS number	Biocidal product group
Linalool	201-134-4	78-70-6	19
Geraniol	203-377-1	106-24-1	18, 19
Benzyl benzoate	204-402-9	120-51-4	2, 18
Eugenol	202-589-1	97-53-0	Not given
Farnesol	225-004-1	4602-84-0	Not given
(R)-p-mentha-1,8-diene	227-813-5	5989-27-5	12
Citriodiol/mixture of cis- and trans-p-menthane-3,8 diol	255-953-7	42822-86-6	1, 2, 19
Citral	226-394-6	5392-40-5	Not given
Pine ext.	304-455-9	94266-48-5	10
TANACETUM CINERARIIFOLIUM FLOWER EXTRACT	289-699-3	89997-63-7	18
Citrus oils (main component: limonene)	several	various	
Clove oil (main component: eugenol (83.8 %), caryophyllene (12.4 %))	/	8000-34-8	

Product groups(According to Biocide Directive 98/8/EC)

- 1 Human hygiene biocidal products
- 2 Private area and public health area disinfectants and other biocidal products
- 3 Veterinary hygiene biocidal products
- 10 Masonry preservatives
- 12 Slimicides
- 18 Insecticides, acaricides and products to control other arthropods
- 19 Repellents and attractants

The above illustrates that the consumer is exposed to fragrance substances from a wide variety of cosmetic products, other consumer products, pharmaceuticals and occupational exposures.

All these exposures are of importance in the context of contact allergy as it is not the source of exposure that is critical for both induction and elicitation, but the cumulative dose per unit area.

10.3. Exposures related to particular anatomical sites

Contact allergy to fragrances most often causes dermatitis of the hands, face and axillae. Axillary involvement has been shown to be statistically related to fragrance allergy (9). It is recognised that the axillary skin is a problematic area as it is moist, occluded and is easily irritated. Moreover, facial eczema is a common manifestation of fragrance allergy (3, 47). There is an association between fragrance allergy and hand eczema or aggravation of hand eczema (13-15). Vehicles may influence elicitation capacity of an allergen and the presence of detergents (surfactants) as in hand cleaning products may increase the clinical response by a factor of 4-6 (261). Men using wet shaving as opposed to electric razors have an increased risk of being fragrance allergic (17), most likely due to microtraumata and to the presence of surface active substances in shaving foam.

In use tests, the upper arm has been shown to be more sensitive than the forehead and lower arm (262). The axillae, neck and face are more sensitive than the upper arms (10). The threshold of elicitation may vary depending on the volatility of the substance (263). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (264).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (74). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (265). Following this, eczema may appear, or be worsened by, the use of a variety of product types including other cosmetics, household products, industrial products and flavours.

The association between contact allergy to fragrance ingredients and certain anatomical sites, which mirrors exposure to fragrance-containing products on these anatomical sites, has been described in several publications (266, 267), see above. However, due to the potential confounding effect of other factors, at least on some anatomical sites, an adjusted analysis will provide a more valid impression of the association between certain anatomical sites and contact allergy to fragrance ingredients. As an adjusted, multifactorial analysis relies on: (i) a substantial number of observations (patients tested); and (ii) an outcome prevalence not too close to 0%, such an approach has, hitherto, been limited to FM I.

In a paper published 2001, data from the IVDK in terms of patch test reactions to FM I and relevant clinical and demographic information of the patients tested (n=57,779) was studied by Poisson regression analysis (90). Risk was quantified by the prevalence ratio, which can be interpreted as an estimate of relative risk, i.e. the factor by which the risk of being sensitised to FM I (in this example) is to be multiplied (RR > 1: elevated risk; or RR < 1: reduced risk) if a certain "risk factor" is present, compared to those patients in whom this risk factor is not present (the reference category) (general aspects of such analyses are discussed in (268)). In the analysis, potential risk factors and confounders, respectively, including occupation, year of patch testing (to address a possible time trend), sex, age, past or current atopic dermatitis, in addition to anatomical site. The relevant part of Table 3 of (90) is reproduced below.

Table 10-9: Result of a Poisson regression analysis of patients tested with the Fragrance Mix between January 1992 and December 1998, considering two alternative outcomes – part I: non-occupational factors

Attribute	Prevalence (%)	At least + (11.5%)		At least ++ (4.0%)	
		PR	95% CI	PR	95% CI
Age:					
≤30	26.7	1.00	Reference	1.00	Reference
>30–44	23.8	1.42	1.31 to 1.53	1.61	1.40 to 1.84
>44–58	25.6	1.67	1.55 to 1.80	1.90	1.66 to 2.16
>58	23.9	1.93	1.77 to 2.10	2.07	1.79 to 2.39
Sex (female)	64.5	1.29	1.21 to 1.37	1.18	1.07 to 1.31
Main site:*					
Trunk	2.9	1.00	Reference	1.00	Reference
Hands	29.9	1.24	1.07 to 1.46	1.28	0.98 to 1.67
Arm	3.8	1.23	1.01 to 1.49	1.19	0.86 to 1.65
Face	15.2	1.20	1.03 to 1.42	1.13	0.86 to 1.48
Neck	1.4	1.39	1.10 to 1.75	1.31	0.88 to 1.94
Feet	2.8	1.26	1.02 to 1.55	1.19	0.84 to 1.68
Leg	8.7	1.59	1.36 to 1.89	1.50	1.14 to 1.99
Axilla	0.9	2.77	2.20 to 3.46	2.73	1.87 to 4.00
Other site	8.9	0.66	0.55 to 0.80	0.48	0.35 to 0.67

*Additionally controlled for several more sites—none of these associated with a significantly increased or decreased risk.

Compared to the trunk, which was arbitrarily chosen as the reference category, all other anatomical sites are associated with an increased risk of being sensitised to FM I (significantly if the lower limit of 95% CI is > 1). Most evidently, dermatitis of the axilla(e) is strongly associated with contact allergy to FM I, presumably due to the application of deodorants. Furthermore, the part of the table shown above illustrates a strong, positive age gradient, i.e. the older patients are, the more likely they are to be sensitised to FM I, the risk being almost double when comparing the oldest with the youngest age group. This observation is in concordance with a bivariate (unadjusted) association between age and contact allergy to FM I found in another study (89). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (269): (i) an even stronger age gradient; and (ii) no particular association to axillary dermatitis (included in the “other” category) was found (Table 10-10).

Table 10-10: Association between selected risk factors and positive patch test to *Myroxylon pereirae* resin. For full model see (269). Risk quantified with the prevalence ratio (PR) with accompanying 95% confidence interval (CI).

Factor	PR	95% CI
Atopic dermatitis, past or present	1.02	(0.95-1.10)
Female sex	1.13	(1.06-1.20)
Site		
Trunk	1.00	(reference)
Hand or Arm	1.03	(0.94-1.12)
Foot or Leg	1.76	(1.61-1.92)
Head or Neck	0.94	(0.86-1.03)
“Other” site	0.72	(0.64-0.81)
Missing site	1.07	(0.97-1.19)
Age		
30 years and younger	1.00	(reference)
31 to 44	1.92	(1.73-2.12)
45 to 58	2.87	(2.61-3.16)
58 or older	3.85	(3.49-4.25)

10.4. Conclusion

There are various modes of exposure to fragrances, including not only products used for their scent, such as perfumes and eau de toilette, after shaves, and deodorants, but also types of products where scent is an added feature, such as other cosmetic categories (including wipes), topical pharmaceuticals, household products, and products encountered in the occupational setting.

Consumer exposure can change over time, both qualitatively and quantitatively.

Different routes of exposure are reflected by certain anatomical sites affected: deodorants are associated with axillary dermatitis, the axillary skin being particularly vulnerable to sensitisation due to occlusion, maceration and irritation. However, while sensitisation and initial disease may follow a distinct pattern, later less specific exposures, e.g. via hand creams, cleaning lotions etc. may be sufficient to cause allergic contact dermatitis.

11. Dose-response relationships and thresholds

The dose-response relationship between exposure to contact allergens and induction of allergy, i.e. sensitisation, is well established in animal models and by experiments in healthy volunteers (270). It seems that not only the dose per unit area of allergen (271), but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (272). The induction of contact allergy is an immunological process (type IV-allergy), which is without any clinical symptoms. In the case of continued exposure or re-exposure with a sufficient dose of allergen, elicitation will occur. Elicitation is an inflammatory response (eczema) with clinical symptoms of erythema, induration and in some cases vesicles. Studies of the elicitation response are normally done in patients with an allergy to the substance in question. Different provocation models exist (see chapter 11.2.1). Elicitation experiments in healthy human volunteers following the induction have only rarely been performed (273) and may be considered a less valid model than patient studies. The reason is that following experimental induction, the level of sensitivity may not be at the same level as in a real life situation and that individuals who have actually acquired the disease are a more relevant endpoint to study.

Knowledge of the dose-response relationship provides an opportunity to establish levels of exposure which are safe for the majority of individuals. In the following chapter, the use of different data and models for the establishment of such safe levels in relation to fragrance ingredients are explored. The focus will be on those chemicals, which have been identified in chapter 7.1 as established contact allergens in humans and which have already given rise to a significant number of published cases (category 3 or more): cinnamal, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, isoeugenol. Limonene and linalool are considered in chapter 5 as their ability to cause sensitisation depends on air oxidation, and hydroxyisohexyl 3-cyclohexene carboxaldehyde is considered in chapter 4.3.2 and 11.4.

11.1. Induction

A model for dermal sensitisation quantitative risk assessment (QRA) has been developed and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments, mainly the local lymph node assay (LLNA) (see chapter 8.1). A set of safety factors are applied for inter-individual differences, for vehicle effects and for use considerations, stated to give rise to a safety margin from 10 to 1000 (274). In this way, a so-called “acceptable exposure level” is derived. The exposure to an allergen in different types of products should be below this level. The restrictions, which have been introduced by the fragrance industry based on the QRA model, are given in Table 11-1 for some important product categories.

The IFRA guidelines give concentration limits for 11 product categories (http://www.ifraorg.org/en-us/standards_1, last accessed 2011-11-02), three of which are mentioned in Table 11-1. These three products have the lowest concentrations except for lip products, which give a slightly lower concentration limit.

Table 11-1: Current IFRA restrictions based on induction experiments.

Fragrance chemicals	IFRA guideline ¹		
	Deodorant (%)	Hand cream (%)	Perfume (%)
Cinnamal	0.02	0.05	0.05
Cinnamyl alcohol	0.1	0.4	0.4
Citral	0.05	0.3	0.6
Coumarin	0.13	0.8	1.6
Eugenol	0.2	0.5	0.5
Farnesol	0.11	0.6	1.2
Geraniol	0.4	2.8	5.3
Hydroxycitronellal ²	0.2	1.0	1.0
Isoeugenol ²	0.01	0.02	0.02

Notes: 1) Exposure per mg/cm²/day is based on 8.5 mg/cm²/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.
 2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

The SCCP evaluated this methodology (275) as well as its application to three model fragrance substances.

It was, among other things, concluded that:

“The data provided show that the application of the dermal sensitisation QRA approach would allow increased exposures to allergens already known to cause allergic contact dermatitis in consumers. The model has not been validated and no strategy of validation has been suggested. There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer.”

and that:

“Identification of safe levels of exposure to existing substances known to cause allergic contact dermatitis in the consumer should be based on clinical data and/or elicitation low-effect levels. Currently, these are the only methods which have proven efficient in reducing/preventing existing problems of sensitisation/allergic contact dermatitis in the consumer.”

11.2. Elicitation

11.2.1. General considerations

A response in terms of elicitation of allergic contact dermatitis by application of the (suspected) allergen under standardised conditions is the outcome of interest of the routine diagnostic procedure for suspected contact allergy, the patch test. While the patch test procedure is largely standardised, it is optimised as a diagnostic tool for contact allergy. Thus exposure conditions are not comparable to actual exposures occurring in the daily life or working environment of the patient, which often involve long-term, repeated and low-dose contact with the allergen. Here, procedures such as the repeated open application test (ROAT) or provocative use test are often used, because they reflect actual exposure much better and can be used, for instance, to validate the current clinical relevance of a positive PT reaction.

Generally, exposure of a sensitised patient to a set of graded doses (quantity/area) of the suspected allergen, i.e. threshold testing, will allow not only quantitative diagnosis of the presence or absence of specific contact sensitisation but will additionally provide evidence on the intensity (degree) of sensitisation. This may have important individual consequences in terms of everyday or occupational exposures being capable (or not) of eliciting allergic contact dermatitis. However, beyond the individual perspective, clinical dose-response data collected from sensitised individuals provide a valuable estimate of the usual doses/unit area resulting in a positive, allergic response in a certain proportion of sensitised persons, e.g. 10, 50 or 90%. Maximum concentration levels can be derived, which are safe in terms of eliciting allergic reactions in only a defined low percentage of sensitised persons. As such data will always be based on small samples, the precision of the estimate should be considered, and therefore results are preferably given with confidence intervals.

A statistically significant relationship between threshold concentrations in the ROAT and patch test has been found, on analysing results from different allergens (see Table 11-2) (276), but the dose of allergen per unit area per application needed to elicit a reaction in the two study methods is not the same. A translation factor between the two methods has been suggested for non-volatile substances: $ED_{xx}(ROAT) = 0.0296 \cdot ED_{xx}(\text{patch test})$ based on testing nickel and methyl dibromo glutaronitrile (276). Based on this the eliciting dose per application in an open test is 33 times lower than in the patch test. In practice it means that the cumulative dose in a ROAT (in $\mu\text{g}/\text{cm}^2$) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in $\mu\text{g}/\text{cm}^2$) for a given number of responders (see Figure 11-1). For a given cut-off point the elicitation dose determined by patch testing will be higher than determined by ROATs.

Table 11-2: Spearman's rank correlation between the threshold concentration in the patch test and the repeated open application test for three allergens.

Allergen	Number of patients	Correlation coefficient	P-value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011

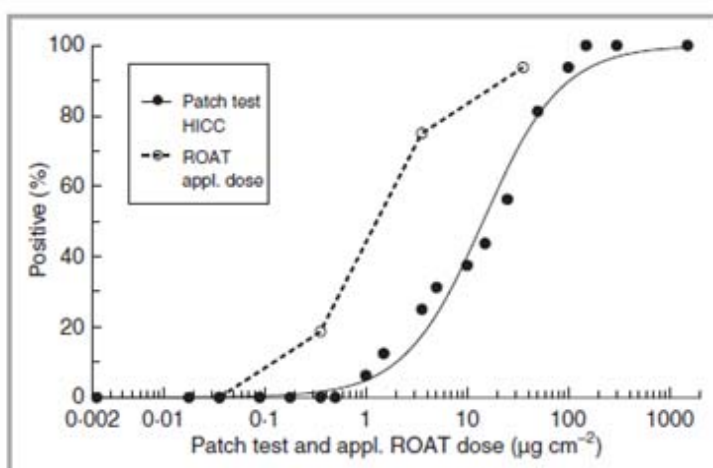


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded application as in the patch test.

In the translation between methods, evaporation needs to be taken into consideration for volatile substances. The experience, based on a study of the fragrance ingredient HICC and using the results from the literature on isoeugenol, is that if the same equation is used as for non-volatile substances, the response in the ROAT will be overestimated by a factor 3 to 4. Thus, the translation factor would be 0.1060 instead of 0.0296, but this needs to be confirmed by other fragrance allergens. This implies that for the fragrance ingredients tested, the eliciting dose per application in a ROAT was 9.4 times lower than the patch test compared to a 33 times lower dose for non-volatile substances (276). This needs to be confirmed by studying other fragrance allergens. Thus, according to these experiments, the dose ($\mu\text{g}/\text{cm}^2$) eliciting a response in threshold patch testing will be at most 33 times higher than established in the ROAT if an identical vehicle is used.

Volatility effects in skin sensitisation

The potency of volatile skin sensitisers can be underestimated, to an extent depending on how rapidly it evaporates, by assays such as the LLNA in which the test substance is applied topically to exposed healthy skin without occlusion. Such sensitisers present a greater sensitisation risk to consumers when the skin is occluded by clothing and/or compromised, than when healthy non-occluded skin is exposed.

Volatility at physiological temperature, say 40°C , is represented by the vapour pressure p_{40} at that temperature. This is related to the boiling point T_B by the Clapeyron-Clausius equation, which can be written (277):

$$\text{Log}(p_{40}) = - (T_B - 40)\text{Tr}/2.303RT$$

Where p is in atmospheres, T_B is in $^\circ\text{C}$, R is the gas constant, Tr is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to $22 \text{ cal}\cdot\text{deg}^{-1}$ for many organic compounds) and T is physiological temperature in degrees absolute ($= 313$ for 40°C).

It has been shown, in experiments where evaporation from a glass slide is measured under simulated LLNA conditions, that 2-hexenal ($T_B = 146\text{-}149^\circ\text{C}$, $p_{40} = 17 \text{ mmHg}$) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal ($T_B = 248^\circ\text{C}$, $p_{40} = 0.5 \text{ mmHg}$), more than 90% remains after 1 hour (278). In agreement with these findings, cinnamal fits a QSAR relating LLNA EC3 to reactivity, whereas the EC3 for 2-hexenal is higher (lower potency) than predicted from its reactivity.

The above is only a partial rationalisation, since different solubilities in different vehicles will influence the tendency to evaporate, according to Henry's law.

11.2.2. Studies on specific fragrance ingredients

Studies concerning chloroatranol/atranol, cinnamal, hydroxycitronellal, hydroxyisohexyl 3-cyclohexenecarboxaldehyde and isoeugenol have been identified. These are summarised in Annex III.

Overview of results

In four studies dummy deodorants spiked with a single fragrance allergen in realistic use concentrations have been used to study elicitation responses, unscented deodorants were used as control products in paired designs. The deodorants were used by patients sensitised to the fragrance allergen in question as well as a healthy control group

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(without fragrance allergy) (102,103,104,279). Between 76 and 100% of the sensitised individuals reacted to the deodorants spiked with allergen, isoeugenol, cinnamal, hydroxycitronellal and hydroxyisohexyl 3-cyclohexene carboxaldehyde, and none of the controls (Table 11-4).

Table 11-3: Overview of results of deodorant provocation investigations with different allergens. Frequency in % of test groups, which reacted at different doses of allergen applied in a roll-on deodorant in the axilla, is given in the table.

Dose in ppm in deodorant	Isoeugenol	Cinnamal (1)	Cinnamal (2)	Hydroxycitronellal	HICC
0	0	0	0	0	0
63	23				
100			11		
200	69				64
320		25	55	57	
600					85
630	76				
1000		75	88	71	
1800					100
3200		100		100	
No. test persons	13	8	9	7	14
No. of control persons	10	20		7	10
% control persons, who reacted	0	0		0	0
Exposure according to study should be:	< 63 ppm	<100 ppm		<320 ppm	< 200 ppm
Reference	(279)	(103)		(104)	(102)

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Eleven studies concerning dose-response results of the five allergens listed above were identified, including the above mentioned studies of deodorants. An overview of the results of the studies concerning thresholds is given in Table 11-4. In Annex III the details of each study are given.

Table 11-4: Overview of threshold results from clinical studies.

“Observed” means that the proportion was actually observed in the study while “estimated” means that the value is derived from a fitted curve, i.e. is interpolated.

Chloroatranol			
ROAT			Ref.
In ethanol 92 % positive	0.025 µg/cm ²	observed	(238)
In ethanol 100% positive	0.125 µg/cm ²	observed	(238)
PATCH TEST			

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ED10%	0.0004 µg/cm ²	estimated	(238)
ED50%	0.0045 µg/cm ²	estimated	(238)
Cinnamal			
ROAT			
In ethanol no effect	0.02%	observed	(101)
In ethanol 44 % positive	0.1%	observed	(101)
In ethanol 72 % positive	0.8%	observed	(101)
Deodorant matrix 11% positive	0.26 µg/cm ² (0.01%)	observed	(103)
Deodorant matrix 41% positive	0.84 µg/cm ² (0.032%)	observed	(103)
Deodorant matrix 82% positive	2.63 µg/cm ² (0.1%)	observed	(103)
PATCH TEST			
ED50%	96 µg/cm ²	estimated	(101)
No effect level	0.4 µg/cm ² (0.01%)	observed	(101)
No effect level	NG (0.002%)	observed	(103)
HICC			
ROAT			
In a cream base ED10%	4.9 µg/cm ²	interpolated	(105)
In a perfume (ethanol) ED10%	1.2 µg/cm ²	interpolated	(105)
In ethanol 61% positive	15.3 µg/cm ² (3.4-22.2)	observed	(224)
In ethanol 89% positive	126.2 µg/cm ² (40.5-226.2)	observed	(224)
In ethanol/water no response	0.0357 µg/cm ²	observed	(263)
In ethanol/water ED10%	0.064 µg/cm ²	estimated	(263)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm ² (median)	observed	(102)
PATCH TEST			
ED10% (95% CI)	0.662 µg/cm ² (0.052-2.35)	estimated	(263)
ED10%	0.75 µg/cm ²	estimated	(102)
ED10%	0.9 µg/cm ² 29 (7-69) ppm	estimated	(224)
ED50% (95% CI)	11.1 µg/cm ² (3.41- 33.1)	estimated	(263)
ED50% (95% CI)	18.3 µg/cm ² (3.41- 33.1)	estimated	(102)
ED50% (95% CI)	20 µg/cm ² 662 (350-1250) ppm	estimated	(224)
No effect level	<0.0022 µg/cm ²	observed	(263)
Hydroxycitronellal			
ROAT			
Deodorant matrix 57 % positive	0.94 µg/cm ² (0.032%)	observed	(104)
Deodorant matrix 71 % positive	2.94 µg/cm ² (0.1%)	observed	(104)
Deodorant matrix 100 % positive	9.40 µg/cm ² (0.32%)	observed	(104)
PATCH TEST			

No effect level	<0.00012 % (=0.036 µg/cm ²)* (*calculated)	observed	(104)
Isoeugenol			
ROAT			
in ethanol 63% positive	5.6 µg/cm ²	observed	(100)
in ethanol 42% positive	2.2 µg/cm ²	observed	(264)
in ethanol 67% positive	9.0 µg/cm ²	observed	(264)
Deodorant matrix 23 % positive	0.167 µg/cm ²	observed	(279)
Deodorant matrix 69 % positive	0.53 µg/cm ²	observed	(279)
Deodorant matrix 77 % positive	1.67 µg/cm ²	observed	(279)
PATCH TEST			
ED50% (in petrolatum)	32 µg/cm ²	estimated	(100)
No effect (in ethanol)	<0.0005% (0.15 µg/cm ²)	observed	(264)
No effect (in petrolatum)	<0.4 µg/cm ²	observed	(100)

Summary of results for specific fragrance ingredients

Chloroatranol (constituent of *Evernia prunastri*)

In ROAT a dose of 0.025 µg/cm² to 0.125 µg/cm² in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm².

Cinnamal

In ROAT a dose of 0.26 µg/cm² gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm².

The ED50 in patch testing was 96 µg/cm².

HICC

In ROAT a dose of 0.0357 µg/cm² gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm² to 1.2 µg/cm². The dose in a cream base was 4.9 µg/cm².

In ROAT a dose of 15.3 µg/cm² to 126.2 µg/cm² in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm².

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm² gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm².

The no-effect level in patch testing was below 0.036 µg/cm².

Isoeugenol

In ROAT a dose of 2.2 µg/cm² a response in 42% and 9.0 µg/cm² in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm² caused a response in 23% and 77% reacted to 1.67 µg/cm².

The ED50 in patch testing was 32 µg/cm².

The no-effect in patch testing was below 0.15 µg/cm².

Elicitation levels have been studied for cinnamal, isoeugenol and hydroxycitronellal which are established contact allergens in humans and which already have given rise to a significant number of cases (> 100, see chapter 7). Further HICC has been studied extensively, but is considered in a separate section (chapter 11.3) of this opinion. It is however not possible to derive a safe threshold directly from the data of cinnamal, isoeugenol and hydroxycitronellal. The main reasons are that many of the test subjects reacted to all the tested doses in ROAT, which is a simulation of every day exposures. Thus it was not possible to determine the dose only eliciting responses in a few, e.g. 10% of the subjects and that only a limited number of exposure scenarios were studied.

The studies have covered few product types: hydro-alcoholic products, e.g. perfumes and deodorant roll-on matrix. The vehicle is one of many factors which influence the thresholds of allergic reactions. Also the presence of irritants and other allergens can influence the elicitation level. This means that the currently available studies do not cover all the relevant exposure scenarios. However, taking into account that dose-response investigations in sensitised patients are very complex to perform, it is not likely that much more data will become available in the near future. It is therefore necessary to exploit the full pool of elicitation data, also covering chemicals other than fragrance ingredients, to derive a more general threshold which could be used when no or insufficient data exist to set a specific threshold for a substance of concern.

General thresholds

The methodology of the different experiments has varied to some extent as different anatomical sites of exposure have been employed, different vehicles, exposure periods and cut-off points. The reason is that the studies have been performed to investigate various clinical and scientific aspects of allergic contact reactions and not for formal regulatory requirements. Some studies are small and for this reason the precision of the estimates of thresholds is limited. In spite of this, the results of the various experiments are reasonably uniform, except for chloroatranol which had very low threshold reactions, and show that low concentrations may elicit allergic reactions.

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent “meta-analysis” of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED₁₀ at patch testing varied by a factor of 7 from the lowest to the highest value and the median was 0.82 µg/cm² if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 µg/cm² if included (see Table 11-6 and Figure 11-2 below: (280)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (280) and guinea pig QSAR evidence (281). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of 0.8 µg/cm² for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED₁₀ value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions: 0.8 µg/cm² (280).

- Exposure doses and exposure areas from SCCS notes of guidance 7th revision (282) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (274).

Equation:

Safe concentration in product = (Generally safe exposure level ($0.8 \mu\text{g}/\text{cm}^2$)/daily exposure to product ($\mu\text{g}/\text{cm}^2/\text{day}$)) x 100 (for %).

Table 11-5: Concentration limits in different product types based on $0.8 \mu\text{g}/\text{cm}^2$ allergen as a 'generally safe exposure level', if specific dose-response data are unavailable.

	Estimated daily exposure level (g) (Table 3 SCCS NoG)	Mean exposed skin surface (cm^2) (Table 2 SCCS NoG)	Exposure $/\text{cm}^2/\text{day}$ in grams	Exposure $/\text{cm}^2/\text{day}$ in μg ($1\text{g} = 1 \times 10^6 \mu\text{g}$)	Concentration limit in product % in product: (GEL/daily exposure) x 100
Body lotion	7.82 g	15,670 cm^2	0.000499	499	0.16%
Face cream	1.54 g	565 cm^2	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 cm^2	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221 ¹⁾	2210	0.04%

Note: 1) $2.21 \text{ mg}/\text{cm}^2/\text{day}$ from Technical dossier Quantitative Risk Assessment.

The estimated daily use of the various product categories in Table 11-5 are based on the SCCS Notes of Guidance (see above), except for perfume, for which no value is given. This value is taken from the Technical Dossier on Quantitative Risk Assessment from RIFM.

Generally the estimated use of different products is higher in the IFRA/RIFM assessments than in SCCS Notes of Guidance.

Table 11-6: Overview of dose-response studies and thresholds for eight allergens, after (280).

ED₁₀ patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (283), MCI/MI (Kathon TM CG) (284), nickel (285), methylidibromo glutaronitrile (MDBGN) (286), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (102, 224, 263), isoeugenol (264, 279) and formaldehyde (287). The shaded values were considered as outliers.

Study	Number of patients	ED₁₀ ($\mu\text{g}/\text{cm}^2$)	95 % interval
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59

Study	Number of patients	ED ₁₀ (µg/cm ²)	95 % interval
Nickel 1999	26	7.49	2.42–14.5
Nickel 2005	13	0.74	0.066–2.38
Nickel 2007	20	0.82	0.13–2.37
Cobalt 2005	11	0.44	0.033–1.3
Chromium	17	1.04	0.0033–5.55
Isoeugenol 2001	24	1.48	0.22–4.74
Isoeugenol 2005	13	0.23	0.0073–1.32
HICC 2003	18	0.85	0.062–3.26
HICC 2007	14	1.17	0.043–5.05
HICC 2009	17	0.66	0.052–2.35
MDBGN 2004	19	0.025	0.00021–0.19
MDBGN 2008	18	0.50	0.052–1.69

Note: The ED₁₀ value is the concentration which elicits an allergic reaction in 10% of a group of sensitised individuals under patch test conditions.

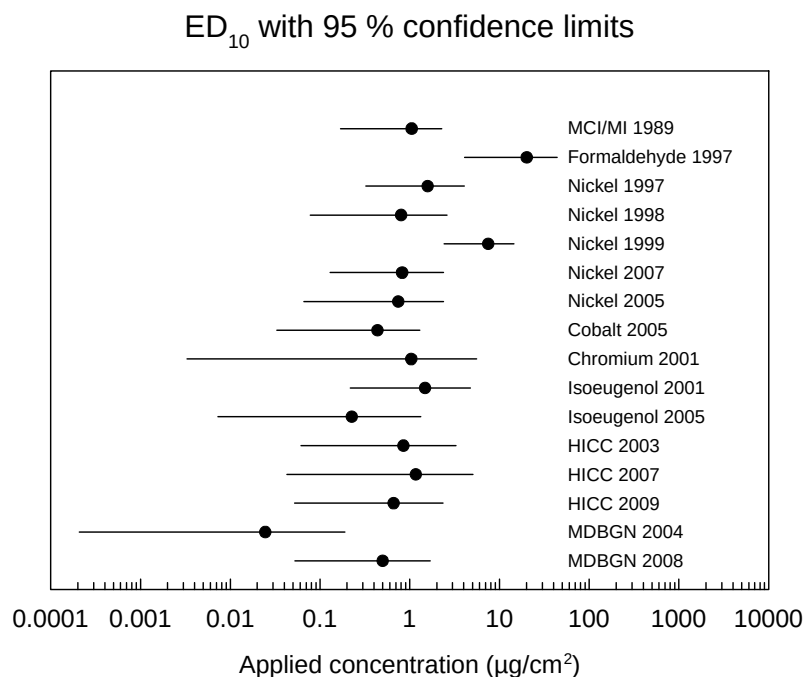


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

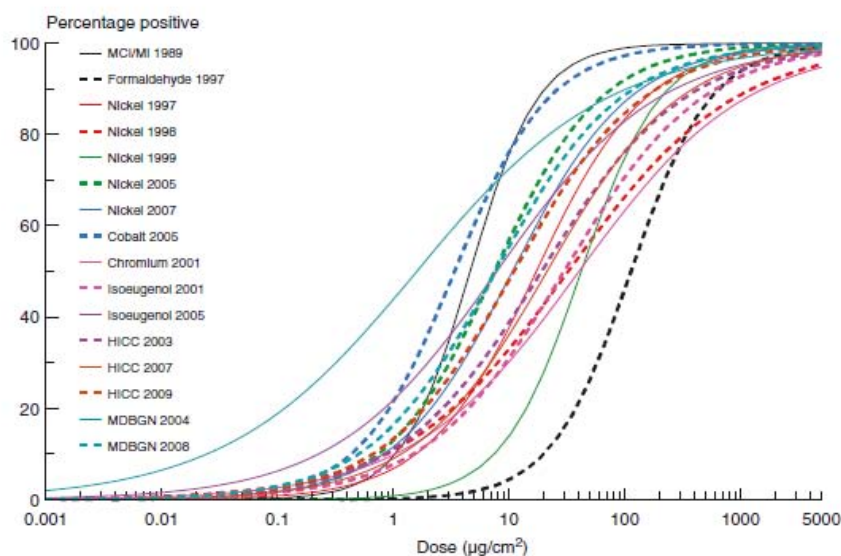


Figure 11-3: The fitted dose-response curves from the studies in Table 11-6, which are the basis for estimation of the ED10 value, after (280).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was $0.8 \mu\text{g}/\text{cm}^2$. In the model data for the fragrance substances isoeugenol and HICC was included. The two studies on isoeugenol and the three studies on HICC gave an average ED10 value of $0.85 \mu\text{g}/\text{cm}^2$ and $0.89 \mu\text{g}/\text{cm}^2$ with a range 0.23-1.48. This means that even if the model was used for these substances individually the result would be very similar to the general threshold value.

The data from cinnamal and hydroxycitronellal studies was not incorporated in the model because: (i) serial dilution patch testing was done in petrolatum for cinnamal, making the dosing less exact; (ii) and only seven patients participated in the hydroxycitronellal study, while a criteria for inclusion in the model was ten participants (280).

According to the above calculations, a limit of $0.8 \mu\text{g}/\text{cm}^2$ for the product types of most importance for fragrance allergy corresponds to concentrations of 100 to 400 ppm (0.01-0.04%) for deodorants, perfume spray, hand and face lotions. For body lotion the general threshold was 0.16%. However, it does not seem meaningful in the context of contact allergy to distinguish between different types of creams, as a body cream would be applied with the hands and the relevant parameter in contact allergy is dose per area skin and not total dose.

A general threshold would have to take into consideration the uncertainties in quantification of exposure and safe thresholds as well as the possibilities of aggregate exposures and exposure to chemically similar substances. Therefore in setting one general threshold the product category carrying the highest risk of sensitisation and elicitation, which is deodorants, was chosen to drive the generation of the threshold. This means that a threshold of $0.8 \mu\text{g}/\text{cm}^2$ is equal to 0.01% or 100 ppm (see Table Table 11-1 and the related text), the lowest of the threshold values derived.

The approach taken by the SCCS is based on scientific evidence published in peer-reviewed journals (283)(284)(285)(286)(102, 224, 263)(264, 279)(287) in the past 20 years. The meta-analysis deriving the general threshold limit at $0.8 \mu\text{g}/\text{cm}^2$ limit has been published (280) in a peer-reviewed journal. The use of threshold limits based on elicitation data is a well established methodology which has been applied (with success) in EU to prevent further cases of induction and elicitation (primary and secondary prevention) in the case of nickel allergy, chromium in cement, chromium in shoes in

Germany, dimethyl fumurate in consumer items and also in part in IFRA guidelines e.g. concerning HICC.

The elicitation threshold model is based on 16 studies of 8 allergens, two of which are fragrance ingredients. It includes data from moderate to extreme allergens with a median EC3 value of 1.2.

The 11 fragrance allergens to which the limit is suggested to apply range from extreme to moderate with median EC3 value of 4.8, although in the case of coumarin an EC3 value could not be established.

Thus in general the potency profile of the fragrance substances of concern is not very different from those included in the model to provide the suggested general safe threshold.

The approach is targeting the relevant end-point, namely, allergic contact dermatitis. The mere consideration of potency of the allergen, according to the LLNA (EC3), is insufficient in identifying the size of the problems of contact allergy/allergic contact dermatitis. Additional information is needed from clinical and epidemiological studies, exposure assessment and dose-elicitation studies. For instance, the elicitation thresholds of e.g. HICC (EC3: 17.1) and isoeugenol (EC3: 0.54) are very similar (0.85 µg/cm² and 0.89 µg/cm², respectively) despite very different potencies. Both are frequent causes of contact allergy.

It should be noted that the general threshold is only suggested to be used for substances of concern if no specific data of sufficient quality exist to set an individual safe threshold. In cases where specific data of sufficient quality are available, these data should be used to set an individual safe threshold.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (288) and in a reduction in morbidity, i.e. elicitation (289). Another example is restriction of chromium VI in cement (290).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens (239). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most

frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (291)) (for more studies see chapter 4.3.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (69). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (105). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (102).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (292). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see 11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: "The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material" (293). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (293).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/ antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcohols for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcohols for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02

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Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.

QRA Quantitative risk assessment.

* Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the pre-consultation version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany (294)) and 2003-2011 (Danish contact dermatitis group (295)), respectively. The first analysis identified a slight decrease, which was considered “not overwhelming in absolute terms”, namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

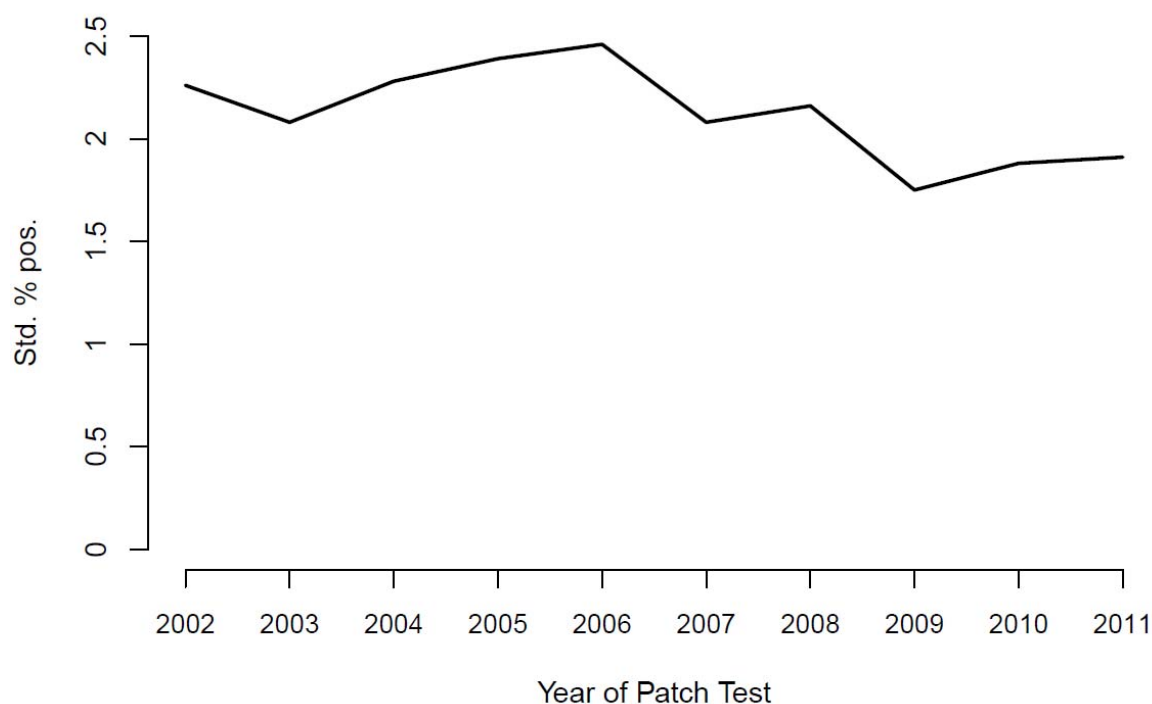


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

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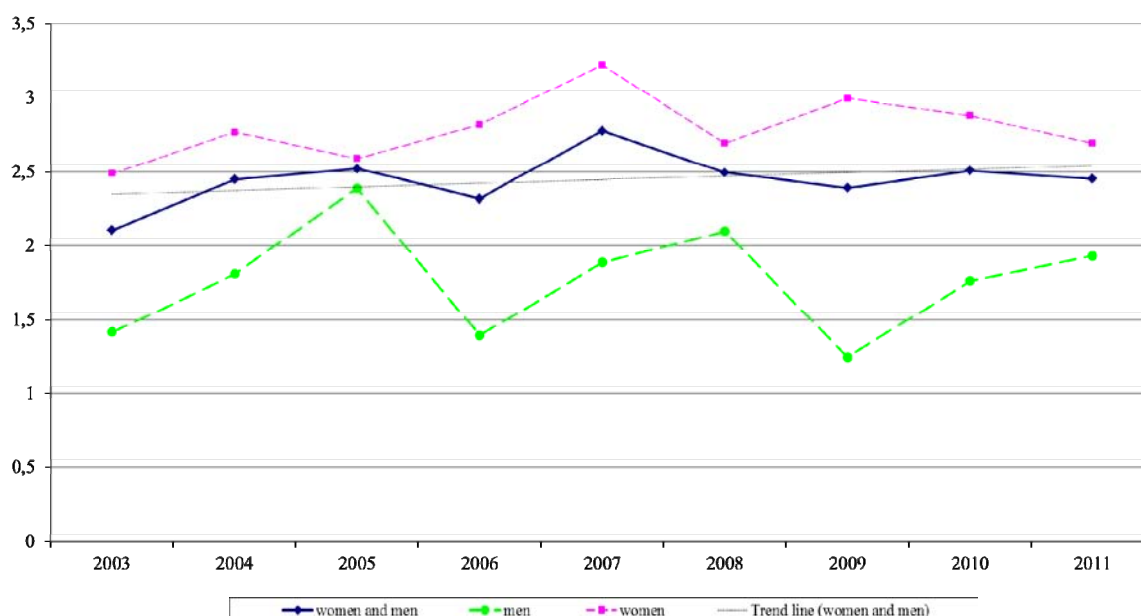


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: "There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."
- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, "safe use" means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of $0.8 \mu\text{g}/\text{cm}^2$ was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.

- It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.
- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.
- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.
- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents, despite efforts to reduce the allergen content (296).

12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up to 10% of the European population carry mutations, which impair 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 prehapten, 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 prehapten and prohaptens to increase the possibility to diagnose allergy from these type of substances.
- There is a need for more experimental research to further establish the impact of the behaviour of fragrance substances when applied on the skin (including factors such as volatility, autoxidation, skin penetration, reactivity in skin and bioactivation).

12.2. Non-human studies

- Several studies in the industry submission (164) were of insufficient quality, not following the OECD guidelines.
- In some cases it was found that either very few concentrations points had been used in LLNAs, or concentrations were insufficient for achieving a 3-fold increase of the SI.

A sufficient number of doses (concentrations) should be applied in LLNAs (at least 5) so that interpolation (for deriving an EC3 value) can rely on more than two or three actual data points to be more reliable. SCCS therefore suggests a change in the OECD guideline 429. (It is important to remember that the production of unreliable data is a waste of animals.) Moreover, the maximum concentration should be high enough to achieve a > 3-fold increase in SI, as far as this is possible with the substance/vehicle combination chosen.

- Data on experimental results are often not published, but available only on file in the companies having performed the tests. Access to such results would be important for the scientific community, e.g. in the context of REACH, or independently, either to the public domain, or to a Public Trustee.
- The OECD guideline 429 recommends several vehicles. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus, as an additional control, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.
- As long as no validated *in vitro* method exists, more research is needed. Until one or more method(s) have been decided to fulfil the requirements for substituting *in vivo* testing, the *in vivo* testing for prediction of skin sensitisation has to be used.
- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances. Therefore, further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is needed to increase the safety for the consumer, i.e. experimental studies which include air oxidation and bioactivation.
- Further experimental investigations of the sensitisation potential of fragrance substances are needed to determine the impact of the volatility of the substance as well as the effect of the vehicle on skin penetration/absorption and reactivity.
- From a clinical perspective it is important for the individual who is sensitised to one fragrance substance to know if they must also avoid other fragrance substances that can cause allergic contact dermatitis due to cross-reactivity with the original sensitiser. Prediction of risks for cross-reactivity requires sound application of theoretical principles in combination with well-designed experimental studies. This is a field that has not been studied very much so far and needs to be focused on much more in the future.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods as possible. Prediction of different activation pathways should be included.
- Effect estimates such as proportions of sensitised humans or animals, or mean stimulation indices, EC3 values and other derivations should ideally be accompanied by an interval estimate (confidence interval) to address precision (297).

13. Opinion

Contact allergy to fragrances is a common, significant and relevant problem in Europe. The studies since the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 26 fragrance allergens, identified by the SCCNFP, are still relevant fragrance allergens for consumers because of their exposure from cosmetic products. Additional exposure to many of these 26 fragrance allergens also occurs from the use of other consumer products, such as detergents, toys, etc. Some of these fragrance substances are also used as preservatives.

The overall trend of fragrance contact allergy appears to have been stable for the last 10 years, as some causes of fragrance allergy have decreased and others increased. From the few population-based studies, it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. This is based on the limited testing with eight common fragrance allergens (FM I) out of the approximately 2500 fragrance ingredients listed in CosIng and indicative of the substances that may be present in fragrance compounds. However, the real prevalence of contact allergy to fragrance substances may be higher if the testing were to be performed with the full spectrum of fragrance allergens, including oxidised substances, where relevant.

Among eczema patients in the European population, around 16% are sensitised to fragrance ingredients. The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Contact sensitisation, and its clinical manifestation, allergic contact dermatitis, can be prevented if the exposure to known contact allergens is reduced or abolished (primary prevention). Experiences so far, have indicated that not all substances that later turned out to be significant contact allergens after human exposure, were predicted by experimental studies, e.g. the preservative methyldibromo glutaronitrile and the fragrance chemical HICC. Thus, a significant exposure of the population may occur before a substance is established as an important contact allergen in man.

Elicitation of allergic contact dermatitis occurs when a consumer sensitised to a certain substance is re-exposed to the substance in question. Prevention at this stage, termed secondary prevention, can be achieved if use of the allergen in products is eliminated or reduced to a tolerable level (general prevention), or if the patients succeed in avoiding all sources of exposure (individual prevention). Ingredient listing of individual fragrance allergens has been shown to be an important tool to enable consumers with an identified allergy to reduce/avoid relevant exposures. Moreover, ingredient listing is also of great importance to ensure that an adequate diagnosis of fragrance contact allergy can be made without undue delay. If the information given on the presence of fragrance allergens is incomplete, diagnosis of fragrance contact allergy may be missed.

The SCCNFP, in its 1999 opinion, identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the European Cosmetics legislation (298) as ingredient labelling of these 26 fragrance substances (Annex III, entries 67-92). However, safe use concentrations for these substances in cosmetic products have not yet been determined and much new evidence concerning fragrance allergy has been published since 1999. The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature up to October 2010. This review addresses the issue of contact allergy to fragrance substances, including natural extracts and updates the list of fragrance allergens relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of: (i) established fragrance allergens; (ii) likely fragrance allergens; and (iii) possible fragrance allergens. The review also includes fragrances, which on modification by oxidation or by enzyme mediated processes, can produce allergens. Available dose-response data have been

examined to answer whether safe thresholds can be established for the most frequent fragrance allergens.

13.1. Question 1

Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?

In order to answer this question, the SCCS has used clinical and epidemiological data to identify known fragrance allergens. These were categorised as *established contact allergens in humans* (see Table 13-1).

Where sufficient animal evidence was present, these substances were categorised as established contact allergens in animals (Table 13-2). For a number of other fragrance substances, combinations of limited clinical data together with SAR considerations have been applied to indicate likely fragrance allergens in man (Table 13-3). Finally, SAR has also been applied to substances that lack human data to identify fragrance chemicals that have the structural potential to be contact allergens. Substances with insufficient human data were also considered as possible fragrance allergens. For these further tests (experimental/clinical data) are required (Table 13-4).

Table 13-1: Established contact allergens in humans.

For categorisation of importance (+ to +++) see chapter 7.1. Allergens of special concern are substances where between 100 and 1,000 cases (+++) and more than 1,000 (+++++) have been published. These are set in bold. Fragrance substances identified as allergens in the 1999 opinion of SCCNFP (1) are marked with an asterisk.

“ox.” = oxidised; “non-ox.” = non-oxidised; “r.t.” = rarely tested (see chapter 7)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
Individual chemicals		
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL *	122-40-7	++
AMYL CINNAMYL ALCOHOL *	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISE ALCOHOL *	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL *	100-51-6	++
BENZYL BENZOATE *	120-51-4	++
BENZYL CINNAMATE *	103-41-3	++
BENZYL SALICYLATE *	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL *	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL*	104-55-2	+++
CINNAMYL ALCOHOL*	104-54-1	+++
CITRAL*	5392-40-5	+++
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN*	91-64-5	+++
(DAMASCENONE) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL*	97-53-0	+++
FARNESOL*	4602-84-0	++ - +++
GERANIOL*	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
HYDROXYISOHEXYL CARBOXALDEHYDE (HICC)* 3-CYCLOHEXENE	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL*	107-75-5	+++
ISOEUGENOL*	97-54-1	+++
alpha-ISOMETHYL IONONE*	127-51-5	++
(DL)-LIMONENE*	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL*	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-	67801-20-1	++ (r.t.)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CYCLOPENTENYL)PENT-4-EN-2-OL		
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	+
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++
Natural extracts		
CANANGA ODORATA and Ylang-ylang oil	83863-30-3; 8006-81-3	+++
CEDRUS ATLANTICA BARK OIL	92201-55-3; 8000-27-9	++
CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
CITRUS AURANTIUM AMARA FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
CITRUS BERGAMIA PEEL OIL EXPRESSED	89957-91-5	+ (r.t.)
CITRUS LIMONUM PEEL OIL EXPRESSED	84929-31-7	++
CITRUS SINENSIS (syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED	97766-30-8; 8028-48-6	++
CYMBOPOGON CITRATUS / SCHOENANTHUS OILS	89998-14-1; 8007-02-1; 89998-16-3	++
EUCALYPTUS SPP. LEAF OIL	92502-70-0; 8000-48-4	++
EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL	8000-34-8	+++
EVERNIA FURFURACEA EXTRACT*	90028-67-4	+++
EVERNIA PRUNASTRI EXTRACT*	90028-68-5	+++
JASMINUM GRANDIFLORUM / OFFICINALE	84776-64-7; 90045-94-6; 8022-96-6	+++
JUNIPERUS VIRGINIANA	8000-27-9;	++

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
	85085-41-2	
<i>LAURUS NOBILIS</i>	8002-41-3; 8007-48-5; 84603-73-6	++
<i>LAVANDULA HYBRIDA</i>	91722-69-9	+ (r.t.)
<i>LAVANDULA OFFICINALIS</i>	84776-65-8	++
<i>MENTHA PIPERITA</i>	8006-90-4; 84082-70-2	++
<i>MENTHA SPICATA</i>	84696-51-5	++
MYROXYLON PEREIRAE	8007-00-9;	++++
<i>NARCISSUS SPP.</i>	diverse	++
<i>PELARGONIUM GRAVEOLENS</i>	90082-51-2; 8000-46-2	++
<i>PINUS MUGO/PUMILA</i>	90082-72-7 / 97676-05-6	++
<i>POGOSTEMON CABLIN</i>	8014-09-3; 84238-39-1	++
<i>ROSE FLOWER OIL (ROSA SPP.)</i>	Diverse	++
SANTALUM ALBUM	84787-70-2; 8006-87-9	+++
TURPENTINE (oil)	8006-64-2; 9005-90-7; 8052-14-0	++++
VERBENA ABSOLUTE	8024-12-2	++

Table 13-2: Fragrance substances categorised as established contact allergens in animals.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Individual chemicals			
Allyl phenoxyacetate	7493-74-5	none	3.1
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0	none	4.3
CYCLAMEN ALDEHYDE	103-95-7	none	22
Dibenzyl ether	103-50-4	none	6.3
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	limited	7.5
trans-2-Hexenal	6728-26-3	none	2.6
2-Hexylidene cyclopentanone	17373-89-6	none	2.4
HEXYL SALICYLATE	6259-76-3	negative	0.18
p-Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	none	9.5

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Isocyclocitral	1335-66-6	none	7.3
α -Methyl cinnamic aldehyde	101-39-3	none	4.5
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0	none	16.4
METHYLUNDECANAL	110-41-8	none	10
2-Methoxy-4-methylphenol	93-51-6	none	5.8
4-Methoxy- α -methyl benzenpropanal	5462-06-6	none	23.6
METHYL OCTINE CARBONATE	111-80-8	limited	2.5
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	none	8.1
PHENYLACETALDEHYDE	122-78-1	limited	3
Natural extracts			
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	none	35.4

Table 13-3: Fragrance substances categorised as likely contact allergens by combination of evidence.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
AMBRETTOLIDE	7779-50-2	limited	none	+
CARVACROL	499-75-2	limited	none	+
Citrus paradisi §	8016-20-4	none	R43	n.a.
CUMINALDEHYDE	122-03-2	limited	none	+
CYCLOPENTADECANONE	502-72-7	limited	none	+
trans-trans-delta-DAMASCONE	71048-82-3	limited	none	+
2,4-dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	none	R43	+
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	limited	none	+
ETHYL VANILLIN	121-32-4	limited	none	+
HELIOTROPINE	120-57-0	limited	none	+
ISOAMYL SALICYLATE	87-20-7	limited	none	++
ISOLONGIFOLENEKETONE	33407-62-4	limited	none	+
Longifolene §	475-20-7	none	R43	+
Mentha arvensis §	68917-18-0	none	R43	n.a.
METHOXYCITRONELLAL	3613-30-7	limited	none	+
METHYL CINNAMATE	103-26-4	limited	none	++
METHYLIONANTHEME	55599-63-8	limited	none	+

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
5-METHYL-alpha-IONONE	79-69-6	limited	none	+
MYRCENE	123-35-3	limited	none	++
MYRTENOL	515-00-4	limited	none	+
NEROL	106-25-2	limited	none	++
Nerolidol (isomer not specified)	7212-44-4	limited	none	++
NOPYL ACETATE	128-51-8	limited	none	+
PHYTOL	150-86-7	limited	none	+
RHODINOL	6812-78-8	limited	none	+
trans-ROSE KETONE-5	39872-57-6	limited	none	++

§ Substances/natural mixtures were classified as R43, according to the submission by IFRA. The evidence on which this classification was based was not available to the SCCS, so the validity of classification cannot be assessed. Nevertheless, the four substances/substance mixtures should be treated as *likely contact allergens*.

n.a.: not applicable (natural mixture)

Table 13-4: Fragrance substances categorised as possible contact allergens.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
Individual chemicals				
CYCLOHEXYL ACETATE	622-45-7	limited	none	0
ETHYLENE DODECANEDIOATE	54982-83-1	limited	none	0
HYDROXYCITRONELLOL	107-74-4	limited	none	0
METHOXYTRIMETHYLHEPTANOL	41890-92-0	limited	none	0
METHYL p-ANISATE	121-98-2	limited	none	0
METHYL DIHYDROJASMONATE	24851-98-7	limited	none	0
PHENETHYL ALCOHOL	60-12-8	limited	none	0
PHENYLPROPANOL	122-97-4	limited	none	0
AMYL CYCLOPENTANONE	4819-67-4	negative	none	+
BENZYL ACETATE	140-11-4	negative	none	+
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	negative	none	+
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	negative	none	+
alpha-IONONE	127-41-3	negative	none	+
beta-IONONE	79-77-6	negative	none	+
METHYL IONONE (mixture of	1335-46-2	negative	none	+

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC value (min; %)	SAR
isomers)				
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	negative	none	+
alpha-TERPINYL ACETATE	80-26-2	negative	none	+
CITRONELLYL NITRILE	51566-62-2	none	none	++
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0	none	none	+
DECANAL	112-31-2	none	none	++
DIHYDROMYRCENOL	18479-58-8	none	none	+
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6	none	none	++
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6	none	none	+
GERANYL ACETATE	105-87-3	none	none	++
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0	none	none	+
IONONE isomeric mixture	8013-90-9	none	none	+
ISOBERGAMATE	68683-20-5	none	none	+
METHYL DECENOL	81782-77-6	none	none	+
TRICYCLODECENYL PROPIONATE	17511-60-3	none	none	+
OXACYCLOHEXADECENONE	34902-57-3	none	none	++
VERDYL ACETATE	2500-83-6/ 5413-60-5	none	none	+
trans-beta-Damascone	23726-91-2	none	none	+
gamma-Damascone	35087-49-1	none	none	+
Citronellal	106-23-0	none	none	++
Phenethyl salicylate	87-22-9	none	none	++
Natural extracts				
ACORUS CALAMUS ROOT OIL	84775-39-3	Limited	none	
CEDRUS DEODARA WOOD OIL	91771-47-0	Limited	none	
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Limited	none	
CITRUS TANGERINA ...	223748-44-5	Limited	none	
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Limited	none	
ILLICIAM VERUM FRUIT OIL	84650-59-9	Limited	none	
LAVANDULA SPICA	97722-12-8	Limited	none	

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
LITSEA CUBEBA	90063-59-5	Limited	none	
PELARGONIUM ROSEUM	90082-55-6	Limited	none	
SALVIA spp.	Diverse	Limited	none	
TARGETES PATULA	91722-29-1	Limited	none	
THYMUS spp.	84929-51-1	Limited	none	
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	Limited	none	

Regarding the above categorisation of fragrance substances, the following aspects need to be considered when interpreting an outcome other than established contact allergen in humans:

- If human evidence is negative, there is still a potential sensitisation risk, as in this set of substances the number of (consecutive) patients tested was low, i.e. up to a few hundred.
- If EC3 values are given as higher (>) than a certain value (see 8.3), an exact EC3 could not be established, as the substance had been tested in too low concentration(s). In these cases, the substances have not been categorised as 'established contact allergen in animals'.
- For SAR, the categories of prediction are: non-sensitiser (0); possible-sensitiser (+); predicted sensitiser (++) and not predictable (n.p.). (For details see Table 9-3 and Table 9-4). SAR predictions are only considered when human and animal data are limited or missing.
- Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I to this opinion, these substances have not further been evaluated.

Fragrance ingredients listed in Table 13-1 clearly have caused disease in man, and based on the clinical experience alone, these 82 substances were classified as established contact allergens in humans, 54 individual chemicals and 28 natural extracts (mixtures of chemicals), including all 26 fragrance allergens identified by SCCNFP in 1999. For a number of other substances, no patch test data were available, but positive animal data, obtained by a validated guideline method (LLNA) addressing hazard, indicate that a – yet not quantified – risk for humans is very likely to exist, given sufficient exposure. In other cases only in a relatively small number of patients has been tested positively ('limited human evidence'). Here, combination with SAR analyses corroborates the conclusion that these substances, too, are sufficiently qualified to be regarded as 'likely fragrance allergens'.

Of those 82 substances identified as established contact allergens in humans, 12 chemicals (listed in Table 13-5) and eight natural extracts are considered of special concern as they have given rise to at least 100 reported cases. These substances pose a particularly high risk of sensitisation to the consumer and are further considered in the answer of question 2. One substance, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), was shown to be the cause of allergic contact dermatitis in more than 1500 reported cases since 1999. The number of cases is only those reported in scientific publications, and therefore the actual number of cases is severely under-estimated.

Table 13-5: Established fragrance contact allergens of special concern (single chemicals only).

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)

*including their respective esters

The established contact allergens in animals (Table 13-2) and the likely contact allergens, identified based on a combination of limited evidence from man together with positive SAR predictions (Table 13-3), are predicted to cause disease in man given sufficient exposure.

Information on the presence of all the substances given in Table 13-1, Table 13-2 and Table 13-3 in cosmetic products is important in order to enable aimed testing of patients with contact dermatitis and to diagnose fragrance allergy without delay. Further, this information is important to the sensitised consumer as it will enable them to avoid cosmetic products, which they may not tolerate.

Substances given in Table 13-4 are possible contact allergens and further data are required to judge if these are contact allergens in humans and give rise to contact allergy in consumers.

Conclusions - Question 1

The studies since the SCCNFP Opinion on fragrance allergy in consumers (1) have confirmed that the fragrance allergens currently listed in Annex III, entries 67-92 are still relevant fragrance allergens for the consumers from their exposure to cosmetic products.

The review of the clinical and experimental data shows that many more fragrance substances than those identified in the SCCNFP opinion of 1999 have been shown to be sensitisers in humans. A comprehensive list of established contact allergens in humans is given in Table 13-1.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking.

Additionally, limited human and/or animal evidence together with structure activity relationship analysis suggests that other fragrance ingredients may be a cause of concern with regard to their potential of causing contact allergy in humans.

Ingredient listing is important in clinical practice for the management of patients who are allergic to one or more of the listed fragrance chemicals. It is also important for the

patients in order to avoid future exposure to fragrance contact allergens which they may not tolerate.

The SCCS considers that those substances itemised in Table 13-1, Table 13-2 and Table 13-3 represent those fragrance ingredients that the consumer should be made aware of when present in cosmetic products.

Substances known to be transformed (e.g. hydrolysis of esters) to known contact allergens should be treated as equivalent to these known contact allergens. The combined concentration of the alcohol and its ester must be considered regarding exposure. Important indicative, but not exhaustive, examples include isoeugenol and its esters, geraniol and its esters, eugenol and its esters, and linalool and its esters.

13.2. Question 2

Can the SCCS establish any threshold for their safe use based on the available scientific data?

Dose-response relationships exist between exposure to contact allergens and the proportion of consumers who will become sensitised to an allergen (i.e. induction), as well as the proportion who will suffer from allergic contact dermatitis (elicitation). For a number of recognised contact allergens in man, dose-elicitation studies on sensitised individuals are available. These studies indicate that it is in principle possible to derive exposure levels that the majority of sensitised individuals will tolerate. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy and limit the risk of induction.

Among the established chemical fragrance allergens, 12 were identified as posing a high risk of sensitisation to the consumer (Table 13-5), i.e. more than 100 reported cases. For these substances, limitation of exposure would help to protect sensitised consumers from developing allergic contact dermatitis.

In cases where specific data of sufficient quality on threshold levels for a particular allergen are available, these data should be used to set an individual safe threshold. However, when such quality data are not available and a substance has been identified to pose a high risk of sensitisation to the consumer, a general threshold limit can be applied.

Dose-response studies have been performed with only four of these fragrance substances (HICC, isoeugenol, cinnamal and hydroxycitronellal). In addition, such a study has also been performed on chloroatranol, a potent allergen in *Evernia prunastri* and *Evernia furfuracea*. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.

If no adequate data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 µg/cm² has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 µg/cm² under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

The model providing the general threshold of 100 ppm has been based on single substances only and no general safe level for the natural extracts of concern can be

identified, but the maximum use concentration applies to the identified fragrance allergens also when present in the natural extract.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of more than 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I), which will severely underestimate the actual prevalence in the population. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. The SCCP concluded in 2003 that 200 ppm of HICC would be tolerated by the majority of sensitised individuals and this level of exposure would have a low potential to induce sensitisation (241). Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Chloroatranol and atranol are the main allergenic components of *Evernia prunastri* and *Evernia furfuracea*. The SCCS concluded in 2004 (239) that these should not be present in cosmetic products, due to their exceptionally high sensitisation potential. Attempts to effectively reduce the content of these compounds in “oak moss abs.” (300) have largely failed to reduce contact allergy to *Evernia prunastri* and *Evernia furfuracea* and the data presented in this opinion show that the number of cases remains high.

Conclusions - Question 2

There are two components to the safety of fragrance ingredients in terms of contact allergy. First, the need to eliminate or reduce induction of contact allergy (primary prevention), which, when it occurs, is life long. Secondly, the need to eliminate or reduce elicitation reactions (secondary prevention) on the skin of those individuals who are already sensitised. Human dose elicitation experiments have hitherto been performed only for a very small number of substances. It is unlikely that more of these studies will be performed due to experimental and subject recruitment difficulties.

For individual substances, no levels that could be considered safe for the majority of consumers could be established from the available data.

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 µg/cm² (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

Such a threshold based on elicitation levels in sensitised individuals will be sufficiently low to protect both sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy.

The SCCS is of the opinion that for substances identified as posing a high risk to the consumer and for which no individual thresholds could be derived (Table 13-5), the general threshold of 0.01% would limit the problem of fragrance allergy in the consumer significantly.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the above identified fragrance allergens also when present in the natural extract. This will also reduce the risk of sensitisation and elicitation from natural extracts.

It is important to stress that this general threshold, although limiting the problem, does not preclude that the most sensitive segment of the population may react upon exposure to these levels. Hence, this threshold does not remove the necessity for providing information to the consumer concerning the presence of the fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, in 2003 the SCCP suggested that levels of up to 200 ppm would be tolerated by the majority of sensitised individuals. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized. The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to allergenic constituents, despite efforts to reduce the allergen content (296). The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

13.3. Question 3

Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation, and thus increasing the risk of sensitisation.

Experimental and clinical studies have shown that there are fragrance substances that act as prehaptens, i.e. their sensitisation potency is markedly increased by air exposure due to oxidation (autooxidation). Non/low-sensitising compounds are thereby transformed into more potent sensitisers. Limonene, linalool, linalyl acetate, alpha-terpinene and geraniol have all been identified as prehaptens. These fragrance substances are common in scented cosmetics as well as in household products. The clinical studies show that the exposure to allergens formed due to autooxidation causes significant contact allergy in consumers. Patch testing with oxidised limonene and oxidised linalool shows that these substances rank among the most common contact allergens.

In the SAR analyses performed in this work by the SCCS, fragrance compounds with structural alerts that indicate that they are possible prehaptens have been identified (Table 9-1, Table 9-2). In such cases further thorough investigations are needed. It is also important to investigate the stability of the primary oxidation products (the hydroperoxides) formed from various structures of fragrance compounds. The stability of these compounds can have great impact on the sensitisation potency of the oxidised compound as they are strong sensitisers. However, the secondary oxidation products (aldehydes and epoxides) can also be important sensitisers depending on the overall structure of the compound as was demonstrated for oxidised geraniol.

Air oxidation of prehaptens can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autooxidation rate depends not only on the compound itself, but also on its purity. The prevention of autooxidation using antioxidants

needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation. As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

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

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 µg/cm² (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

Compounds that are bioactivated by metabolising enzymes to haptens are referred to as prohaptens. Established prohaptens of clinical importance are cinnamyl alcohol, geranial, geraniol, eugenol, isoeugenol and alpha-terpinene.

Table 13-6: Known prehaptens and prohaptens.

Fragrance substance	Activation by air oxidation	Bioactivation (oxidation)	Bioactivation (hydrolysis)
Cinnamyl alcohol		x	
Eugenol		x	
Eugenyl acetate		x	x
Geranial	x	x	
Geraniol	x	x	
Geranyl acetate	x	x	x
Isoeugenol		x	
Isoeugenyl acetate		x	x
Limonene	x		
Linalool	x		
Linalyl acetate	x		
alpha-Terpinene.	x	x	

When bioactivation occurs, the risk of cross-reactivity should be considered. An increased complexity in the cross-reactivity pattern is obtained when a compound could act both as a prehaptens and a prohaptens. For instance, it is known that cinnamyl alcohol and cinnamal can cross-react due to the formation of common sensitising substances. The same applies to geraniol and citral.

In case derivatives of a fragrance substance are used, it must be taken into account that the derivative could be transformed into the parent or a cross-reacting compound. For such derivatives the same rules as for the corresponding parents should apply, unless the

stability of the derivative has been demonstrated. In particular, hydrolysis of esters to the corresponding alcohols can cause cross-reactions. Acetate esters of eugenol, isoeugenol and geraniol are frequently used in cosmetics.

To be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests.

Activation of individual compounds to various haptens increases the risks of cross-reactivity between chemicals and also causes difficulties in prediction of these risks. Prediction of risks requires sound application of theoretical principles in combination with well designed experimental studies. Based on the acquired knowledge, qualified suggestions using structure activity relationship (SAR) regarding many fragrance substances have been made (Table 9-1 to Table 9-3). However, as the stability of formed oxidation products (mainly hydroperoxides) is important for the sensitisation potency, the SAR hypotheses must be followed by experimental investigations for the actual compounds.

Conclusions - Question 3

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation. Fragrances with published data showing the formation of sensitising compounds by autoxidation, bioactivation or both include the following (see also Table 13-6).

Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation are limonene, linalool, and linalyl acetate.

Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation are cinnamyl alcohol, eugenol, isoeugenol and isoeugenyl acetate.

Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation are geraniol and alpha -terpinene.

A fragrance substance that sensitises without activation but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is geranial (one isomer of citral).

In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

The possibility to reduce the sensitisation potency by preventing air oxidation is important also for a direct acting hapten or prohaptens, if a further activation by air oxidation to more allergenic compounds has been shown.

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

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

The substances presented above are based on current knowledge and should be seen as indicative and illustrative of the general problem. As substances with structural alerts for acting as pro- and/or prehapten are quite common among the fragrance substances listed (see Tables 9-1 and 9-2), the possibility for activation to generate new potent allergens should be considered.

The SCCS is of the opinion that substances known to be transformed (e.g. by oxidation either via air oxidation or via bioactivation) to known contact allergens should be treated as equivalent to these contact allergens, i.e the same restrictions and other regulatory requirements should apply, unless specific data exist that allow for an individual assessment. Important indicative examples include limonene, linalool, linalyl acetate, geraniol, geranial, alpha-terpinene, eugenol, isoeugenol and cinnamyl alcohol.

14. List of abbreviations

ACD	Allergic contact dermatitis
alc.	Alcohol (as vehicle)
CI	Confidence interval
CLP	Classification, labelling and packaging
coloph.	Colophonium
DCs	Dendritic cells
EC	European Commission
ESSCA	European Surveillance System on Contact Allergies
EDT	Eau de toilette
EDP	Eau de perfume
EU	European Union
FM	Fragrance mix
GC	Gas chromatography
GPMT	Guinea pig maximisation test
HICC	Hydroxyisohexyl 3-cyclohexene carboxaldehyde
HRIPT	Human repeat insult patch test
IFRA	International Fragrance Association (www.ifraorg.org)
IVDK	Information Network of Departments of Dermatology (www.ivdk.gwdg.de)
INCI	International Nomenclature on Cosmetic Ingredients
LCs	Langerhans cells
LLNA	Local lymph node assay
MPR	<i>Myroxylon pereirae</i> resin
NACDG	North American Contact Dermatitis Group
OECD	Organization of Economic Co-operation and Development
ox.	oxidised
pet.	Petrolatum (as vehicle)
ppm	parts per million (10000 ppm = 1%)
PPV	Positive predictive value
PR	Prevalence ratio
PT(ed)(ing)	Patch test(ed) (ing)
QMM	Quantitative mechanistic model
QRA	Quantitative risk assessment
(Q)SAR	(Quantitative) structure activity relationship
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
RIFM	Research Institute for Fragrance Materials (www.rifm.org/)

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ROAT	Repeated open application test
SC	Single constituents (of one of the fragrance mixes)
SCCS	Scientific Committee on Consumer Safety
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SCCP	Scientific Committee on Consumer Products
UK	United Kingdom
US(A)	United States (of America)
UV	Ultraviolet

15. References

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Annex I - Catalogue of fragrance allergens**Contents**

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Single chemicals

Often, results with the single constituents of the FM I or, yet more rarely, FM II, are presented in one paper. As the main ordering of this annex is by allergen, core information on these studies is presented in a tabular format and referenced by a unique acronym in the single sections, to avoid redundancy. Regarding nomenclature, terms which are often not officially an INCI Name but Perfuming Name as listed by CosIng are used. "Current Regulation" refers to the EU Cosmetics Directive only.

Table 55: Background information on studies reporting results with (all) single constituents of the FM I (**amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, isoeugenol, EVERNIA PRUNASTRI**)

Reference	Country	Study period, Patients	Comments by reviewers
Larsen 2002 c (1)	7 industrial countries worldwide	Prior to 2002 n=218 patients with known contact allergy to fragrance ingredients	Test concentrations identified as non-irritating in serial dilution testing in 20 healthy volunteers
Utrecht 1999 (2)	Utrecht, The Netherlands	1994-1998 n=757 patients with suspected ACD to cosmetics	All patients tested with FM I and single constituents
Sheffield 1999 (3)	UK	1994-1995 n=744, 40 of these positive to FM I and tested with single constituents	
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
Hungary 2002 (5)	Hungary, multicentre study,	1998-1999, n=3604 patients	recruitment not clear, presumably consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients
IVDK 2010 (7)	Germany, Switzerland and one centre in	2005-2008 n=36961 tested with FM I, n=4167 with FM II and	

	Austria	all SC	

Table 56: Results of PTing with single constituents of the FM I in patients positive to the FM I (as percent)

N(pos) to FM I, ref.	<i>Evernia prun.</i>	Isoeu g.	Hydroxy citron.	Cinna mal	Cinnamy I alcohol	Eugen ol	Gera-niol	Alpha-amyl cinnam al
N=160 (5)	13.1%	14.8 %	2.5%	8.1%	20.6%	8.8%	7.5%	5.0%
N= 991 (8)	18.4%	11.2 %	10.1%	6.1%	6.1%	6.6%	4.6%	2.4%
N=50 (2)	19.6%	14.3 %	8.9%	8.9%	7.1%	5.4%	2.7%	0%
n=40 Sheffield 1999 (3)	30%	20%	2.5%	12.5 %	10%	5%	0%	0%
N=226 Coimbra 2000 (9)	22.1%	19.9 %	6.6%	13.3 %	7.9%	14.6 %	8.4%	4.4%
N=655 IVDK 2010 (7)	29.8%	18.0 %	12.8%	11.6 %	9.6%	6.7%	4.7%	2.8%

Table 57: Background information on studies reporting results with (all) single constituents of the FM II (**citronellol, citral, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), Farnesol, alpha-Hexyl-cinnamic aldehyde**)

Reference	Country	Study period, Patients	Comments by reviewers
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
EU 2005 (10)	6 European centres	10/2002 – 06/2003, n=1701	Applied in consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients

IVDK 2010b (11)	Germany, Switzerland and one centre in Austria	2005-2008 n=35633 tested with FM II, n=2217 with all SC	

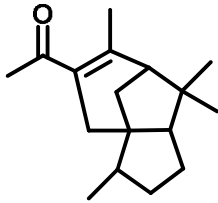
Table 58: Background information on studies reporting results with several fragrance compounds not, or only partly, corresponding to mixes (later created) or with essential oils

Reference	Country	Study period, Patients
deGroot 2000 (12)	The Netherlands (multicentre)	09/1998-04/1999 n=1825 consecutive patients
An 2005 (13)	South Korea (multicentre)	04/2002 – 06/2003 n=422 consecutive patients
Sugiura 2000 (14)	Nagoya, Japan	1990-1998 n=1483 patients with suspected cosmetic dermatitis
Frosch 1995 (15)	11 European depts.	Prior to 1995 n=1069 consecutive patients
Frosch 2002 a (16)	6 European depts.	10/1997-10/1998 n=1855 consecutive patients
Frosch 2002 b (17)	6 European depts.	Prior to 2002 n=1606 consecutive patients
Coimbra 2000 (9)	Portugal	07/1989-06/1999 n=226 with FM I SC n=67 also with other fragrances
Larsen 1977 (18)	US	1977 n=20 “perfume-sensitive patients”
Larsen 2001 (19)	worldwide multicentre	? (prior to 2001) n=178 patients with known contact allergy to fragrance ingredients
Belsito 2006 (20)	North American (5 US, 1 Canadian) depts.	2003 n=1603 patients
NACDG 2009 (21)	US and Canada	2005-2006 n= 4454 patients
Wöhrl 2001 (22)	“FAZ” clinic Vienna	1997-2000 n=747 of 2660 consecutive patients tested with special series
EECDRG 1995 (15)	European, multicentre	Different fragrances, tested in 2 concentrations, in sets of about 100 patients each in different centres
Goossens 1997 (23)	Leuven, Belgium	1978-1987 n=111 “Japanese perfume series” (highly selected patients)

Opinion on fragrance allergens in cosmetic products

Reference	Country	Study period, Patients
Malten 1984 (24)	Dutch multicentre	N=182 patients with suspected cosmetic dermatitis tested with 22 fragrance compounds
DeGroot 1985 (25)	Dutch	N=179 patients with suspected cosmetic dermatitis tested with 16 fragrance compounds
Rudzki 1976 (26)	Warsaw, Poland	N=200 consecutive patients
Rudzki 1986 (27)	Warsaw, Poland	N=86 patients of 299 (of 5315) patients with positive reaction to FM I tested with essential oils series
Santucci 1987 (28)	Rome, Italy	N=1500 consecutive patients; n=63 reacting positively to FM I re-tested with extended fragrance series
Nakayama 1974 (after (29))	Japan	N=183 patients with cosmetic dermatitis
IVDK 2010c (30)	Germany, Switzerland and one centre in Austria	15682 patients tested with at least one essential oil in different test series
Trattner/David (31)	Tel Aviv, Israel	N=641 consecutive patients

Catalogue of single chemicals evaluated

ACETYLCEDRENE	
CAS # 32388-55-9	
EC # 251-020-3	
1-[(3R,3aR,7R,8aS)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl]-ethanone	
Other names 1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-, [3R-(3α,3αβ,7β,8α)]-Ethanone; 1H-3a,7-Methanoazulene, Ethanone deriv.; Acetyl-α-cedrene; Lixetone; Vertofix	

Current regulation: /

Clinical

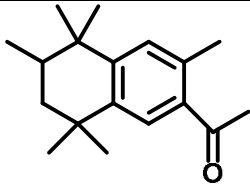
data:

In the Frosch 2002 a study, a total of 0.2% had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction to 1% and none to 5% "Vertofix ®" in pet., tested in 100 consecutive patients in Stockholm, were observed (15). In a case report, a 28-year-old patient with axillary dermatitis after using 2 different deodorants tested positive not only to HICC, but also to acetyl cedrene (tested 10.8% in diisopropylene glycol (20 healthy controls negative) (32). In this case report it is stated that "Acetyl cedrene (Vertofix Coeur) is a complex reaction mixture of which a principal constituent is methyl cedryl ketone".

Additional

information:

Acetyl cedrene (Vertofix®, IFF) is a complex mixture obtained from cedar wood oil by the acetylation of terpenes. The principal component of acetyl cedrene is methyl cedryl ketone (CAS 32388-55-9). It is a "top 100" substance (IFRA, pers. comm.2010)

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	
CAS # 21145-77-7	
EC # 216-133-4 / 244-240-6	
1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone	
AHMT (perfume), AHTN, Extralide, Fixolide, Musk tonalid, NSC 19550, Tentarome, Tetralide, Tonalid, Tonalide.	

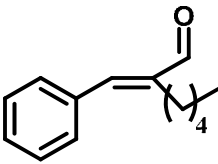
Current regulation: Annex III, part 1, entry 182

Clinical

data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Tonalide ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

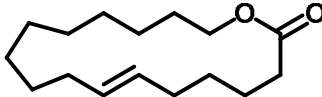
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; Jasmine aldehyde; NSC 6649; Pentylcinnamaldehyde; α-Amyl-β-phenylacrolein; α-Amylcinnamal; α-Amylcinnamaldehyde; α-Pentylcinnamaldehyde	

Current regulation: Annex III, part 1, entry 67

Clinical data:
In the “background information” section of the 1999 opinion (33), amyl cinnamal (synonymous: alpha amyl cinnamaldehyde) has been classified as frequently reported contact allergen because it has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=4, i.e., 0.2% (95% CI: 0.1 – 0.5%) positive reactions to this compound (1% pet.) in 2062 consecutively PTed patients (4). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed (6). The Larsen 2001 study yielded 2.3% positive reactions in 178 patients with known contact allergy to fragrance ingredients (test concentration: 5% pet.) (19). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to amyl cinnamal (22). The IVDK 2010 study, 0.26% (95% CI: 0 – 0.60%) of 1214 consecutively tested patients reacted to the compound, while 0.61% (95% CI: 0.36 – 0.86%) of 4375 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7).

Additional information:
It is a “top 100” substance and classified as R43 (IFRA, pers. comm. 2010).

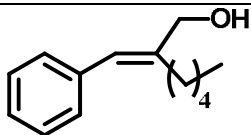
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

Current regulation: /

Clinical data:
The Larsen 2001 study, using omega-6-hexadecenlactone (HDL, 5% pet.) as test concentration, diagnosed 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Ambrettolide is 1 of 2 components of Ambrette seed oil (obtained from *Hibiscus abelmoschus* L., *Malvaceae*) responsible for the musk odour. In Surburg/Panten, the compound has the chemical name (Z)-7-hexadecen-16-olide (or Hexadec-7-en-16-olide according to CosIng), CAS 123-69-3 (34).

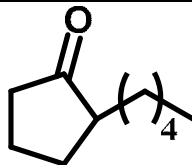
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol,	
2-Benzylidene- (6Cl,8Cl)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

Current regulation: Annex II, Part 1, entry 74

Clinical data:
In the “background information” section of the 1999 opinion, amyl cinnamyl alcohol is mentioned to cross-react with amyl cinnamal. Moreover, this compound has been identified as a cause of allergic reactions in a notable number of persons with eczema from the use of cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.4% (95% CI: 0.1 – 0.7%) positive reactions in 1977 consecutively PTed patients (4). The IVDK 2010 study, 0.79% (95% CI: 0.54 – 1.04%; percentages standardised for age and sex) of 5650 patients PTed reacted to the compound (7). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6).

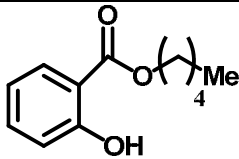
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

AMYLCYCLOPENTANONE	
CAS # 4819-67-4	
EC # 225-392-2	
2-Pentylcyclopentanone	
2-Pentyl-1-cyclopentanone; 2-Pentylcyclopentanone; 2-Pentylcyclopenten-1-one; 2-n-Amylcyclopentanone; 2-n-Pentyl cyclopentanone; Delphone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

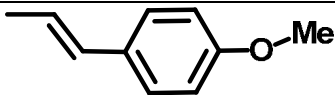
AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

Current regulation: /

Clinical data:

In the Frosch 2002 a study, a total of n=3 (0.2%) had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% amyl salicylate and 1 positive reaction to 5% amyl salicylate were observed in 100 consecutive patients patch tested in Stockholm (15).

Additional information:
A RIFM review is available (36). It is a “top 100” substance (IFRA, pers. comm.2010)

trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, EINECS); E-Anethole (INCI); 1-Methoxy-4-[(1E)-1-propenyl]benzene; (E)-1-Methoxy-4-(1-propenyl)-benzene (CosIng); NSC 209529; trans-1-(4-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)propene; trans-1-p-Anisylpropene; trans-4-(1-Propenyl)anisole; trans-Anethol; trans-Anethole; trans-p-Anethole; trans-p-Methoxy-β-methylstyrene	

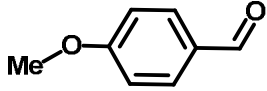
Current regulation: /

Clinical data:

A case of a 64 year old patient, who developed severe cheilitis and a loss of taste has been described (37). Both were reversible after the cessation of use of previous toothpastes. The patch test was strongly positive to anethole (isoform not given) 5% pet.; this was found an ingredient of the causative toothpaste. Two cases of occupational allergic contact dermatitis occurring in a traditional cake factory due to anise oil have been described, both testing (strongly) positive to anise oil (5% o.o.) and anethole (5% pet.) (38).

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). trans-Anethole can be purified from star anise oil (34, 39), see 3.2., and is the main component of anise, star anise

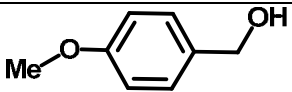
and fennel oils (38)

ANISALDEHYDE	
CAS # 123-11-5	
EC # 204-602-6	
4-Methoxy-benzaldehyde	
p-Methoxybenzaldehyde; p-Anisaldehyde; 4-Anisaldehyde; Aubepine; Crategine; NSC 5590; Obepin; p-Anisic aldehyde; Anisic aldehyde; p-Formylanisole.	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

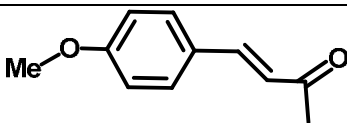
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy- α -hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methoxy)phenyl]methanol; p-(Methoxyphenyl)methanol; p-Anisalcohol; p-Anisyl alcohol; p-Methoxybenzyl alcohol	

Current regulation: Annex III, part 1, n° 80

Clinical data:
In the “background information” section of the 1999 opinion, anisyl alcohol is classified as “less frequently reported allergen”; 2 studies were identified where 3 and 4 cases, respectively, with cosmetic dermatitis due to contact allergy to anisyl alcohol had been reported (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded $n=1$, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2004 consecutively PTed patients, patch test concentration: 1% pet. (4). Similar results were obtained in the following period, with $n=1$ (and $n=3$ irritant and $n=6$ doubtful) reactions in 986 patients tested with 1% in pet. (30). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). This test concentration has been regarded as relatively high by Hostynek and Maibach (40). The test concentration of Anisyl Alcohol has been further validated by Bruze et al. and 10% in pet was recommended as a non-irritant concentration for routine investigations (40a).

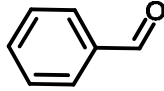
Additional information: /

ANISYLIDENE ACETONE	
CAS # 943-88-4	
EC # 213-404-9	
4-(4-Methoxyphenyl)-3-Buten-2-one	
1-(p-Methoxyphenyl)-1-buten-3-one; 4-(4-Methoxyphenyl)-3-buten-2-one; 4-(p-Methoxyphenyl)-3-buten-2-one; 4-Methoxybenzalacetone; 4-Methoxybenzylideneacetone; 4-Methoxystyryl methyl ketone; 4'-Methoxybenzylideneacetone; Anisalacetone; Methyl p-methoxystyryl ketone; NSC 31752; NSC 7946; p-Anisalacetone; p-Methoxybenzalacetone; p-Methoxybenzylideneacetone; p-Methoxystyryl methyl ketone	

Current regulation: Annex III, part 1, n° 443

Clinical data:
In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to anisylidene acetone 2% pet. (24)

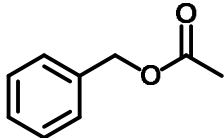
Additional information: /

BENZALDEHYDE	
CAS # 100-52-7	
EC # 202-860-4	
Benzaldehyde	
Artificial Almond Oil; Benzaldehyde FFC; Benzenecarbonal; Benzenecarboxaldehyde; Benzoic acid aldehyde; Benzoic aldehyde; NSC 7917; Phenylformaldehyde; Phenylmethanal	

Current regulation: /

Clinical data:
 In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions to benzaldehyde 5% pet. (22). The IVDK 2010 study, 6 weak positive reactions were observed, i.e., 0.16% (95% CI: 0.03 – 0.29%; percentages standardised for age and sex) of 2820 patients PTed reacted to the compound (7). A review is available in the Int. J. Toxicol. (41). In the case of a 19 year old pastry maker, Seite-Bellezza et al. report on immediate reactions to MP, cinnamal and benzaldehyde (tested at 5% pet.) subsiding after a few hours, in line with the patient's history (42).

Additional information: /

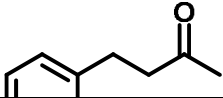
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl acetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

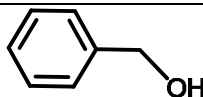
Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl acetate in pet., tested in 100 consecutive patients in Odense, DK, were observed (15). Benzyl acetate is a component of several natural mixtures, for example a major constituent of Narcissus abs., and a minor constituent of Jasmine abs. (17).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

BENZYL ACETONE	
CAS # 2550-26-7	

EC # 219-847-4	
4-Phenyl-2-butanone	
4-Phenylbutan-2-one (REACH, EINECS); Benzylacetone; Methyl 2-phenylethyl ketone; Methyl phenethyl ketone; NSC 44829; NSC 813M; Phenethyl methyl ketone; 1-Phenyl-3-butanone; 2-Phenylethyl methyl ketone	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (43).	

BENZYL ALCOHOL	
CAS # 100-51-6	
EC # 202-859-9	
Phenylmethanol	
Benzyl alcohol; (Hydroxymethyl)benzene; Benzenecarbinol; Benzylic alcohol; NSC 8044; Phenylcarbinol; Benzenemethanol; Phenylmethyl alcohol; Sunmorl BK 20; TB 13G; α-Hydroxytoluene; α-Toluenol	
Current regulation: Annex III, part 1, n° 45; Annex VI, part1, n ° 34	

Clinical data:
In the “background information” section of the 1999 opinion, benzyl alcohol is classified as allergen frequently causing allergic reactions. It has been found to cause allergic reactions in 1.2 to 15% of patients with eczema from cosmetic products (33). A CIR expert panel review is available in the Int. J. Toxicol. (44).

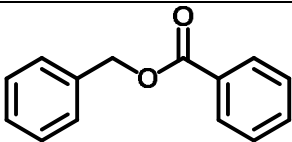
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

Both in terms of case reports (45-47) and clinical epidemiology data (0.22 % [95% CI: 0.16 – 0.28%] positive tested with benzyl alcohol in the context of a “topical drugs” series, n=26448 (7)) the relevance of this alternative exposure is highlighted. In a study from Alicante, Spain, 86 selected patients were tested with benzyl alcohol, yielding 2 positive reactions (48).

After application of saline soaks preserved with benzyl alcohol onto his stasis dermatitis, a 53 year old patient developed a rash, which was, according to test results obtained by J. D. Guin and J. Goodman, at least partly due to an immediate hypersensitivity to benzyl alcohol, as verified by an intense urticarial reaction at the test site lasting several days (49). According to 2 cases reported by A. A. Fisher, PT-proven, relevant delayed type hypersensitivity is not associated with immediate reactions in scratch or intradermal tests (50). D. W. Shaw describes a patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications (51). Another contribution points to covert exposures to benzyl alcohol even in products labelled “fragrance free” (52) probably because benzyl alcohol is used as preservative, or an essential oil containing benzyl alcohol is used as cosmetic ingredient.

Additional information:

Benzyl alcohol is a component of several natural mixtures, including Myroxylon pereirae resin, which have been used for extraction, but is nowadays synthesised (53). It is permitted in certain foodstuffs (liquors: < 100 mg/l, sweets and cakes: < 250 mg/kg) under the coding “E 1519” (http://www.zusatzstoffe-online.de/zusatzstoffe/317.e1519_benzylalkohol.html), last accessed 2009-11-27). In addition to being a fragrance compound (which may be used, even in relatively high concentration, to scent topical medications (54)), benzyl alcohol is used as antioxidant in topical therapeutics or cosmetics. The German “Rote Liste” (<http://www.rote-liste.de>, last accessed 2009-11-11), for instance, lists 205 specialties containing benzyl alcohol. Benzyl alcohol may be used up to 1.0% as a preservative in cosmetic products according to the Cosmetic Directive 76/768/EEC

BENZYL BENZOATE	
CAS # 120-51-4	
EC # 204-402-9	
Benzyl benzoate	
Benzyl ester benzoic acid; Ascabin; Ascabiol; Benylate; Benzyl benzenecarboxylate; Benzyl benzoate; Benzyl phenylformate; Benzylets; Colebenz; NSC 8081; Nicca Sunsolt LM 7EX; Novoscabin; Pelemol B66; Peruscabin; Phenylmethyl benzoate; Scabagen; Scabanca; Scabcare BB; Scabide; Scabiozon; Scobenol; Vanzoate; Venzonate	

Current regulation: Annex III, part 1, n° 85

Clinical data:

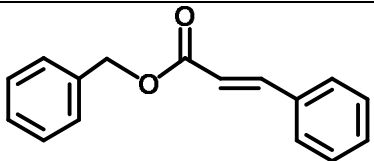
In the “background information” section of the 1999 opinion, benzyl benzoate is classified as “less frequently reported allergen”; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2003 consecutively PTed patients, test concentration 1% pet. (4). In the subsequent period (2005-2008), n=1062 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). Thus, the pooled proportion of positive patch test reactions is 1 / 3385 (0.03%, exact upper 1-sided 95% CI: 0.14%)

Additional information:

Benzyl benzoate naturally occurs in MP resin and ylang-ylang oil. Nowadays it is synthesised and used for a variety of purposes (53). These include use as a scabicide (one brand specialty on the German market, using a concentration of 10% for children and 25% for adults), possibly with some differences among European countries. In France, a combination of benzyl benzoate 10% and sulfiram 2% is reported to be used most often (55). Hausen et al. review the older literature and mention a study identifying 1 sensitised patient in 73 patients treated for scabies (details not given) (53). According to the mandatory factsheet (see PDF “benzylbenzoate_infosheet_DE.pdf”) dermatitis after anti-scabies treatment is “rare”, in a range between 1:1000 and 1:10000.

It is a “top 100” substance (IFRA, pers. comm.2010).

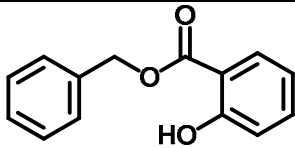
<i>BENZYL CINNAMATE</i>	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

Current regulation: Annex III, part 1, n° 81

Clinical data:
In the “background information” section of the 1999 opinion, benzyl cinnamate (synonymous: benzyl 3-phenyl-2-propenoate, cinnamein) is classified as “less frequently reported allergen”; one study of patients with contact allergy to cosmetic products was identified and further a study where benzyl cinnamate associated with contact sensitisation to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2042 consecutively PTed patients, test concentration 5% pet. (4). The IVDK 2010 study, n=4 weak positive were observed, amounting to 0.12% (95% CI: 0 – 0.25%; percentages standardised for age and sex) of 2872 patients PTed reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, using the same test concentration, were observed in 320 patients (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22).

Additional information: A RIFM review is available (56).

BENZYL SALICYLATE	
CAS # 118-58-1	
EC # 204-262-9	
Benzyl 2-hydroxybenzoate	
Salicylic acid, Benzyl ester; Benzoic acid, 2-Hydroxy-, phenylmethyl ester; Benzyl o-hydroxybenzoate; NSC 6647	

Current regulation: Annex III, part 1, n° 75

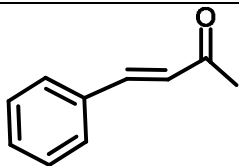
Clinical data:

In the “background information” section of the 1999 opinion (33), benzyl salicylate is classified among the frequent allergens, with 0.2 to 10% of patients with eczema from cosmetic products testing positively. In one study, benzyl salicylate accounted for 75% of reactions to commercial products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=2, i.e. 0.1% (95% CI: 0.01 – 0.4%) positive reactions in 2041 consecutively PTed patients (test concentration 1% pet.) (4). The IVDK 2010 study, 2 of 3775 patients PTed reacted weakly positive to the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at 2% pet. (6). In the deGroot 2000 study, 10 of 1825 consecutive patients tested positive to benzyl salicylate (2% pet.), of these, 3 were not detected by the FM I (12). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22). Trattner/David found 2 positive cases in 641 consecutive eczema patients (31). In a study from Alicante, Spain, 86 selected patients were tested with benzyl salicylate, yielding 2 positive reactions (48).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010). A RIFM review is available, including internal results on, e.g. HRIPT, and a review of LLNA results, where benzyl salicylate is classified as “weak” allergen (57).

BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; Acetocinnamone; Benzalacetone; Benzylideneacetone; Methyl 2-phenylvinyl ketone; Methyl phenylvinyl ketone; Methyl styryl ketone; Methyl β-styryl ketone; NSC 5605; Styryl methyl ketone	

Current regulation: Annex II, n° 356

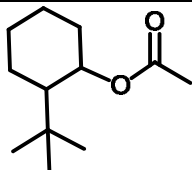
Clinical

data:

In the Malten 1984 study, none of 182 patients displayed a positive PT reaction to

benzylidene acetone 0.5% pet. (24).

Additional information: /

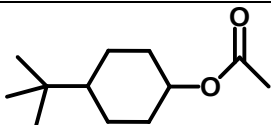
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Verdox ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (58).

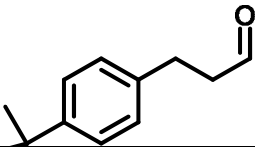
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinal A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; NSC 163103; Oryclone, Oryclone special, Oryclon extra; p-t-BCHA; p-tert-Butylcyclohexyl acetate; para-tert-Butylcyclohexyl acetate; PTBCHA; Velvetone; Verbeniax; Vertenex; Vertinate; Vertopol; Ylanate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Vertenex ®” in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (59).

p-tert -Butyldihydrocinnamaldehyde	
CAS # 18127-01-0	
EC # 242-016-2	

4-(1,1-Dimethylethyl)-benzenepropanal	
p-tert-Butyl-hydrocinnamaldehyde; 3-(4-tert-Butylphenyl)propanal; Bourgeonal; p-tert-Butyldihydrocinnamaldehyde	

Current regulation: III/155

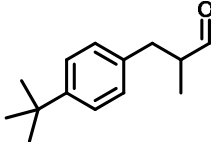
Clinical data: /

Additional

information:

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=39132

BUTYLPHENYL METHYLPROPIONAL (Lilial®)	
CAS # 80-54-6	
EC # 201-289-8	
3-(4-tert-Butylphenyl)-2-methylpropanal	
p-t-Butyl-α-methylhydrocinnamic aldehyde; 2-(4-tert-Butylbenzyl)propionaldehyde (REACH, EINECS); 4-(1,1-Dimethylethyl)-α-methyl-benzenepropanal; Hydrocinnamaldehyde, p-tert-Butyl-α-methyl-; ($\pm$)-2-Methyl-3-(4-tert-butylphenyl)propanal; 2-Methyl-3-(4-tert-butylphenyl)propanal; 2-[(4-tert-Butylphenyl)methyl]propanal; 3-(4-tert-Butylphenyl)-2-methylpropanal; 3-(p-tert-Butylphenyl)-2-methylpropionaldehyde; 3-(p-tert-Butylphenyl)isobutylaldehyde; 4-(1,1-Dimethylethyl)-α-methylbenzenepropanal; 4-tert-Butyl-α-methylhydrocinnamic aldehyde; Lilestralis; Lilial; Lysmeral; NSC 22275; lilestral; p-tert-Butyl-α-methylhydrocinnamaldehyde; p-tert-Butyl-α-methylhydrocinnamic aldehyde; pt-Bucinal; α-Methyl-p-tert-butylhydrocinnamaldehyde; β-Lilial	

Current regulation: Annex III, part 1, n° 83

Clinical data:

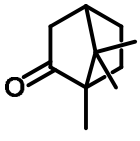
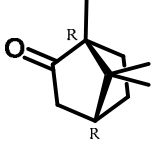
In the “background information” section of the 1999 opinion, lilial is classified as “less frequently reported allergen”; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were considered to possibly have been false positive (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002a study yielded 0.2% positive reactions to Lilial® (10% pet.) among the 1855 consecutive patients tested (16). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2004 patients consecutively tested (4). The IVDK 2010 study, 0.62% (95% CI: 0.04 – 1.21%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In the deGroot 2000 study, 9 of 1825 consecutively tested patients had a positive reaction to lilial® (5%

pet.) (12). Lilial® has been identified as constituent of perfumes used by a patient, causing ACD (60).

Additional information:

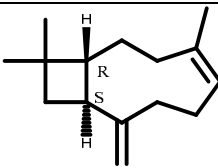
It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2
CAS # 76-22-2 / 464-49-3	
EC # 207-355-2 / 200-945-0	
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (76-22-2) (1R,4R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (464-49-3)	
76-22-2: DL-Bornan-2-one (REACH, EINECS); 2-Bornanone; Bornan-2-one, INCI name according to CAS; CAMPHOR/DL-bornan-2-one; Camphor; (±)-Camphor; DL-Camphor; 1,7,7-Trimethylnorcamphor; 2-Camphanone; Alphanon; Borneo camphor; Root bark oil; Spirit of camphor 464-49-3: (1R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one; (1R,4R)-(+)-Camphor; (+)-2-Bornanone; (+)-Camphor; (1R)-(+)-Camphor; (1R)-Camphor; (1R,4R)-(+)-Camphor; (R)-(+)-Camphor; (R)-Camphor; Camphor; D-Camphor; D-(+)-Camphor; Alcanfor; Japanese camphor.	 464-49-3

Current regulation: /

Clinical data:
From the UK, a case of allergic contact dermatitis after application of Earex ® ear drops due to rectified camphor oil (tested 10% pet.) was reported (61). Application of a liquid rubefacient of Asian origin caused allergic contact dermatitis in a 58-year-old patient, according to the positive PT result with 10% camphor ("alcaonfor") in pet. due to this ingredient (62). In the US, a case of contact dermatitis due to "Vics VapoRub" has been reported (63).

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

beta-CARYOPHYLLENE	
CAS # 87-44-5	
EC # 201-746-1	
(1R,4E,9S)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	
(E)-(1R,9S)-(-)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; [1R-(1R*,4E,9S*)]-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; (-)-(E)-Caryophyllene; (-)-Caryophyllene; (-)-E-Caryophyllene; (-)-trans-Caryophyllene; (-)-β-Caryophyllene; (E)-Caryophyllene; Caryophyllene; Caryophyllene B; NSC 11906; l-Caryophyllene; trans-Caryophyllene; β-Caryophyllen; β-Caryophyllene; (-)-β-Caryophyllene	

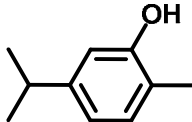
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% positive reactions to caryophyllene (5% pet.) in 1606 consecutive were observed (17).

Additional information:

beta-Caryophyllene autoxidizes at air exposure. As the primary oxidation products, the hydroperoxides, are very unstable and immediately form epoxides with low sensitizing capacity, the increase in allergenic activity caused by autoxidation is comparably low (64). A multicenter study identified 0.5% positive reactions to oxidized *beta*-caryophyllene (3.0% pet.) in 1511 consecutive patients (65). Of these, 2 patients (0.1%) reacted to the major oxidation product (caryophyllene oxide) (3.9% pet.).

CARVACROL	
CAS # 499-75-2	
EC # 207-889-6	
2-Methyl-5-(1-methylethyl)-phenol	
2-Hydroxy-1-methyl-4-(1-methylethyl)benzene; 2-Hydroxy-p-cymene; 2-Methyl-5-(1-methylethyl)phenol; 2-Methyl-5-isopropylphenol; 3-Isopropyl-6-methylphenol; 5-Isopropyl-2-methylphenol; 5-Isopropyl-o-cresol; 6-Methyl-3-isopropylphenol; Antioxine; Dentol; Isopropyl o-cresol; Isothymol; NSC 6188; p-Cymen-2-ol	

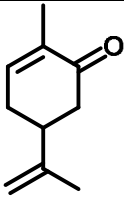
Current regulation: /

Clinical data:

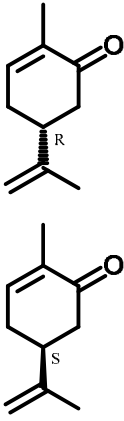
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an “excited back syndrome” and are thus of limited evidence (25). Meynadier et al. ¹¹ patch tested 28 patients with contact allergy to fragrance ingredients using 2% carvacrol in pet. Positive reactions were observed in 3 of 28 patients (after (66)).

Additional information:

Carvacrol is derived from p-cymene by sulfonation followed by alkali fusion. Carvacrol can also be derived from savory, thyme, marjoram, oregano, lovage root, and Spanish origanum oil (66). Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001, according to (66)).

CARVONE	
CAS # 99-49-0 / 6485-40-1 / 2244-16-8	
EC # 202-759-5 / 229-352-5 / 218-827-2	
2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (99-49-0)	

¹¹ Meynadier, J. M., J. Meynadier, J. L. Peyron, and L. Peyron. 1986. Clinical forms of skin manifestations in allergy to perfume. *Ann. Dermatol. Venerol.* 113:31–39.

(5R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (6485-40-1) (5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (2244-16-8)	
99-49-0: p-Mentha-6,8-dien-2-one; (±)-Carvone; 2-Methyl-5-isopropenyl-2-cyclohexenone; 5-Isopropyl-2-methyl-2-cyclohexen-1-one; Carvone; DL-Carvone; Karvon; Limonen-6-one; NSC 6275; p-Mentha-1(6),8-dien-2-one 6485-40-1: R)-(-)-p-Mentha-6,8-dien-2-on); (-)-(5R)-Carvone; (-)-(R)-Carvone; (-)-Carvone; (-)-p-Mentha-6,8-dien-2-one; (4R)-(-)-Carvone; (R)-(-)-Carvone; (R)-Carvone; L-(-)-Carvone; L-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; l-Carvone 2244-16-8: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone	

Current regulation: /

Clinical data:

Cases of allergic contact cheilitis due to L-carvone in toothpastes have been reported (67-69). In an earlier study, 15 of 541 (2.8%) of consecutive PT patients tested also with L-Carvone (5% pet.) exhibited positive reactions, which were (i) associated with positive PT results to *Compositae* mix and (ii) mostly were not considered clinically relevant. Upon re-testing with lower concentrations (2% and 1% pet.) only 2 of 8 patients thus tested were positive (70).

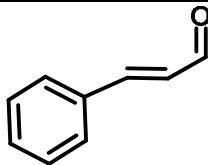
“Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis on the head. She had used a hair conditioner with a “mint” scent, and the dermatitis resolved when she discontinued using this product. While the manufacturer would not confirm carvone as an ingredient, the clinical course, patch-test results, and ingredient list strongly suggest that this was a relevant allergen in this case of allergic contact dermatitis”¹²

Additional information:

D-Carvone occurs in caraway seed oil and dill oil in a concentration of up to 60%. L-Carvone is a component of the oil from *Mentha spicata* (spearmint).

R-Carvone is identified as a secondary oxidation product in autoxidized limonene (71). However, it is not a major allergen in this oxidation mixture and only one of 30 patients with known contact allergy to oxidized R- limonene reacted when tested with carvone (3% pet.) (72). Experimental findings in guinea pigs show no cross reactivity between R- and S carvone, but both enantiomers were found to be equally strong sensitizers (73).

¹² <http://www.ncbi.nlm.nih.gov/pubmed/20233552>

CINNAMAL	
CAS # 104-55-2	
EC # 203-213-9	
3-Phenyl-2-propenal	
Cinnamaldehyde; 3-Phenyl-2-propen-1-al; 3-Phenyl-2-propenaldehyde; 3-Phenylacrolein; 3-Phenylacrylaldehyde; 3-Phenylpropenal; Abion CA; Benzylideneacetaldehyde; Cassia aldehyde; Cinnacure; Cinnamal; Cinnamic aldehyde; Cinnamite; Cinnamyl aldehyde; NSC 16935; NSC 40346; Phenylacrolein; Zimtaldehyde; β-Phenylacrolein	

Current regulation: Annex III, part 1, n° 76

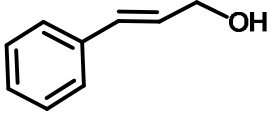
Clinical data:
In the “background information” section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.0% (95% CI: 0.6 – 1.6%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.6% (95% CI: 0.5 – 3.6%) had positive reactions to cinnamal (6). In a study by the North American Contact Dermatitis Group, no significant trend of cinnamal contact sensitisation in the consecutive patients analysed was observed between 1984 (5.9% pos.) and 2000 (3.6% pos.); tested at 1% pet. (74). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded 1.7% positive reactions. In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.9% positive reactions (22). The NACDG study found 3.1% positive reactions in 4435 patients tested (21). The IVDK 2010 study, 1.43% (95% CI: 0.67 – 2.18%) of 1214 consecutively tested patients reacted to the compound, while 2.64% (95% CI: 2.16 – 3.13%) of 4527 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=7 reacted positively to cinnamal (48).

While, in addition to typical ACD due to contact sensitisation, immediate reactions to some fragrance compounds (and MPR, see below) are observed not infrequently, such immediate type reactions may rarely be very severe (anaphylaxis) and possibly immunologically mediated, as illustrated by the case of a 42 year old nurse with anaphylaxis (maximum grade of contact urticaria syndrome) 20 min after application of cinnamal (75). Following industrial use as “odour masking” agent, cinnamal caused occupational ACD in an exposed worker (76).

Additional information:

A specific RIFM review is available (77); another RIFM review addresses several cinnamic compounds (78).

CINNAMYL ALCOHOL	
CAS # 104-54-1	
EC # 203-212-3	
3-Phenyl-2-propen-1-ol	

Opinion on fragrance allergens in cosmetic products

Cinnamyl alcohol; 1-Phenyl-3-hydroxy-1-propene; 1-Phenylprop-1-en-3-ol; 3-Hydroxy-1-phenylprop-1-ene; 3-Phenyl-2-propenol; 3-Phenylallyl alcohol; Cinnamic alcohol; NSC 623440; NSC 8775; Styrene; Styryl alcohol; Styryl carbinol; γ -Phenylallyl alcohol	

Current regulation: Annex III, part 1, n° 69

Clinical data:

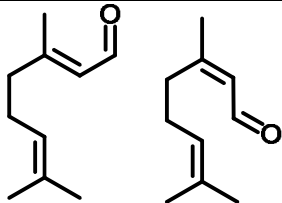
In the “background information” section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to cinnamyl alcohol, tested at 2% pet., i.e., twice the commonly used concentration (6). As test concentrations of up to 5% are apparently non-irritating (de Groot et al. after (33)), the latter data can be regarded as valid. In the An 2005 study, 13 of 422 consecutive patients, i.e., 3.1%, had positive reaction (13) (test concentration 2%). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 0.73% (95% CI: 0.17 – 1.30%) of 1214 consecutively tested patients reacted to the compound, while 2.36% (95% CI: 1.89 – 2.83%) of 4502 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to cinnamyl alcohol (48).

Additional information:

In a recent experimental study protein-cinnamal adducts were detected in skin homogenates treated with cinnamal and cinnamyl alcohol but not with alpha-amyl cinnamal. This suggests that there is a common hapten involved in cinnamal and cinnamyl alcohol sensitization, in line with the observation of a marked concordance upon patch testing (7, 79), and that metabolic activation (to cinnamal) is involved in the latter. Conversely, there does not appear to be a common hapten for cinnamal and alpha-amyl cinnamal (80), again in line with the observations in the IVDK 2010 study (7).

A RIFM review is available (81)

CITRAL	 Citral = isomeric mixture of Geranial and Neral
CAS # 5392-40-5	
EC # 226-394-6	
3,7-Dimethyl-2,6-octadienal	
3,7-Dimethyl-2,6-octadien-1-al; Citral; Citral PQ Extra; Lemarome N; Lemsyn GB; NSC 6170	

Current regulation: Annex III, part 1, n° 70

Clinical data:

In the “background information” section of the previous opinion (33), citral is classified as frequent allergen, causing about 1% allergic reactions in consecutive PT patients, and being a proven cause of contact allergic reactions in 2.6% patients with eczema from

cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002 a study yielded 1.1% positive (and 1.3% doubtful) reactions among the 1855 consecutive patients tested (16). In a study on 586 consecutive patients with hand eczema it has been noted that citral (2% pet.) not only caused (mostly weak) positive PT reactions, but far more often irritant reactions (n=82 vs. n=28). It was hypothesised that this very property could contribute to citral's sensitising potential (82). In the EU 2005 study, 12 of 1701 patients (0.7%, 95% CI: 0.4 – 1.2%) reacted positively to 2% citral in pet. (10). The IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2021 consecutively PTed patients; 10 of 13 citral positive patients also reacted positively to geraniol (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 multicentre study, 19 of 1825 consecutive patients tested positively to citral (2% pet.), 4 of whom did not react positively to the FM I (12). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reaction (13) (test concentration 2%). In the Malten 1984 study, neral at 1% in pet. yielded 2.6% positive reactions in 182 patients (24). In a study from Alicante, Spain, 86 selected patients were tested with citral, yielding 2 positive reactions (48).

Citral in a lip salve has been reported to have caused longstanding, recurrent allergic contact cheilitis in a 30 year old female patient, diagnosed by a strong positive reaction to the FM II, followed by a strong positive reaction to citral (83).

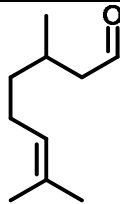
Additional information:

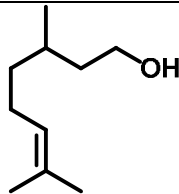
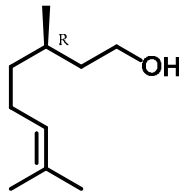
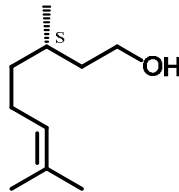
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

Geranial forms oxidation product with increased sensitizing capacity both via spontaneous autoxidization at air exposure and via metabolic oxidation (Hagvall L. Thesis 2009: <http://hdl.handle.net/2077/18951>).

Geranial and neral have been identified as secondary oxidation products when geraniol autoxidizes (84). They have also been identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLAL	
CAS # 106-23-0	
EC # 203-376-6	
3,7-Dimethyl-6-octenal	
(±)-Citronellal; 2,3-Dihydrocitral; 3,7-Dimethyloct-6-en-1-al; Citronellal; NSC 46106; Rhodinal; dl-Citronellal; β-Citronellal	
Current regulation: /	
Clinical /	data:
Additional information: A compound of essential oils of citrus fruits, namely grapefruit, but also contained in “citronella oil” and oil of Melissa.	

CITRONELLOL			
CAS # 106-22-9 / 1117-61-9 / 7540-51-4			
EC # 247-737-6 / 214-250-5 / 231-415-7			
3,7-Dimethyl-6-octen-1-ol (106-22-9); (3R)-3,7-Dimethyl-6-octen-1-ol (1117-61-9); (3S)-3,7-Dimethyl-6-octen-1-ol (7540-51-4)			106-22-9
106-22-9: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronello 950; DL-Citronellol; Dihydrogeraniol; NSC 8779; Rodinol; dl-Citronellol; β-Citronellol			
1117-61-9: (R)-3,7-Dimethyl-6-octen-1-ol; (R)-(+)-3,7-Dimethyl-6-octen-1-ol; (+)-(R)-Citronellol; (+)-Citronellol; (+)-β-Citronellol; (3R)-(+)-β-Citronellol; (R)-(+)-Citronellol; (R)-(+)-β-Citronellol; (R)-Citronellol; (R)-β-Citronellol; D-Citronellol; d-Citronellol	1117-61-9	7540-51-4	
7540-51-4: (-)-3,7-Dimethyl-6-octen-1-ol; (-)-(S)-Citronellol; (-)-Citronellol; (-)-β-Citronellol; (S)-(-)-Citronellol; (S)-(-)-β-Citronellol; (S)-3,7-Dimethyl-6-octen-1-ol; (S)-Citronellol; (S)-β-Citronellol; L-Citronellol; l-Citronellol			

Current regulation: Annex III, part 1, n° 86

Clinical data:

In the “background information” section of the 1999 opinion, citronellol is classified as “less frequently reported allergen”; with few cases of contact allergy reported in the literature (33).

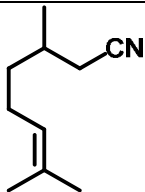
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronello“ (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%, 95% CI: 0.06 – 0.6%) reacted positively to 1%

citronellol in pet.; at the same concentration, n=23 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2003 patients consecutively PTed (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at only 2% pet. (6). The Larsen 2001 study yielded 5.6% positive reactions to l-citronellol (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Citronellol autoxidizes spontaneously in contact with air in the same way as linalool forming allergenic primary oxidation products, hydroperoxides (AT Karlberg, personal communication, 2011).

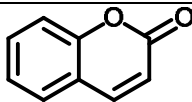
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLYL NITRILE	
CAS # 51566-62-2	
EC # 257-288-8	
3,7-Dimethyl-6-octenenitrile	
3,7-Dimethyl-6-octenenitrile (REACH, EINECS, INCI); Agrunitril; Agrunitrile; Citronellyl nitrile	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010)

COUMARIN	
CAS # 91-64-5	
EC # 202-086-7	
2H-1-Benzopyran-2-one	
1,2-Benzopyrone; 2-Chromenone; 2-Propenoic acid, 3-(2-hydroxyphenyl)-, δ -lactone; 5,6-Benzo-2-pyrone; Benzo- α -pyrone; Coumarinic anhydride; NSC 8774; Rattex; Tonka bean camphor; cis-o-Coumarinic acid lactone; o-Hydroxycinnamic acid lactone	

Current regulation: Annex III, part1, n° 77

Clinical data:

In the “background information” section of the previous opinion (33), coumarin is classified as frequent allergen, causing allergic reactions in about 0.4 – 0.8% in consecutive PT patients, and causing contact allergic reactions in 0.8-10% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the EU 2005 study, none of the

1701 patients reacted positively to 5% coumarin in pet., while 7 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2020 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 study, 13 of 1825 consecutive patients reacted positively to coumarin (5% pet.) (12).

V. Mutterer et al. present the case of a 44 year old patient in whom coumarin was identified as culprit allergen by controlled ROAT testing with 1%, after having caused dermatitis by the use of a deodorant containing coumarin at 0.23% and an EdT (90).

Additional information:

Coumarin is found in several plant families, including *Melilotus* and *Galium*, e.g., *Galium odoratum* (sweet woodruff), however, also in oil of lavender, lovage and others (53).

Researchers from INSERM and “Rhodia Organique, Lyon , France” observed that pure coumarin is not an allergen in the LLNA, however, commercially available materials, containing “contaminants” (3,4-dihydrocoumarin, 6-chlorocoumarin and 6,12-epoxy-6H,12H-dibenzo[b,f][1,5] dioxocin, were identified as weak and moderate sensitisers, resp. (91).

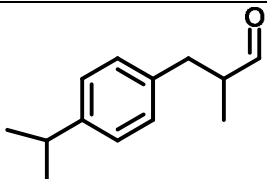
Coumarin is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CUMINALDEHYDE	
CAS # 122-03-2	
EC # 204-516-9	
4-(1-Methylethyl)-benzaldehyde	
4-Isopropylbenzaldehyde; p-Isopropylbenzaldehyde; 4-(Propan-2-yl)benzaldehyde; 4-Isopropylphenylcarboxaldehyde; Cumaldehyde; Cumic aldehyde; Cuminal; Cuminaldehyde; Cuminic aldehyde; Cuminyaldehyde; NSC 4886; p-Cumic aldehyde; p-Isopropylbenzaldehyde; p-Isopropylbenzenecarboxaldehyde	

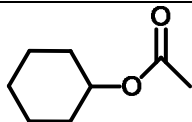
Current regulation: /

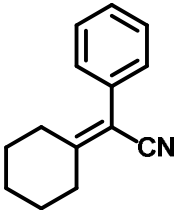
Clinical data:
The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 15% PT preparation of cuminaldehyde (25).

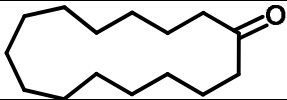
Additional information: ...

CYCLAMEN ALDEHYDE	
CAS # 103-95-7	
EC # 203-161-7	
α-Methyl-4-(1-methylethyl)-benzenepropanal	
p-Isopropyl-α-methyl-hydrocinnamaldehyde; 2-Methyl-3-(4-isopropylphenyl)propionaldehyde; 2-Methyl-3-(p-isopropylphenyl)propionaldehyde; 3-(4-Isopropylphenyl)-2-methylpropanal; 3-(p-Isopropylphenyl)-2-	

methylpropionaldehyde; methylpropionaldehyde(REACH, EINECS); methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl- α - methylhydrocinnamaldehyde; methylethyl)benzenepropanal; isopropylhydrocinnamaldehyde	3-p-Cumenyl-2- 4-Isopropyl- α - α -Methyl-4-(1- α -Methyl-p-	
Current regulation: ...		
Clinical /		data:
Additional information: It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).		

CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	
Current regulation: /	
Clinical data:	
In the Larsen 2002 c study, 0.5% positive reactions among 218 patients with know contact allergy to fragrance ingredients were observed (1).	
Additional information: A RIFM review is available (92).	

<i>alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE</i>	
CAS # 10461-98-0	
EC # 423-740-1	
α-Cyclohexylidenebenzeneacetonitrile	
alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ 1 α -Phenyl- α -Cyclohexaneacetonitrile; 2-Cyclohexylidene-2-phenylacetonitrile; NSC 408284; Peonile (REACH)	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

CYCLOPENTADECANONE	
CAS # 502-72-7	

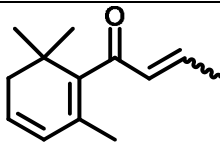
Opinion on fragrance allergens in cosmetic products

EC # 207-951-2	
Cyclopentadecanone	
CPE 218; Exaltone; NSC 63900; Normuscon; Normuscone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, n=3, i.e., 1.7% positive reactions were observed to the compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: ...

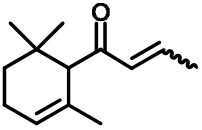
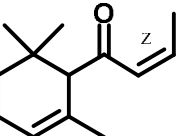
DAMASCENONE	
ROSE KETONE-4 (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-(2-butenoyl)-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-crotonyl-1,3-cyclohexadiene; Rose ketone # 4	

Current regulation: Annex III, part1, n° 160 (max. conc. 0.02%)

Clinical data: /

Additional information:

RIFM reviews are available (93, 94), quoting 1 negative, and 2 positive (2 of 37, 1 of 50 volunteers) HRIPTs with damascenone based on 2 LLNA, the EC3 values were calculated as 1.24% and 1.22%, respectively (94).

<i>alpha-DAMASCONE (TMCHB)</i>	 43052-87-5
CAS # 43052-87-5 / 23726-94-5	
EC # x / 245-845-8	
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-2-enone (43052-87-5); (2Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one (23726-94-5)	
43052-87-5: 2,6,6-Trimethyl-1-crotonyl-2-cyclohexene; α -Damascone	 23726-94-5
23726-94-5: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)- α -Damascone; cis- α -Damascone	

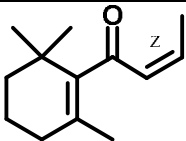
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

Clinical data:
In the Frosch 2002 b study, n=8 (0.5%) mostly strong positive PT reactions to consecutive patients were noted using a mixture of alpha and beta damascene, 0.1% pet. each (17). In human sensitisation experiments, after epicutaneous induction with 30% 1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enone (TMCHB, CAS # 43052-87-5) with adjuvant, to enhance response to this weak sensitiser, 8 of 30 patients were elicited by a challenge with 3% TMCHB 2 weeks later (95).

Additional information:

The former CAS # refers to alpha-Damascone or 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one. The latter CAS # refers to the identified ingredient cis-alpha-Damascone or (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, the content of which is restricted (SCCS-opinion 0392/00).

A RIFM review is available on alpha-damascone (96), quoting a number of partly positive HRIPT and other human studies, as well as different animal experiments. In 1 LLNA reported, an EC3 value of 3.3% was found. Another RIFM review is available for cis-alpha-damascone (97), supplying, however, no data on sensitisation.

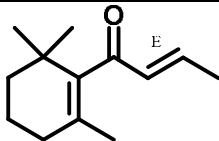
<i>cis-beta-DAMASCONE</i>	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)- β -Damascone	

Current regulation: Annex III, part 1, n° 162 (max. conc. 0.02%)

Clinical data:
Regarding results of the Frosch 2002 b study, see under alpha-damascone.

Additional information:

A RIFM review is available (98), citing several negative and one positive HRIPTs, and a number of – mostly positive – animal experiments.

<i>trans-beta-DAMASCONE</i>	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)- β -Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans- β -Damascone; β -Damascone	

Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (99), citing 2 negative HRIPT and 1 negative maximisation test, and a number of positive animal experiments (the EC3 value, based on 1 LLNA, was found to be 2.4%).

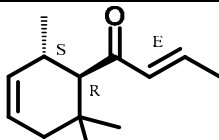
<i>delta-DAMASCONE</i>	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ-Damascone	

Current regulation: Annex III, part 1, n° 161 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (100), citing several positive HRIPT and 1 negative HRIPT. Cross sensitisation to alpha- and beta-damascone was demonstrated in 3 sensitised subjects. 2 LLNA studies are reported on, yielding EC3 values of 5.19% and 9.6%, resp.

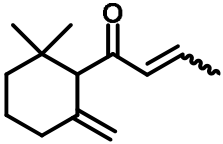
<i>trans-trans-delta-DAMASCONE</i>	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1 α (E),2 β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans- δ -Damascone; δ -Damascone; trans, trans- δ -Damascone	

Current regulation: Annex III, part 1, n° 165 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (101), citing 1 positive HRIPT (2/15 with 1%).

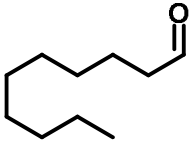
<i>gamma-DAMASCONE</i>	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

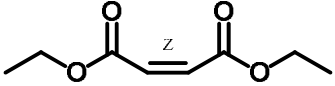
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

<i>DECANAL</i>	
CAS # 112-31-2	
EC # 203-957-4	
n-Decanal	
Capraldehyde; Capric aldehyde; Caprinaldehyde; Caprinic aldehyde; Decaldehyde; Decanaldehyde; Decyl aldehyde; Decylic aldehyde; NSC 6087; n-Decaldehyde; n-Decyl aldehyde	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

<i>DIETHYL MALEATE</i>	
CAS # 141-05-9	
EC # 205-451-9	
(2Z)-Diethyl but-2-enedioate	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

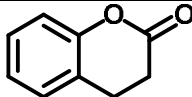
Current regulation: Annex II, n° 426

Clinical

data:

In the Malten 1984 study, 3.2% of 182 patients displayed a positive PT reaction to diethyl maleate 0.1% pet. (24). In this study, it has been noted that “in the max. test and clinically this is a strong sensitiser having caused patch test sensitisation (42%)”

Additional information: /

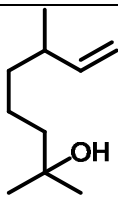
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ -lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

Current regulation: Annex II, n° 427

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dihydrocoumarine 5% pet. (24).

Additional information: /

DIHYDROMYRCENOL	
CAS # 18479-58-8	
EC # 242-362-4	
(±)-2,6-Dimethyloct-7-en-2-ol	
1,1,5-Trimethyl-6-heptenol; 2,6-Dimethyl-7-octen-2-ol; 3,7-Dimethyl-1-octen-7-ol; 2,6-Dimethyl-7-octen-2-ol (INCI)	

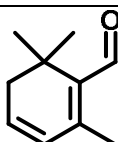
Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (103), listing 2 negative HRIPTs and 1 negative human maximisation test.

It is a “top 100” substance (IFRA, pers. comm.2010).

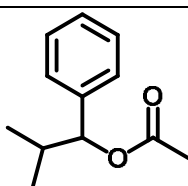
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

Additional information:

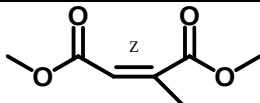
A RIFM review quotes one positive HRIPT (5 of 53) and one negative HRIPT (0 of 54) (93).

DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	
CAS # 151-05-3	
EC # 205-781-3	
2-Methyl-1-phenylpropyl acetate	
Benzeneethanol, α,α-dimethyl-, acetate; Phenethyl alcohol, α,α-dimethyl-, acetate; 1,1-Dimethyl-2-phenylethyl acetate; 2-Methyl-1-phenyl-2-propyl acetate; 2-Methyl-1-phenylpropan-2-yl acetate; Benzyl dimethylcarbinol acetate; Benzyl dimethylcarbinyl acetate; Dimethylbenzylcarbinol acetate; Dimethylbenzylcarbonyl acetate; NSC 46123; α,α-Dimethylphenethyl acetate	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% positive PT reactions to consecutive patients were noted (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and one to 5% DMBCA in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

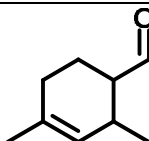
<i>DIMETHYL CITRACONATE</i>	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

Current regulation: Annex II, n° 431

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dimethylcitraconate 12% pet. (24). In this paper, a human maximisation test positive in “4/44” is quoted.

Additional information: ...

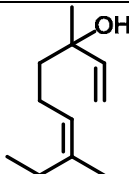
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

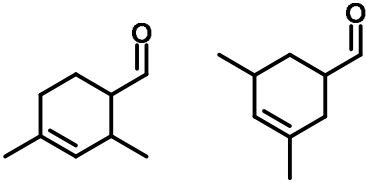
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyllinalool	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (104), citing 1 negative human maximisation test (n=25).

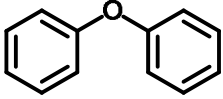
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde 3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
Hivertal; Vertocitral	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 2.3% positive PT reactions were observed with the isomer mixture, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

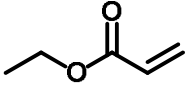
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryn JK-EB; Diphenyl ether; Diphenyl oxide; NSC 19311; Oxybisbenzene; Phenoxybenzene; Phenyl oxide	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

ETHYL ACRYLATE	
CAS # 140-88-5	
EC # 205-438-8	
Ethyl 2-propenoate	
Acrylic acid ethyl ester (6CI,8CI); 2-Propenoic acid ethyl ester; Ethyl 2-propenoate; Ethyl acrylate; Ethyl acrylic ester; Ethyl propenoate; NSC 8263	

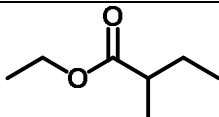
Current regulation: Annex II, n° 435

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In the NACDG 2009 multicentre study, 0.9% of

consecutive patients (n=4428) had a positive PT reaction (21).

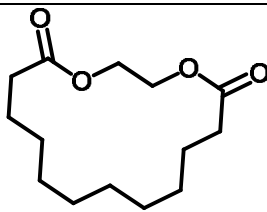
Additional information: /

ETHYL 2-METHYLBUTYRATE	
CAS # 7452-79-1	
EC # 231-225-4	
Ethyl 2-methylbutyrate	
Butyric acid, 2-methyl-, ethyl ester (6CI,7CI,8CI); (±)-Ethyl 2-methylbutanoate; 2-Methylbutanoic acid ethyl ester; 2-Methylbutyric acid ethyl ester; Ethyl 2-methylbutanoate; Ethyl 2-methylbutyrate; Ethyl α-methylbutyrate; NSC 1103	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

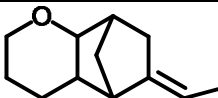
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

Current regulation: /

Clinical data:

In the Larsen 2002 c study on 218 patients with known contact allergy to fragrance ingredients, this compound caused 0.9% positive PT reactions at 5% pet. (1).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	
CAS # 93939-86-7	
EC # 300-376-9	
6-Ethylideneoctahydro-5,8-methano-2H-1-benzopyran	

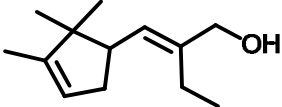
Current regulation: /

Clinical

data:

In the Larsen 2001 study, no positive PT reactions were observed with this compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

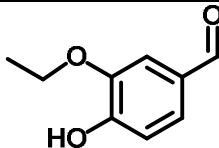
Additional information: /

2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	
CAS # 28219-61-6	
EC # 248-908-8	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol; 2-Ethyl-4-(2',2',3-trimethylcyclopent-3'-enyl)but-2-enol; Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

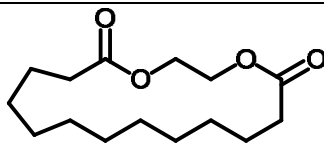
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde ethyl ether; 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).

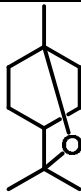
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

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	

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6171; Terpan; p-Cineole	

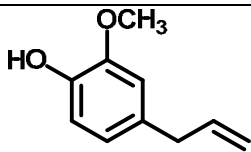
Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010).

See also ***EUCALYPTUS SPP. LEAF OIL***; eucalyptol is the major ingredient there (up to 85%), but found in significant quantities also in a number of other essential oils (see 3.2).

EUGENOL	
CAS # 97-53-0	
EC # 202-589-1	
2-Methoxy-4-(2-propen-1-yl)-phenol	
Other names: 4-Allyl-2-methoxy-phenol; 1-Allyl-4-hydroxy-3-methoxybenzene; 2-Hydroxy-5-allylanisole; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-(2'-propenyl)phenol; 2-Methoxy-4-[2-allyl]phenol; 2-Methoxy-4-allylphenol; 3-(3-Methoxy-4-hydroxyphenyl)propene; 3-(4-Hydroxy-3-methoxyphenyl)-1-propene; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; Allylguaiacol; Bioxeda; Caryophyllic acid; Dentogum; Eugenig acid; Eugenol; NSC 209525; NSC 8895; p-Allylguaiacol; p-Eugenol	

Current regulation: Annex III, part 1, n° 71

Clinical data:

In the “background information” section of the previous opinion (33), eugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.2% in consecutive PT patients and accounting for 4 to 16% of reactions to the FM I. Allergic reactions had been observed in 0.7 – 20% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). F. Giusti et al. examined 1754 consecutive patients tested with eugenol 1% pet. in addition to the baseline series, 09/1998 - 01/2000. 21 patients (1.2%) reacted positively to eugenol (106). In the An 2005 study, 8 of 422 consecutive patients, i.e., 1.9%, had positive reaction (13) (test concentration 2%). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 2.5% positive reactions (22). The IVDK 2010 study, 0.44% (95% CI: 0.04 – 0.84%) of 1214 consecutively tested patients reacted to the compound, while 1.57% (95% CI: 1.19 – 1.95%) of 4801 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to eugenol (48).

Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material was reported from India (108); however, occasional cases are also reported from Europe (109). Occupational exposure to eugenol / zinc oxide type dental restorative material, which is apparently less frequently used nowadays, may lead to occupational sensitisation to eugenol, as illustrated by a case report (110).

Additional information:

Eugenol is the main component (80-95%) of clove oil, but also found in citronella oil, pimento leaf oil and cinnamon bark oil (see section 3.2).

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

FARNESOL

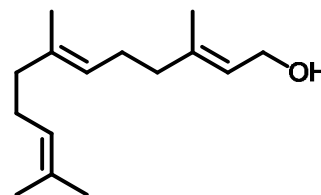
CAS # 4602-84-0

EC # 225-004-1

3,7,11-Trimethyl-2,6,10-Dodecatrien-1-ol

Farnesol; 3,7,11-Trimethyl-2,6,10-dodecen-1-ol; FCI 119a; Farnesyl alcohol; NSC 60597; Nikkosome

Current regulation: Annex III, part 1, n° 82



Clinical data:

In the “background information” section of the 1999 opinion, farnesol is classified as “less frequently reported allergen”; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive PT reactions to MPR (33).

Since the last SCCNFP-opinion of 1999, farnesol is used not only for its scent, but also for its (slight) antimicrobial activity, useful, for instance, in deodorants. Thus, axillary dermatitis is a relatively typical presentation (111). In a multicentre study based on 1997/98 PT data, 0.5% positive reactions in consecutive patients were noted (Frosch 2002 a (16)). Farnesol is included in the FM II. In the original publication on single constituents of the FM II, 6 of 1701 consecutive patients reacted positively to farnesol 5%, ie., 0.35% (95% CI: 0.13 – 0.77%) (10). In a study on consecutive patients tested in 2003, 38 of 4238 patients had positive reactions to farnesol 5% pet. (0.9%, 95% CI: 0.6 – 1.2%) (4)(IVDK 2007). (A paper on farnesol previously published by the IVDK (112) presents results included in this later analysis.) In a series from Nagoya, Japan, 1.1% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at 5% pet.) (14). In the Groningen 2009 study, 0.9% (95% CI: 0.2 – 2.7%) had positive reactions (6).

Additional information:

“Farnesol is an acyclic primary sesquiterpene alcohol found in essential oils such as lemongrass, citronella, tuberose blossom, sandalwood and orange blossom” (23). A RIFM review is available (113).

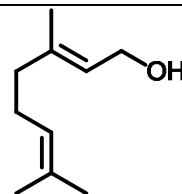
GERANIOL

CAS # 106-24-1

EC # 203-377-1

(2E)-3,7-Dimethyl-2,6-octadien-1-ol

(E)-3,7-Dimethyl-2,6-octadien-1-ol; (E)-Geraniol; (E)-Nerol; 3,7-Dimethyl-trans-2,6-octadien-1-ol; Geraniol; Geranyl alcohol; Lemonol; MosquitoSafe; NSC 9279; trans-3,7-Dimethyl-2,6-octadien-1-ol; trans-Geraniol; β-Geraniol



Current regulation: Annex III, part 1, n° 78

Clinical data:

In the “background information” section of the previous opinion (33), geraniol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.4% in consecutive PT patients and accounting for 3 to 7% of reactions to the FM I. Allergic reactions had been observed in 1.2 – 30% of patients with

eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at 2%, i.e. twice the usual concentration (6). In a series from Nagoya, Japan, 0.3% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at the unusually high concentration of 5% pet.) (14). In the 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).

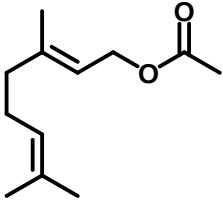
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman 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. Geraniol and neral together with hydroperoxide have been identified as oxidation products when geraniol autoxidizes (84). Geraniol and neral were also identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

A review is available by Hostynek and Maibach (116) and by RIFM (117). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

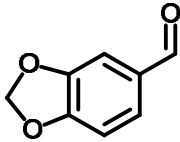
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic acid geraniol ester; Bay pine (oyster) oil; Geranyl acetate; Geranyl ethanoate; NSC 2584; trans-1-Acetoxy-3,7-dimethyl-2,6-octadiene; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-Geranyl acetate; β-Geranyl acetate	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010).

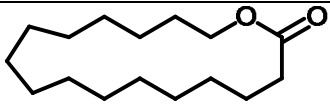
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Heliotropin; Heliotropine; NSC 26826; Piperonaldehyde; Piperonylaldehyde; Protocatechuic aldehyde methylene ether	

Current regulation: /

Clinical data:

In the Frosch 2002 b study, n=2 (0.2%) positive reactions to “piperonal” (1% pet.) and n=6 (0.4%) to “piperonal” (5% pet.), respectively, in 1606 consecutive were observed (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% heliotropine in pet., tested in 106 consecutive patients in Barcelona, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

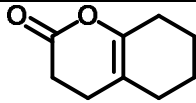
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid; 1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; NSC 33546	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with previously diagnosed contact allergy to fragrance ingredients had a positive PT reaction to this compound, tested 5% pet. (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to 5% “hexadecanolide” (13).

Additional information: /

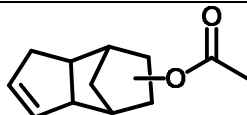
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ -Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ -1,6-2-Oxabicyclo(4.4.0)decen-3-one	

Current regulation: Annex II, n° 1135

Clinical data: /

Additional information:

A RIFM review is available (93), p. S115 ff, citing a number of positive human sensitisation experiments.

3α,4,5,6,7,7α-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxydihydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl acetate; Tricyclodecenyl acetate	

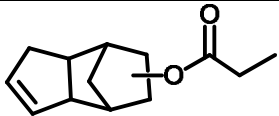
Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 1 to 5% "Cyclacet ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information:

Produced by IFF under the brand name "Cyclacet" (<http://www.iff.com/Ingredients.nsf/0/1C9F2CB39EB1EF6480256993002FBC14>, last accessed 2010-07-08).

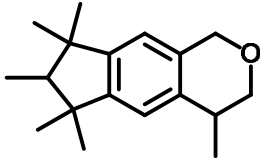
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol propanoate	

3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl propionate; Verdyl propionate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

HEXAMETHYLINDANOPYRAN	
CAS # 1222-05-5	
EC # 214-946-9	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[γ]-2-benzopyran	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyrane; 1,3,4,6,7,8-Hexahydro-4,6,6,8,8,8-hexamethylcyclopenta-2-benzopyran; Abbalide; Galaxolide; Galaxolide 50; Galaxolide 50BB; Galaxolide 50IPM; Galaxolide White; HHCB; Pearlide	

Current regulation: /

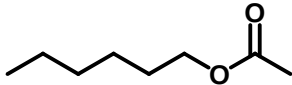
Clinical data:

In the Frosch 2002 a study, n=3 (0.2%) had positive reactions to the compound, tested 10% in isopropyl myristate (with 1 patient reacting positively to the diluent) (16). The Larsen 2001 study, testing with HHCB 7% pet., found 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to “Galaxolide 50”, tested at 5% (13) (test concentration 2% pet.). The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 25% PT preparation of HHCB (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Galaxolide 50 ®” in pet., tested in 100 consecutive patients in Stockholm, were observed (15).

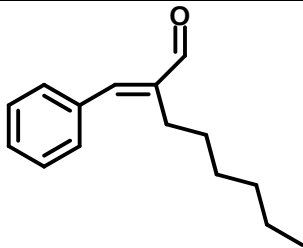
Additional information:

[0403/00 - Opinion concerning Hexahydro-hexamethyl-cyclopenta\(γ\)-2-benzopyran \(HHCB\)](#)

[0610/02 - Opinion on Hexahydro-hexamethyl-Cyclopenta \(γ\)-2-Benzopyran \(HHCB\)](#) (no restrictions) It is a “top 100” substance (IFRA, pers. comm.2010).

HEXYL ACETATE	
CAS # 142-92-7	
EC # 205-572-7	
Hexyl ethanoate	
Acetic acid, hexyl ester, Hexyl alcohol, acetate; 1-Hexyl acetate; Exceed 600; Hexyl acetate; Hexyl ester acetic	

acid;; NSC 7323; n-Hexyl acetate; n-Hexyl ethanoate	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

HEXYL CINNAMAL	
CAS # 101-86-0	
EC # 202-983-3	
α-Hexyl-cinnamaldehyde	
2-(Phenylmethylene)octanal; 2-Hexyl-3-phenyl-2-propenal; 2-Hexylcinnamaldehyde; Hexyl cinnamic aldehyde; NSC 406799; NSC 46150; α-Hexylcinnamaldehyde; α-Hexylcinnamic aldehyde; α-Hexylcinnamyl aldehyde; α-n-Hexyl-β-phenylacrolein; α-n-Hexylcinnamaldehyde	
Current regulation: Annex III, part 1, n° 87	

Clinical data:

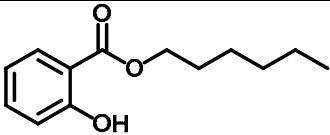
In the “background information” section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as “less frequently reported allergen”; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products were found (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the subsequent EU 2005 study, 2 of 1701 patients had positive reactions to AHCA, and n=16 doubtful or irritant to AHCA at 10% in pet. (10). The IVDK 2007 study yielded n=3, i.e. 0.2% (95% CI: 0.03 – 0.4%) positive reactions in 2019 consecutively PTed patients, using 10% pet. as test concentration (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, using a lower test concentration of 5% pet. (6).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

Hexyl cinnamal is regarded as “a recommended positive control for skin sensitization testing”, e.g., in the context of the LLNA (118).

HEXYL SALICYLATE	
CAS # 6259-76-3	
EC # 228-408-6	
Hexyl-2-hydroxybenzoate	
Salicylic acid, hexyl ester; 1-Hexyl salicylate; Hexyl salicylate; n-Hexyl salicylate	

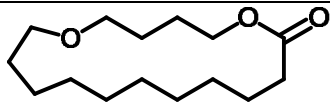
Current regulation: /

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).

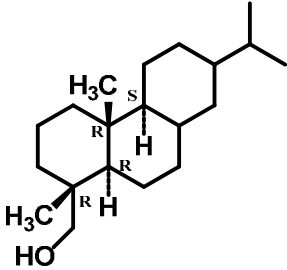
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

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)-phenanthrenemethanol; Tetrahydroabietyl alcohol	1-

Current regulation: AnnexII, n° 440

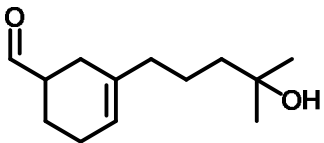
Clinical data:

In the deGroot 2000 study, 17 of 1825 consecutively tested patients had positive reactions to hydroabietyl alcohol (10% pet.) (12).

Additional information:

Commercial hydroabietyl alcohol consists of di- and tetrahydroabietyl alcohol together with non-modified colophony (120)

HYDROXYISOHEXYL	3-CYCLOHEXENE	

CARBOXALDEHYDE (HICC) regioisomers	 51414-25-6
CAS # 31906-04-4 / 51414-25-6	
EC # 250-863-4 / 257-187-9	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (31906-04-4) 3-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (51414-25-6)	

31906-04-4: 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde;
4-(4-Methyl-4-hydroxyamyl)cyclohex-3-ene carboxaldehyde; Lyrall

Current regulation: Annex III, part 1, n° 79

Clinical data:

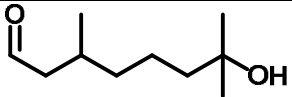
In the “background information” section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 2.7% of the 1855 consecutive patients reacted positively to HICC (5% pet.) (16). In the EU 2005 study, 28 of 1701 patients (1.7%, 95% CI: 1.1 – 2.4%) reacted positively to 5% HICC in pet. (10). In 21325 patients PTed consecutively in the IVDK 2007 study, 2.4% (95% CI: 2.2 – 2.6%) positive reactions were noted to 5% HICC in pet. (4). Similar to other studies, HICC was the most common single fragrance allergen among 320 patients tested in the Groningen 2009 study, with 3.1% (95% CI: 1.5 – 5.7%) positive reactions despite testing with a lower concentration of 2% pet. (6). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded a relatively low prevalence of 0.4% (7 of 1603; exact 95% CI (recalculated): 0.17 – 0.90%) positive reactions with 5% HICC in pet. and even less with lower test concentrations; possible reasons for the much lower prevalence were discussed. The IVDK 2010 study, 2.36% (95% CI: 2.19 - 2.53%) of 37270 consecutively tested patients reacted to HICC (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to HICC (48).

Further clinical data with a focus on quantitative dose-response (see also section 4.3), is discussed in (121).

Among the early case reports, S.A. Hendriks reported the case of a 20 year old patient developing axillary dermatitis after 5 months use of a deodorant containing HICC (122).

Additional information: /

HYDROXYCITRONELLAL	
CAS # 107-75-5	
EC # 203-518-7	
7-Hydroxy-3,7-dimethyl-octanal	

(±)-Hydroxycitronellal; 3,7-Dimethyl-7-hydroxyoctanal; 7-Hydroxy-3,7-dimethyloctanal; 7-Hydroxycitronellal; Citronellal hydrate; Citronellal, hydroxy-; Cyclalial; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguetine principle; NSC

Opinion on fragrance allergens in cosmetic products

406740; Phixia

Current regulation: Annex III, part 1, n° 72

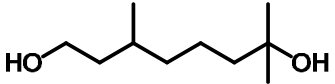
Clinical data:

In the “background information” section of the previous opinion (33), hydroxycitronellal, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.75% in consecutive PT patients and accounting for 6 to 16% of reactions to the FM I. Allergic reactions had been observed in 10 – 45% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.9 – 1.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.2% (95% CI: 0.9 – 4.5%) had positive reactions to this compound, tested at 2% pet., i.e., twice the commonly used concentration (6). The Sugiura 2000 study observed 1% positive PT reactions (test concentration 5% pet.) in 1483 patients tested for suspected cosmetic dermatitis (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 1.17% (95% CI: 0.48 – 1.85%) of 1214 consecutively tested patients reacted to the compound, while 2.95% (95% CI: 2.43 – 3.47%) of 4359 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with hydroxycitronellal, yielding 6 positive reactions (48).

Additional information:

Hydroxycitronellal is a synthetic fragrance, which only recently has been found in a few essential oils, e.g., of a *Narcissus* species and in essential oils of pepper (53)

HYDROXYCITRONELLOL	
CAS # 107-74-4	
EC # 203-517-1	
3,7-Dimethyl-7-octanediol	
2,6-Dimethyl-2,8-octanediol; 3,7-Dimethyl-1,7-octanediol; 3,7-Dimethyloctan-1,7-diol; Citronellol, hydroxy-; Hydroxyciol; Hydroxycitronellol; NSC 406140; NSC 67886	

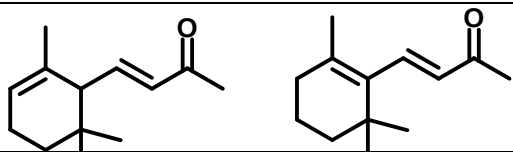
Current regulation: /

Clinical data:

This compound elicited 6.0% positive PT reactions in 218 fragrance sensitive individuals (Larsen 2002 c, (1)).

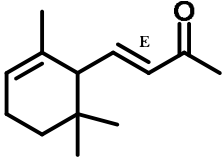
Additional information:

A RIFM review is available, reporting results of a human induction study (maximisation test) in 25 volunteers, yielding no evidence of sensitisation (123).

IONONE isomeric mixture	
CAS # 8013-90-9	
EC # 232-396-8	

Opinion on fragrance allergens in cosmetic products

Ionone	
Irisone, mixture of alpha- and beta ionone	
Current regulation: /	
Clinical data: / (see single isomers)	
Additional information:	
It is a “top 100” substance, further specified with “mixed isomers” (IFRA, pers. comm.2010).	
INCI: “MIXED IONONES”, with CAS # 14901-07-6 / 6901-97-9 / 8013-90-9 (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=35383 , last accessed 2010-07-13).	
A RIFM review is available on “ionone” (124), quoting negative human and experimental results.	

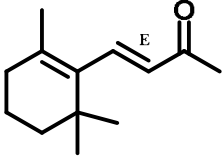
<i>alpha-IONONE</i>	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; 4-(2,6,6-Trimethyl-2-cyclohexenyl)-3-buten-2-one; trans-α-Ionone; α-Cyclocitrylideneacetone; α-Ionone	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% alpha-ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information: A RIFM review is available (125).

<i>beta-IONONE</i>	
CAS # 79-77-6	
EC # 201-224-3	
(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	
(E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; (E)-β-Ionone; Ionone beta; trans-β-Ionone; β-Ionone	

Current regulation: /

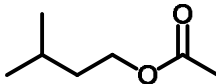
Clinical data:

In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% beta-

ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (126).

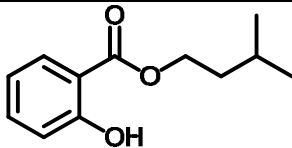
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic acid 3-methylbutyl ester; Acetic acid isopentyl ester; Banana oil; Isoamyl acetate; Isoamyl alcohol acetate; Isoamyl ethanoate; Isopentyl acetate; Isopentyl ethanoate; NSC 9260; Pear oil; i-Amyl acetate; iso-Amyl acetate; iso-Pentyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

In CosIng, it is listed as “solvent” (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=76810>, last accessed 2010-07-13)

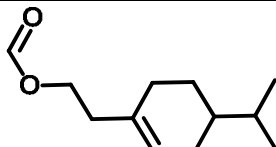
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; NSC 7952	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 50% PT preparation of this compound – this reaction may have been due to an “excited back syndrome” and is thus a limited evidence (25). In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% isoamyl salicylate in pet., tested in 95 consecutive patients, were observed (15).

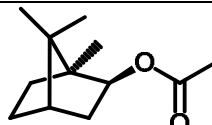
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletely defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

Current regulation: Annex III, part 1, n° 170

Clinical data: /

Additional information: A RIFM review is available (128).

ISOBORNYL ACETATE	
CAS # 125-12-2	
EC # 204-727-6	
(1R,2R,4R)-1,7,7-trimethyl-Bicyclo[2.2.1]hept-2-yl acetate	
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-Trimethyl-, acetate, (1R,2R,4R)-rel- ; Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-; Isoborneol, acetate; (±)-Isobornyl acetate; Isobornyl acetate; NSC 62486; Pichtosin; Pichtosine; exo-Bornyl acetate	

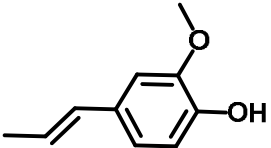
Opinion on fragrance allergens in cosmetic products

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% isobornyl acetate in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ISOEUGENOL	
CAS # 97-54-1	
EC # 202-590-7	
2-Methoxy-4-(1-propen-1-yl)-phenol	
Phenol, 2-methoxy-4-(1-propenyl)- ; Phenol, 2-methoxy-4-propenyl-; 1-(3-Methoxy-4-hydroxyphenyl)-1-propene; 2-Methoxy-4-(1-propenyl)phenol; 2-Methoxy-4-propenylphenol; 3-Methoxy-4-hydroxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-β-methylstyrene; 4-Propenylguaiacol; Isoeugenol; NSC 6769	

Current regulation: Annex III, part 1, n° 73

Clinical data:

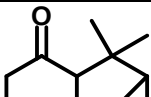
In the “background information” section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been observed in 2 – 25% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.8 – 1.8%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to isoeugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.4% positive reactions (22). At St Johns Institute of Dermatology in London 3636 subjects were patch tested with isoeugenol 2001-2005, 97 of whom were positive. Year-on-year incidence showed an increasing trend, with an overall incidence of 2.67% (129). The IVDK 2010 study, 1.62% (95% CI: 0.87 – 2.38%) of 1214 consecutively tested patients reacted to the compound, while 3.41% (95% CI: 2.90 – 3.92%) of 5747 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=11 reacted positively to isoeugenol (48).

Additional information:

Isoeugenol occurs in a cis- (CAS 5912-86-7) and a trans-isomers (CAS 5932-68-3), the latter dominating in trade products (82-88%) (53).

Isoeugenyl methyl ether caused 7.3% positive reactions in the Larsen 2002 c study (1). A number of derivatives of isoeugenol, such as isoeugenyl acetate, transisoeugenol, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and benzyl isoeugenyl have been examined in 2261 consecutive patients; a varying proportion of positive patch test reactions and a varying proportion of concomitant reactions with isoeugenol have been observed (130). In an earlier study, 5 of 7 patients positive to isoeugenol also displayed positive reactions to isoeugenol acetate (1.2% eth.) (131) (see also section 5 and 6).

ISOLONGIFOLENEKETONE	
CAS # 33407-62-4	

EC # 245-890-3	
1,3,4,6,7,8a-Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	
Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	

Current regulation: /

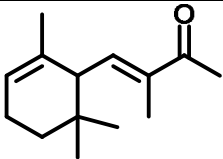
Clinical data:

The Larsen 2001 study identified 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

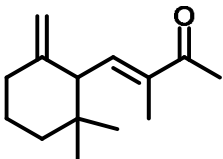
Additional information:

Not listed in CosIng under this CAS #. Other CAS # reported in RIFM review ¹³:

- 29461-14-1 CosIng: INCI name "ISOLONGIFOLENE KETONE EXO";
- 23787-90-8 CosIng: INCI name "ISOLONGIFOLANONE";
- 29461-13-0: CosIng: INCI name "HEXAHYDRO-TETRAMETHYLMETHANONAPHTHALEN-8-ONE".

<i>alpha-ISOMETHYL IONONE</i>	
CAS 127-51-5	
EC 204-846-3	
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	
4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; Cetone Alpha; Isomethyl-α-ionone; NSC 66432; α-Cetone	

Current regulation: Annex III, part 1, n° 90

<i>gamma-Methylionone</i>	
CAS 7388-22-9	
EC /	

According to CosIng, "alpha-ISOMETHYL IONONE" (CAS # 127-51-5) and "gamma-Methylionone" (CAS # 7388-22-99) are synonyms, with one CAS number, and one preferred chemical name. The substance(s) are accordingly treated in the 1999 opinion (33) as one. As this treatment is also found in the literature, both substances are reviewed together.

¹³ Opdyke, D. L. J.; Letizia, C. **Monographs on fragrance raw materials. Isolongifolanone.** Food and Chemical Toxicology (1983), 21(6), 859

Clinical data:

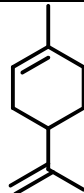
In the “background information” section of the 1999 opinion, “gamma-methylionone” is classified as “less frequently reported allergen”; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

The IVDK 2007 study yielded $n=1$, i.e. 0.1% (95% CI: 0.00 – 0.2%) positive reactions in 2004 consecutively PTed patients (4). In the subsequent period (2005-2008), $n=986$ patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, $n=2$, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In a Korean study with 422 consecutive patients, 2.1% reacted positively to “alpha isomethyl ionone (gamma-methylionone), CAS # 127-51-5”, tested 5% pet. (13)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010) under the label of “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

A RIFM review is available, listing 4 human sensitisation experiments employing different study protocols – all yielding negative results (132). Another review is available by Hostynek and Maibach (133), both referring to “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

(DL)-LIMONENE	
CAS # 138-86-3	
EC # 231-732-0	
1-Methyl-4-(1-methylethenyl)-cyclohexene	
p-Mentha-1,8-diene; (±)-Dipentene; (±)-Limonene; (±)-α-Limonene; 1,8-p-Menthadiene; 1-Methyl-4-(1-methylethenyl)cyclohexene; 1-Methyl-4-isopropenyl-1-cyclohexene; 1-Methyl-4-isopropenylcyclohexene; 1-Methyl-p-isopropenyl-1-cyclohexene; 4-Isopropenyl-1-methyl-1-cyclohexene; 4-Isopropenyl-1-methylcyclohexene; Cajeputen; Cajeputene; Cinen; Cinene; DL-Limonene; Dipenten; Dipentene; Eulimen; Flavor orange; Goldflush II; Kautschin; Limonen; Limonene; NSC 21446; NSC 844; Nesol; Orange X; Orange flavor; PC 560; Roti-Histol; SF 001; dl-Limonene; α-Limonene	

Current regulation: Annex III, part1, n° 88, 167, 168

Clinical data:

In the “background information” section of the 1999 opinion, d-limonene (CAS 5989-27-5) is classified as “less frequently reported allergen in relation to cosmetic exposure”; with contact allergy to oxidised limonene not infrequently reported in the literature (33).

Since 1999, several studies have been performed using limonene where the oxidation state is not given, but intended to be low. In one study, 0.6% positive reactions to limonene (3% pet.) were observed in 1606 consecutive patients (17). The IVDK 2007 study yielded n=3, i.e. 0.1% (95% CI: 0.03 – 0.4%) positive reactions in 2396 patients consecutively PTed with limonene (2% pet.) (4). The IVDK 2010 study, 0.28% (95% CI: 0 – 0.57%; percentages standardised for age and sex) of 1241 patients PTed with dipentene reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed in 320 patients (6).

Regarding selected case reports, a case of a 40 year old citrus fruit picker with work related hand dermatitis and bronchial asthma has been described, who tested extreme positive to DL-limonene (2% pet.), and, less extremely, to citronellol and to the biocide dichlorophene (134). Moreover, limonene is used as a solvent in technical applications and cleaning and can lead to allergic contact dermatitis (e.g., a histopathology technicians (135, 136) or a painter and decorator (137)). In “water-free” hand cleansers it is reported to be used in concentrations around 10 – 20% (137). Wax polishes may contain dipentene and have caused one reported case of occupational ACD in a car mechanic (138). Another case of occupational ACD from dipentene in honing oil has been reported (139). In a case series from Sweden, 2 of 105 car mechanics patch tested for occupational contact dermatitis had positive reactions to oxidised d-limonene (5% pet.) (140).

Additional information:

Limonene is a monocyclic monoterpene existing in two enantiomers: (R)-(+)-limonene (CAS 5989-27-5) and (S)-(-)-limonene (CAS 5989-54-8). Racemic limonene is known as dipentene.

The allergenicity of limonene is closely related to oxidation (71, 72, 141, 142). It has been demonstrated that both enantiomers, R-(+)- and S-(-)-limonene spontaneously autoxidize, and that the primary oxidation products formed, the hydroperoxides, are strong and clinically relevant contact allergens. Among 2411 consecutive patients in a multi-centre European study, 63 (2.6 % [95%CI: 2.0-3.3]) reacted to oxidised (R)-(+)-and/or (S)-(-)-limonene (3.0% pet.) (72). In other multi-studies also, a considerable proportion of patients showed

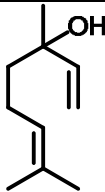
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- between 0.3% and 5.1% of subgroups of 2800 patients in Stockholm and Leuven, depending on test concentration, oxidation state and department(141),
- between 0.3% and 6.5% in 4 different departments in altogether 2273 patients (72, 143).

The primary oxidation products are the major allergens forming specific antigens (Bråred-Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. Contact Dermatitis 2006; 55: 230-237.).

Current IFRA standards emphasise “a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method” (<http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56>, last accessed 2009-11-11). For a more general discussion see section 5.

There is no scientific rationale for the difference in peroxide value allowed for limonene (20 millimoles peroxides per litre) compared to linalool (10 millimoles peroxides per litre). Specific values for hydroperoxides, which are allergens, would be desirable.

LINALOOL	
CAS # 78-70-6 (isomeric mixture)	
EC # 201-134-4; 245-083-6	
See: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=27933	
3,7-Dimethyl-1,6-octadien-3-ol	
(±)-Linalool; 2,6-Dimethyl-2,7-octadien-6-ol; 2-Methyl-1-prenyl-3-buten-2-ol; 3,7-Dimethyl-1,6-octadiene-3-ol; 3,7-Dimethyl-3-hydroxy-1,6-octadiene; L 260-2; Linalol; Linalool; Linalyl alcohol; Linanol; NSC 3789; dl-Linalool; β-Linalool	

Current regulation: Annex III, part 1, n° 84

Clinical data:
In the “background information” section of the 1999 opinion, linalool in non-oxidized form is classified as “less frequently reported allergen”; with 4 cases of contact allergy reported in 2 studies on patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, studies have been performed on contact allergy to linalool, oxidation state not given, but intended to be low. In the Larsen 2002 c study, none of the 218 patients with known contact allergy to fragrance ingredients had a positive reaction to linalool 5% pet., as prepared specially for this study (1). The IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2401 patients consecutively tested with stabilised linalool (10% pet.) (4). The IVDK 2010 study, 1 patient had a weak, and another a ++ reaction among the n=985 patients tested with 10% linalool (stabilised) in pet. (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). The deGroot 2000 study with 1825 consecutively tested patients yielded 3 positive reactions to linalool (12). The DeGroot 1985 study found no positive reactions among 179 patients using a 30 % PT preparation of linalool (25).

Additional information:

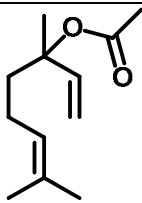
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

2003 in 6 European centres including 1511 consecutive patients, 1.3% showed a positive reaction to oxidized linalool (2.0% pet.) and 1.1% to the hydroperoxide fraction (65). A recent dose-response study in Sweden including 3400 patients in two test centres showed a positive reaction in 5.3% of the 1725 patients tested with oxidized linalool 6% pet. (145).

A review by RIFM is available both regarding linalool (146) and linalool “and related esters” (147). Another review is available by Hostynek and Maibach (148).

It is a “top 100” substance (IFRA, pers. comm.2010).

Additional CAS numbers exist for the single isomers: CAS # 126-90-9 (S-isomer), CAS # 126-91-0 (R-isomer); however, in the studies reviewed the isomeric mixture has been used throughout.

LINALYL ACETATE	
CAS # 115-95-7	
EC # 204-116-4	
3,7-Dimethyl-1,6-octadien-3-yl acetat	
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; Linalool acetate K; (±)-Linaloyl acetate; (±)-Linalyl acetate; 1,5-Dimethyl-1-vinyl-4-hexenyl acetate; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 3-Acetoxy-3,7-dimethyl-1,6-octadiene; Acetic acid linalool ester; Bergamiol; Bergamol; Bergamot mint oil; Linalyl acetate; NSC 2138; dl-Linalool acetate	

Current regulation: /

Clinical data:
In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. (15). In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted (16). Similarly, the RIFM review mentioned quotes a number of studies where no allergic reactions to this compound had been observed, with the exception of one positive reaction in a Dutch study in 1988(149).

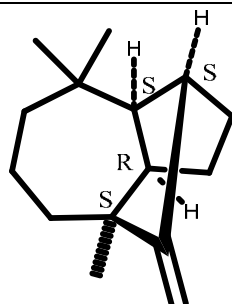
Additional information:

This is the main component of lavender oil (30%), also part of bergamot oil, neroli oil, peppermint oil, lemon oil and jasmine oil (53).

Linalyl acetate autoxidizes spontaneously at air exposure and the major allergens, the hydroperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be metabolically hydrolysed to the corresponding alcohol cross reactions to allergens from oxidized linalool should be possible. This was indicated in a study of lavender oil and oxidised linalyl acetate which elicited positive PT reactions in some patients with known contact allergy to oxidised linalool (n=3) (151).

A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation (152).

It is a “top 100” substance (IFRA, pers. comm.2010).

Longifolene	
CAS # 475-20-7	
EC # 207-491-2	
(1S,3aR,4S,8aS)-Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-; 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1α,3aβ,4α,8aβ)]-; (+)-Longifolene; Junipen; Junipene; Kuromatsuen; Kuromatsuene; Longifolen; NSC 150808; d-Longifolene; α-Longifolene	

Current regulation: /

Clinical data: /

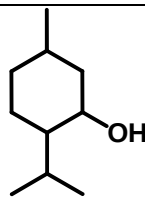
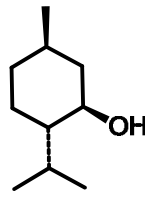
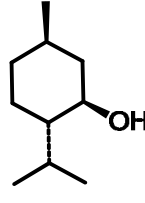
Additional information:

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=77412

This substance is listed in the Register of Flavouring Substances pursuant to Article 3(1) of Regulation EC No. 2232/96 (28 Oct 1996) that lays down a procedure for flavouring substances used or intended for use in or on foodstuffs. Adopted February 23, 1999.

A RIFM review is available citing one negative human maximisation test (n=25) with 10% pet. (153).

MENTHOL	 1490-04-6
CAS # 1490-04-6 / 89-78-1 / 2216-51-5	
EC # 216-074-4 / 239-388-3 / 218-690-9	
5-Methyl-2-(1-methylethyl)-cyclohexanol (1490-04-6) (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol (89-78-1) (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol (2216-51-5)	
Other names: 1490-04-6: Menthol; 1-Methyl-4-isopropyl-3-cyclohexanol; 2-Isopropyl-5-methylcyclohexan-1-ol; 2-Isopropyl-5-methylcyclohexanol; 3-Hydroxy-p-menthane; 5-Methyl-2-(1-methylethyl)cyclohexanol; 5-Methyl-2-isopropylcyclohexanol; Menthyl alcohol; p-Menthan-3-ol 89-78-1: (1a,2β,5a)-5-Methyl-2-(1-methylethyl)-cyclohexanol; cis-1,3,trans-1,4-Menthol; dl-Menthol; (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)cyclohexanol; (±)-Menthol; DL-Menthol; Fisherman's Friend Lozenges; Hexahydrothymol; Menthacamphor; Menthol; Menthomenthol; NSC 2603; Peppermint camphor; Racementhol; Therapeutic Mineral Ice; Thymomenthol; rac-Menthol	 89-78-1
	 2216-51-5

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1 α ,2 β ,5 α)]-5-Methyl-2-(1-methylethyl)-cyclohexanol; (1R,3R,4S)-(-)-Menthol; (-)-Menthol; (-)-Menthyl alcohol; (-)-trans-p-Methan-cis-3-ol; (1R)-(-)-Menthol; (1R,2S,5R)-(-)-Menthol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexan-1-ol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol; (R)-(-)-Menthol; 1R-Menthol; L-Menthol; L-Mentholum; Levomenthol; NSC 62788; l-(-)-Menthol; l-Menthol

Current regulation: /

Clinical data:

Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 10 patients had positive (+ to +++) PT reactions to menthol 5% pet. at D4, mostly reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). In 63 patients positive to the FM I, 1 had a positive PT reaction to menthol, 5% pet., in the Santucci 1987 study (28). The IVDK 2010 study, 1 of 1147 patients tested with 1% menthol in pet. had a weak positive reaction to menthol (7).

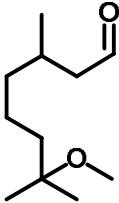
A case of contact allergy to “peppermint and menthol” in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155). Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application of 2% menthol in pet. or 5% peppermint oil in pet., has been reported (156). “A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. During the last two years, the patient had presented dyspnoea, wheezing and nasal symptoms when exposed to mentholated products such as toothpaste and candies. The aetiology was suggested by the history of exposure and diagnosis was established by skin tests and bronchial challenge with menthol. The patient achieved control of symptoms by avoiding menthol and its derivatives.” (157).

Additional information:

Menthol is an ingredient of several essential oils, like peppermint oil, and has been identified as causative allergen in case reports listed above.

Four stereoisomeric forms are known. Natural menthol occurs as L-form (CAS 2216-51-5), trade products are DL-menthol (CAS 1490-04-6). D-form: CAS 89-78-1, racemic: CAS 15356-70-4. Sensitive to light, air and heat (53).

L-menthol and menthol (isomer not specified) are “top 100” substances (IFRA, pers. comm.2010). RIFM reviews are available regarding “menthol” (158), D-menthol (159), L-menthol (160), DL-menthol (161) and menthol, racemic (162). A CIR expert panel review is available (163).

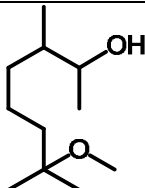
METHOXYCITRONELLAL	
CAS # 3613-30-7	
EC # 222-784-5	
7-Methoxy-3,7-dimethyl-octanal	
7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal	

Current regulation: /

Clinical data:

Nakayama et al. found 1974 (after (29)) 12 “strong positive” and 10 “weak positive” reactions to methoxycitronellal (unknown test concentration), with cross-reactions to hydroxycitronellal (proportion not given), in 183 patients.

Additional information: /

METHOXYTRIMETHYLHEPTANOL	
CAS # 41890-92-0	
EC # 255-574-7	
7-Methoxy-3,7-dimethyl-2-octanol	
3,7-Dimethyl-7-methoxy-2-octanol; Dihydromethoxyelgenol; Elesant; Osyrol	

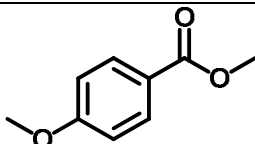
Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.9% of the patients with known contact allergy to fragrance ingredients had a positive PT reaction to this ingredient not reported as allergen previously (1).

Additional information:

A RIFM review is available (128) citing 1 negative maximisation test (n=27).

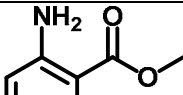
METHYL <i>p</i>-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

Current regulation: /

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to methyl anisate 4% pet. (24).

Additional information: /

METHYL ANTHRANILATE	
CAS # 134-20-3	

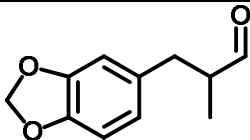
EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; ReJex-iT AP 50; ReJex-iT TP 40; Sunarome UVA; [2-(Methoxycarbonyl)phenyl]amine; o-(Methoxycarbonyl)aniline; o-Aminobenzoic acid methyl ester; o-Carbomethoxyaniline	

Current regulation: /

Clinical data:

In 91 Israeli patients with a positive or doubtful reaction to FM I or MP methyl anthranilate was tested (conc. not given), with a negative result (164).

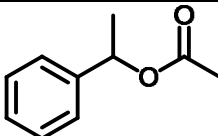
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYLENEDIOXYPHENYL METHYLPROPANAL	
CAS # 1205-17-0	
EC # 214-881-6	
3-(1,3-Benzodioxol-5-yl)-2-methylpropanal	
Hydrocinnamaldehyde, α-methyl-3,4-(methylenedioxy)-; 2-Methyl-3-(3,4-methylenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methylenedioxyphenyl)propionaldehyde; 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal; Heliobouquet; Heliofresh; Heliofan; 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).

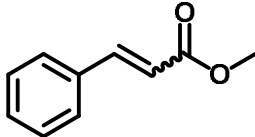
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl 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	

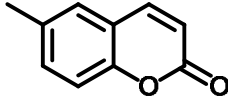
Current regulation: /

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	

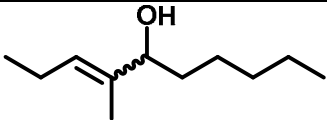
Current regulation: Annex III, part 1, n° 46

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 Portuguese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

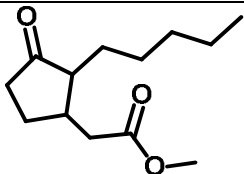
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (170), reporting 1 negative HRIPT (n=50). It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL DIHYDROJASMONATE	
CAS # 24851-98-7	
EC # 246-495-9	
Methyl 2-(3-oxo-2-pentylcyclopentyl) acetate	

Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester; Kharismal; MDJ; Methyl (3-oxo-2-pentylcyclopentyl)acetate; Methyl 3-oxo-2-pentylcyclopentane ethanoate; Hedione

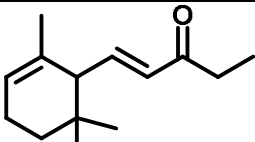
Current regulation: /

Clinical data:

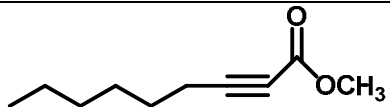
In the Frosch 2002 b study, 3 of 1606 consecutive patients (0.2%) showed positive reactions to hedione (5% pet.) (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% hedione in pet., tested in 100 consecutive patients in Belfast, were observed (15).

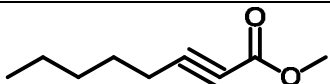
Additional information:

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	

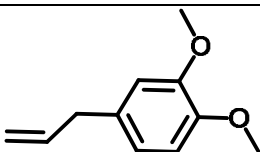
1-(2,6,6-Trimethyl-1-cyclohex-2-enyl)pent-1-en-3-one	
6-Methylionone	
Current regulation: /	
Clinical data:	
See METHYLIONANTHEME for one clinical case report. Regarding methyl ionone gamma, the Frosch 1995 dose-finding pilot study found no positive reaction to 1% and 5% of this substance in pet., tested 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 heptene and methyl octane carbonates; patch testing was strongly positive to both compounds at 1% in MEK (172).	
Additional information: /	

METHYL 2-OCTYNOATE	
CAS # 111-12-6	
EC # 203-836-6	
Methyl oct-2-ynoate	
M2O; Methyl heptin carbonate; Folione; Methyl hept-1-yne-1-carboxylate; Methyl pentylacetylenecarboxylate; NSC 72098; Vert de violette artificiel	
Current regulation: Annex III, part 1, n° 89	

had positive reactions to this allergen, tested at only 2% pet. (6). In a previous case report of a fragrance laboratory assistant with work-related ACD both methyl heptin and methyl octin carbonate had been found sensitisers – probably due to their very similar chemical structure (172). In a recent bi-centric study with 350 eczema patients who were consecutively tested with 1% and 2% M2O in pet.; 0.8% positive reactions were observed. However, in 3 additional cases active sensitisation, with first reactions appearing 2 to 4 weeks after the patch test, and prompt reactions in the 2 cases repeat-patch tested, was observed (174).

Additional information: /

METHYL EUGENOL	
CAS # 93-15-2	
EC # 202-223-0	
1,2-Dimethoxy-4-(prop-2-enyl)benzene	
4-Allylveratrole; Eugenyl methyl ether extra; 1,2-Dimethoxy-4-allylbenzene; 1,3,4-Eugenol methyl ether; 1-(3,4-Dimethoxyphenyl)-2-propene; 1-Allyl-3,4-dimethoxybenzene; 3,4-Dimethoxy-1-(2-propenyl)benzene; 3,4-Dimethoxyallylbenzene; 3-(3,4-Dimethoxyphenyl)propene; 4-Allyl-1,2-dimethoxybenzene; Benzene, 4-allyl-1,2-dimethoxy-; Chavibetol methyl ether; Ent 21040; Eugenol methyl ether; Eugenyl methyl ether; Methyl eugenol ether; Methyl eugenyl ether; Methylchavibetol; NSC 209528; NSC 8900; O-Methyleugenol; Veratrole methyl ether; Veratrole, 4-allyl-	

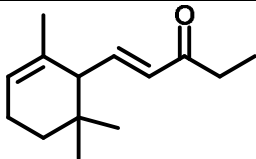
Current regulation: Annex II, 451

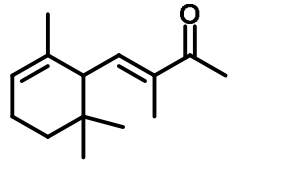
Clinical data:

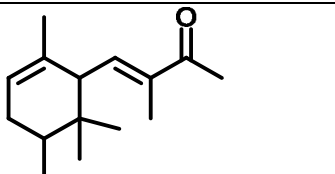
In a previous study by Larsen et al (2002 c), 1.8% of patients with contact allergy to fragrance ingredients reacted positively to this compound (1).

Additional information:

Quote from the SCCS-opinion [0373/00](#): “Methyleugenol should not be intentionally added as a cosmetic ingredient. However, when fragrance compounds containing methyleugenol naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01 % in fine fragrance, 0.004 % in eau de toilette, 0.002 % in a fragrance cream, 0.0002 % in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.” (The reason is genotoxicity and carcinogenicity).

METHYLIONANTHEME	
CAS # 55599-63-8	
EC #	
(1E)-2-Methyl-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-penten-3-one mixt. with (3E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	

8-Methyl- α -ionone-10-methyl- α -ionone mixt.; Iralia Mixture	
Current regulation: ...	
Clinical	data:
One case of ACD has been reported, caused by an E.d.C. (175).	
Additional information:	
Patented by GIVAUDAN SA 1933, is composed of isomeric n-methylionones and iso-methylionones. Methylionone has CAS # 1335-94-0 (not in CosIng) and 1335-46-2 (METHYL α-IONONE ISOMERS); other names: Methyl-α-cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.detail&id=41456, last accessed 2010-07-14).	

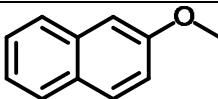
5-METHYL-<i>alpha</i>-IONONE	
CAS # 79-69-6	
EC # 201-219-6	
4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	
Methyl- α -Ionone; 6-Methyl- α -ionone; α -Irone	
Current regulation: /	

Clinical data:

In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to α -irone (10% pet.) (17).

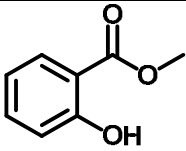
Additional information:

A RIFM review is available (176), citing a (negative) human maximisation test and the study results quoted.

METHYL beta-NAPHTHYL ETHER	
CAS # 93-04-9	
EC # 202-213-6	
2-Methoxynaphthalene	
beta-Naphthyl methyl ether; methyl 2-naphthyl ether; Nerolin (old); NSC 4171; Yara yara; β-Methoxynaphthalene; β-Naphthol methyl ether; β-Naphthyl methyl ether; 2-Methoxynaphthalene; Methyl β-naphthyl ether; 2-Naphthol methyl ether; 2-Naphthyl methyl ether; 6-Methoxy-2-naphthalene	

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

METHYL SALICYLATE	
CAS # 119-36-8	
EC # 204-317-7	
Methyl 2-hydroxybenzoate	
Other names: Salicylic acid, methyl ester; 2-(Methoxycarbonyl)phenol; 2-Carbomethoxyphenol; 2-Hydroxybenzoic acid methyl ester; Analgit; Anthrapole ND; Ben Gay; Exagien; Flucarmit; Methyl ester of 2-hydroxy benzoic acid; Methyl o-hydroxybenzoate; Methyl salicylate; NSC 8204; Wintergreen oil; o-Hydroxybenzoic acid methyl ester; "Oil of wintergreen"	

Current regulation: /

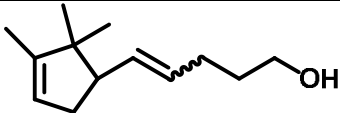
Clinical data:

The deGroot 2000 study yielded 7 positive reactions to methyl salicylate (2% pet.) in 1825 consecutive patients (12).

A case of ACD following the application of a compress bandage containing methyl salicylate has been reported, using 2% "o.o." as PT concentration; the dose per area of methyl salicylate in the occlusive bandage was not reported (177). A similar case was reported in 1977, positive to 2% methyl salicylate in olive oil, with elicitation of pruritus and erythema after oral ingestion of acetyl salicylic acid (178).

Additional information:

A RIFM review is available (179) providing an overview on 3 human sensitisation experiments (e.g., the HRIPT) which were all negative, and clinical data. In a number of older PT studies, positive test results were seen in 6 of 4600, 3 of 183, 3 of 241, 17 of 585, 1 of 70, all employing a test concentration of 2%, usually in pet., according to above review. Methyl salicylate may occur in topical analgesic (OTC) medications, in Germany, for instance, in "Camphopin® Salbe" („Rote Liste 2010").

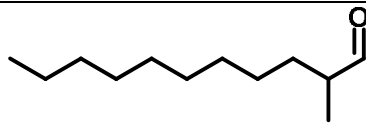
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "MTCP", tested 5% pet. (19). In the An 2005 study, 12 of 422 consecutive patients, i.e., 2.8%, had positive reactions to "ebanol", tested at 5% (13).

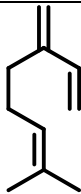
Additional information: /

METHYLUNDECANAL	
CAS # 110-41-8	
EC # 203-765-0	
2-Methylundecanal	
Aldehyde c-12 mna; undecenal, 2-methyl-; 2-Methyl-1-undecanal; Aldehyde M.N.A.; Methyl n-nonyl acetaldehyde; Methylnonylacetaldehyde; NSC 46127	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

MYRCENE	
CAS # 123-35-3	
EC # 204-622-5	
7-Methyl-3-methylideneocta-1,6-diene	
2-Methyl-6-methylene-2,7-octadiene; 7-Methyl-3-methylene-1,6-octadiene; NSC 406264; β-Geraniolene; β-Myrcene	

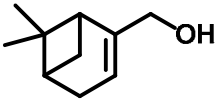
Current regulation: /

Clinical data:

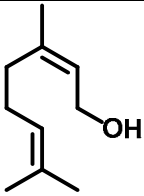
In a clinical study in 6 European centres, including 1511 consecutive patients, 1 patient had a positive reaction to oxidized myrcene (65).

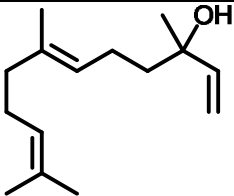
Additional information:

Myrcene autoxidizes spontaneously and rapidly at air exposure. In experimental studies on beta-myrcene an EC3 value of 4.3% was seen for a sample air-exposed 10 weeks (Sköld M. Contact allergy to autoxidized fragrance terpenes (180).

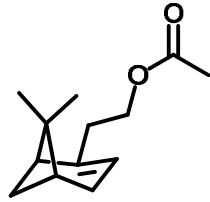
MYRTENOL	
CAS # 515-00-4	
EC # 208-193-5	

(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol
Current regulation: /
Clinical data: /
Additional information:
A RIFM review exists (181), citing 2 of 3 HRIPT studies with 1 case of sensitisation to myrtenol each.

NEROL	
CAS # 106-25-2	
EC # 203-378-7	
(2Z)-3,7-Dimethylocta-2,6-dien-1-ol	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; (Z)-3,7-Dimethyl-2,6-octadien-1-ol; (Z)-Geraniol; (Z)-Nerol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 3,7-Dimethyl-cis-2,6-octadien-1-ol; Nerol 900; Neryl alcohol; cis-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; β-Nerol; cis-geraniol – i.e., isomeric to geraniol	
Current regulation: /	
Clinical data:	
In the Larsen 2002 c study, 6.0% of the fragrance sensitive patients reacted positively to 5% in pet. (1).	
Additional information:	
A RIFM review is available (182) citing (negative) human sensitisation experiments, an older study from Japan and the Larsen 2002 c study (see above).	
Regarding autoxidation studies – see geraniol.	

Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	
Current regulation: /	
Clinical data: /	
Additional information:	

RIFM review is available (183) citing the occurrence of “3 positive reactions in 2273 patients”. Another RIFM review is available on cis-nerolidol (184), mentioning that no data on this compound are available.

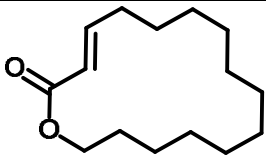
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol acetate; Citroviol; NSC 1286; NSC 404963; Nopol acetate; Nopyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 25% PT preparation of this compound – reactions may have at least partly been due to an “excited back syndrome” and thus a limited evidence (25).

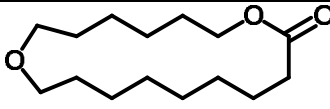
Additional information: /

OXACYCLOHEXADECENONE	
CAS # 34902-57-3	
EC # 609-040-9	
(3E)-Oxacyclohexadec-3-en-2-one	
Globalide; Oxacyclohexadecen-2-one	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

OXALIDE	
CAS # 1725-01-5	
EC # 217-033-3	
1,8-Dioxacycloheptadecan-9-one	
Nonanoic acid, 9-[(6-hydroxyhexyl)oxy]-, o-lactone; 10-Oxa-16-hexadecanolide; Oxalide; Oxalide T	

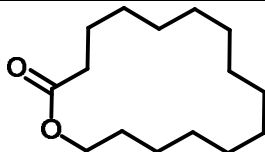
Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "10-oxahexadecanolide", tested 5% pet. (19).

Additional information:

A RIFM review is available (128), citing a negative maximisation test (n=29).

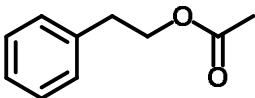
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ -lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; Muskalactone; NSC 36763; Pentadecalactone; Pentadecanolactone; Pentadecanolide; Pentalide; Thibetolide; cpd Supra; ω -Pentadecalactone; angelica lactone; hexaltolide	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). The substance has been used for clinical olfactory testing in the 60ies under the name of exaltolide.

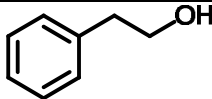
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbiny acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl acetate; β-Phenylethanol acetate; β-Phenylethyl acetate	

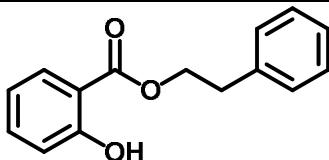
Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). Exposure via plants (*Tanacetum parthenium*) is possible (185).

PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol; β -(Hydroxyethyl)benzene; β -PEA; β -Phenethanol; β -Phenethyl alcohol; β -Phenethylol; β -Phenylethanol; β -Phenylethyl alcohol	
Current regulation: /	
Clinical data:	
The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 25% PT preparation of phenylethyl alcohol (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to this compound, tested 1% pet. in 100 consecutive patients in Odense, DK, was observed (15).	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

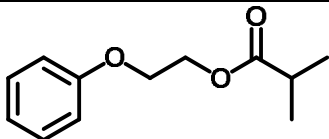
PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201-732-5	
2-Phenylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	

Current regulation: /

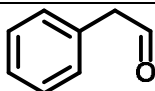
Clinical data: /

Additional information:

A RIFM review exists (186), quoting a negative human maximisation test and a number of animal experiments, including cross-sensitisation experiments with benzyl salicylate. One LLNA study is reported yielding an EC3 value of 2.1%.

PHENOXYETHYL ISOBUTYRATE	
CAS # 103-60-6	
EC # 203-127-1	
2-Phenoxyethyl 2-methylpropanoate	
Isobutyric acid, 2-phenoxyethyl ester; Ethanol, 2-phenoxy-, isobutyrate; 2-Phenoxyethyl isobutyrate; NSC 227210; NSC	

406209; Phenoxyethyl isobutyrate; β -Phenoxyethyl isobutyrate	
Current regulation: /	
Clinical data: /	
Additional information:	
It is a "top 100" substance (IFRA, pers. comm.2010).	

PHENYLACETALDEHYDE	
CAS # 122-78-1	
EC # 204-574-5	
2-Phenylacetaldehyde	
Benzylcarboxaldehyde; Hyacinthin; NSC 406309; Phenacetaldehyde; Phenylacetaldehyde; Phenylacetic aldehyde; Phenylethanal; α-Phenylacetaldehyde; α-Tolualdehyde; α-Toluic aldehyde	

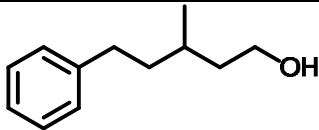
Current regulation: /

Clinical data:

In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to phenylacetaldehyde 2% pet. (24). In a case report, Sanchez-Politta et al. describe a 26-year-old worker in a perfume factory, who suffered from a spill of pure phenylacetaldehyde and became sensitised, as proven by positive patch tests with 0.5%, 1% and 2% (10 healthy controls negative) (187).

Additional information:

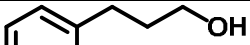
SCCS opinion: [1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assessment" \(QRA: Citral, farnesol and phenylacetaldehyde\)](#)

PHENYLISOHEXANOL	
CAS # 55066-48-3	
EC # 259-461-3	
3-Methyl-5-phenylpentan-1-ol	
3-Methyl-5-phenyl-1-pentanol; 3-Methyl-5-phenylpentanol; 5-Phenyl-3-methylpentanol; Mefrosol; Phenoxanol	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

PHENYLPROPANOL	

Opinion on fragrance allergens in cosmetic products

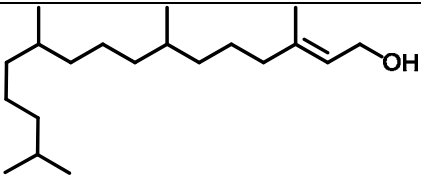
CAS # 122-97-4	
EC # 204-587-6	
3-Phenylpropan-1-ol	
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; NSC 16942; γ-Phenylpropanol; γ-Phenylpropyl alcohol; Phenethyl Carbinol	

Current regulation: /

Clinical data:

The Larsen 2002 c study yielded 0.9% positive reactions in 218 patients with contact allergy to fragrance ingredients (1).

Additional information: ...

PHYTOL	
CAS # 150-86-7	
EC # 205-776-6	
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
Phytol; (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol	

Current regulation: /

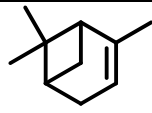
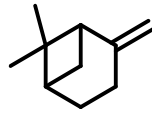
Clinical

data:

/

Additional information:

Phytol is a main constituent of Jasmin abs. with 7.4% reported content (17). In a human maximization study involving 25 subjects, there was one case of contact sensitization to 10% phytol (6900 µg/cm²), applied in petrolatum, as reported in a RIFM review (188).

alpha-PINENE and beta-PINENE	
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	 80-56-8
EC # 201-291-9 (alpha-Pinene; according to CAS service: 219-445-9); EC # 204-872-5 (beta-Pinene; according to CAS service: 245-424-9)	 127-91-3
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (80-56-8)	
6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane (127-91-3)	

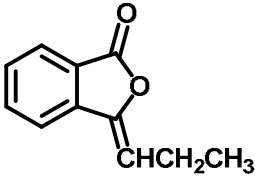
80-56-8: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene 127-91-3: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190; Nopinen; Nopinene; PC 600; PC 600 (pesticide); Pseudopinen; Pseudopinene; Terebenthene; β-Pinene	
Current regulation: Annex III, part 1, n° 130 (Peroxide value less than 10 mmol/L in substance)	

Clinical data:

In 63 patients positive to the FM I, 2 had a positive PT reaction to beta-pinene (and none to alpha-pinene 5% pet.), 1% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 17 of 30 patients reacted positively to alpha-pinene, and 2 to beta-pinene (189). In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear et al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190).

A case report from Zacher and Ippen on 2 patients with allergic contact dermatitis due to bergamot oil (191) describes positive patch test reactions to alpha-pinene and beta-pinene in one, a worker in a perfume factory.

Additional information: /

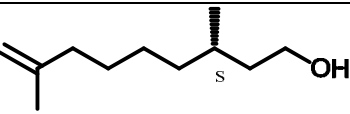
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; Propylidenephthalide; Celeriax; Propylidenephthalide	3-

Current regulation: Annex III, part 1, n° 175

Clinical data:

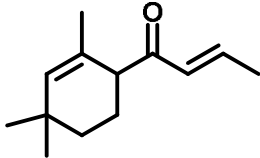
In the Malten 1984 study, 2.6% of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In this paper, "3/25" positive results in human maximisation tests are listed.

Additional information: /

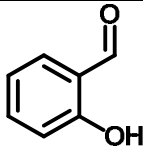
RHODINOL	
CAS # 6812-78-8	
EC # 229-887-4	
(3S)-3,7-Dimethyloct-7-en-1-ol	
Rhodinol; (-)-Rhodinol; α-citronellol; (-)-α-Citronellol; (S)-	

Opinion on fragrance allergens in cosmetic products

α -Citronellol	
Current regulation: /	
Clinical data: / (see below)	
Additional information:	
A RIFM review exists citing a positive HRIPT with several cases of sensitisation, 5 of these proven upon re-challenge, and a negative human maximisation test (192). In a previous RIFM review (128), a Japanese clinical study (source not accessible) is cited: "In patch tests using cosmetics ingredients and fragrance materials on patients with eczema and dermatitis, 5% rhodinol (vehicle not specified) produced one sensitization reaction in 202 patients (Itoh et al., 1988 ¹⁴)"	

trans-ROSE KETONE-5	
CAS # 39872-57-6	
EC # 254-663-8	
(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one	
alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	
Current regulation: Annex III, part 1, n° 159 (max. conc. 0.02%)	

Clinical data: /
Additional information:
A RIFM review is available (193) quoting 2 HRIPT studies: one with 0.2% concentration in DEP in 103 volunteers, and negative result, one with 2% concentration, sensitising 2 of 22 volunteers.

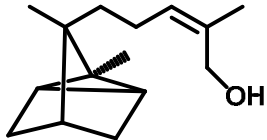
SALICYLALDEHYDE	
CAS # 90-02-8	
EC # 201-961-0	
2-Hydroxybenzaldehyde	
Salicylaldehyde; 2-Formylphenol; NSC 112278; NSC 49178; NSC 83559; NSC 83560; NSC 83561; NSC 83562; NSC 97202; Salicylal; Salicylic aldehyde; o-Formylphenol; o-Hydroxybenzaldehyde	
Current regulation: /	

Clinical data:

¹⁴ Itoh M., Hosono K., Kantoh H., Kinoshita M., Yamada K., Kurosaka R. and Nishimura M. (1988) Patch test results with cosmetic ingredients conducted between 1978-1986. *Nippon Koshohen Kagakkaishi* 12 (1), 27-41.

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to salicylaldehyde was observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to salicylaldehyde 2% pet. (22). The IVDK 2010 study, 0.48% (95% CI: 0.18 – 0.79%; percentages standardised for age and sex) of 2729 patients PTed reacted to the compound (7). An earlier study by Bruze and Zimerson points to possible cross-reactivity between salicylaldehyde and “simple methylol phenols” occurring in synthetic resins based on phenol and formaldehyde (194). Among 24 patients sensitised to resorcinol by application of a wart remover, 2 positive reactions to salicylaldehyde were observed (195).

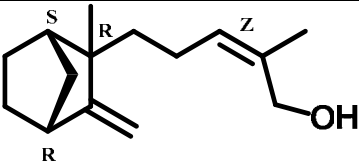
Additional information: Along with other derivatives of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

<i>alpha-SANTALOL</i>	
CAS # 115-71-9	
EC # 204-102-8	
(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.0^{2,6}]hept-3-yl)-2-methylPent-2-en-1-ol	
2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.0 ^{2,6}]heptane, 2-penten-1-ol deriv.; (+)-(Z)-α-Santalol; (+)-α-Santalol; (Z)-α-Santalol; Sandal; Santalol a; cis-α-Santalol; d-α-Santalol	

Current regulation: /

Clinical data: / (see beta-santalol)

Additional information:
Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans.

<i>beta-SANTALOL</i>	
CAS # 77-42-9	
EC # 201-027-2	
(2Z)-2-Methyl-5-[(1S,2R,4R)-2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl]pent-2-en-1-ol	
2-Methyl-5-(2-methyl-3-methylene-2-norbornyl)-2-penten-1-ol; [1S-[1α,2α(Z),4α]]-2-Methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)-2-penten-1-ol; β-Santalol; (-)-(Z)-β-Santalol; (-)-β-Santalol; Santalol b; cis-β-Santalol	

Current regulation: /

Clinical

data:

A RIFM review is available for alpha-santalol (197) and on “santalol” (CAS # 11031-45-1 (198)). The former review cites a Japanese study: “Between April 1979 and August 1990, a total of 3123 male and female patients were patch tested to 2% santalol (.alpha. or .beta. not specified) in petrolatum. Reactions were observed in 47/3123 (1.5%) of the patients. The incidence of positive reactions from 1979 to 1990 was 1.5%. The rate of reactions observed was higher during the earlier period of the patch testing than the later stage (Utsumi et al., 1992)¹⁵.” In another Japanese study cited by the RIFM review “... patch tests were conducted with 0.05–0.5% santalol (specified as santalol 1) in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm² lint with a 2 cm² cellophane disc placed on the lint and then covered with a 4 cm² plaster. Patches were applied to the back, the forearm, and the inside of the upper arm for 24–48 h. Reactions were observed in 15 patients and questionable reactions were observed in 10 patients out of the total 427 participating. A second sample of santalol (specified as santalol 2) was tested on 214 patients. Reactions were observed in three patients and questionable reactions were observed in six patients (Takenaka et al., 1986)¹⁶.” Moreover, “The Mid-Japan Contact Dermatitis Research group (MJDCRG) conducted a 6-year (1976–1981) patch test study on facial dermatoses patients with various fragrance materials. During the year 1979, a total of 327 patients were tested with a mixture of .alpha. and .beta. santalol at concentrations of 10%, 2%, and 1% in white petrolatum. Reactions were observed in 1.5%, 0.6% and 0.6% of the 327 patients tested at concentrations 10%, 2%, and 1%, respectively (MJDCRG, 1984)¹⁷.”

The Goossens 1997 study found 5 of 111 patients positive to “santalol 10% pet.” (isoform not specified) – all sensitised to other fragrance allergens as well (23). In the Larsen 2001 study, patch testing with “2-methyl-5-(2,3-dimethyltricyclo[2.2.1.0(2,6)]hept-3-yl-2-pentenol(.alpha.-form) and 2-methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-3-yl-2-penten-1-ol(beta-form) 5% pet.” (no CAS numbers given) yielded a total of 2 positive reactions among the 178 patients with known contact allergy to fragrance ingredients (19).

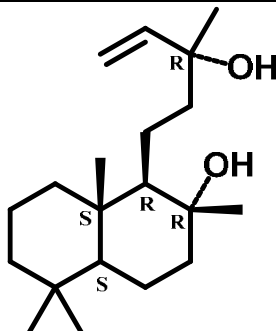
Additional information: “There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)” (A.M. Api, pers. comm., 2010).

Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans

¹⁵ Utsumi, M., Sugai, T., Shoji, A., Watanabe, K., Asoh, S., Hashimoto, Y., 1992. Incidence of positive reactions to sandalwood oil and its related fragrance materials in patch tests and a case of contact allergy to natural and synthetic sandalwood oil in a museum worker. *Skin Research* 34, 209–213

¹⁶ Takenaka, T., Hasegawa, E., Takenaka, U., Saito, F., Odaka, T., 1986. Fundamental studies of safe compound perfumes for cosmetics Part 1. The primary irritation of compound materials to the skin. *Unknown Source*, 313–329.

¹⁷ Mid-Japan Contact Dermatitis Research Group, 1984. Determination of suitable concentrations for patch testing of various fragrance materials. A summary of group study conducted over a 6-year period. *Journal of Dermatology*, 11(1), 31–35.

SCLAREOL	
CAS # 515-03-7	
EC # 208-194-0	
(1R,2R,8aS)-1-[(3R)-3-Hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-2-ol	
(αR,1R,2R,4aS,8aS)-α-Ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1-naphthalenepropanol; [1R-[1α(R*),2β,4αβ,8α]] - α-ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1 Naphthalenepropanol; (13R)-Labd-14-ene-8,13-diol; Sclareol; (-)-Sclareol; [1R-[1.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-.alpha.-vinyldecahydronaphthalene-1-propan-1-ol	

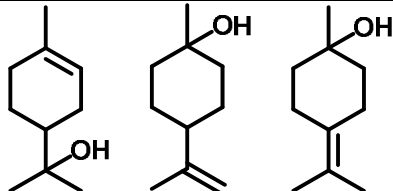
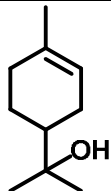
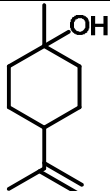
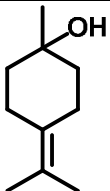
Current regulation: /

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 clinical data, but several maximisation studies, one of which was positive in a few volunteers, which was apparently due to an impurity.

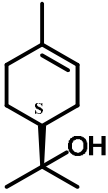
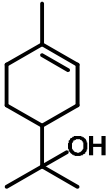
[0986/06 - Opinion on Sclareol \(sensitisation only\)](http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)
(http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

TERPINEOL	
CAS # 8000-41-7	
EC # 232-268-1	
Mixtures of isomers	
Terpineol 318, mixture of terpineol isomers alfa, beta, gamma	 alfa gamma  gamma  beta

Current regulation: /

Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study "Takenaka 1986", finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpineol in pet., tested in 100 consecutive patients in Belfast, were observed (15).

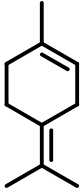
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

<i>alpha-TERPINEOL</i>	 10482-56-1
CAS # 10482-56-1 / 98-55-5	
EC # 233-986-8 / 202-680-6	
2-[(1S)-4-Methyl-1-cyclohex-3-enyl]propan-2-ol (10482-56-1)	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol (98-55-5)	
10482-56-1: (S)-(-)-p-Menth-1-en-8-ol; (-)-α-Terpineol; (S)-(-)-Terpineol; (S)-(-)-α-Terpineol; (S)-α-Terpineol; l-α-Terpineol 98-55-5: p-Menth-1-en-8-ol; (±)-α-Terpineol; 1,1-Dimethyl-1-(4-methylcyclohex-3-enyl)methanol; 1-p-Menthen-8-ol; 2-(4-Methyl-3-cyclohexenyl)-2-propanol; 4-(2-Hydroxy-2-propyl)-1-methylcyclohexene; 8-Hydroxy-p-menth-1-ene; NSC 21449; NSC 403665; PC 593; Pine Oil 593; 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.

<i>Terpinolene</i>	
CAS # 586-62-9	
EC # 209-578-0	
1-Methyl-4-propan-2-ylidenecyclohexene	
p-Mentha-1,4(8)-diene; 1-Methyl-4-(1-methylethylidene)-cyclohexene; 4-Isopropylidene-1-methylcyclohexene; Isoterpinene; Nofmer TP; Terpinolen; Terpinolene; α-Terpinolene; δ-Terpinene	

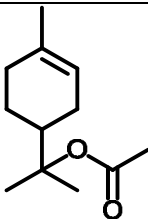
Current regulation: Annex III, part 1, n° 133 (Peroxide value less than 10 mmoles/L in substance)

Clinical data:

A 49-year-old machine cleaner developed occupational contact dermatitis due to the cleaner, which gave a positive patch test result at 1:10 000 in water. Of the ingredients identified by chromatography, only .delta.-3-carene and terpinolene, tested 5% pet.,

gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).

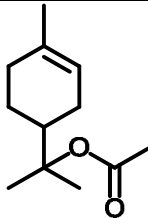
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

TERPINEOL ACETATE (Isomer mixture)	
CAS # 8007-35-0	
EC # 232-357-5	
4-Methyl-1-propan-2-yl-1-cyclohex-2-enyl acetate	
Terpinyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpinyl acetate in pet., tested in 106 consecutive patients in Barcelona, were observed (15)

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

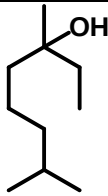
<i>alpha</i>-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate; α-Terpineol acetate; p-Menth-1-en-8-yl acetate; 1-Methyl-1-(4-methylcyclohex-3-enyl)ethyl ethanoate; (±)-.alpha.,.alpha.,4-trimethylcyclohex-3-ene-1-methyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified no positive reactions among 179 patients using a 10% PT preparation of “terpinyl acetate” (25).

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

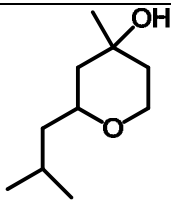
Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	

Current regulation: /

Clinical data:
/

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM

review is available (205) quoting 1 negative human maximisation test.

TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	
CAS # 63500-71-0	
EC # 405-040-6	
4- Methyl-2-(2-methylpropyl)tetrahydro-2H-4-pyranol	
2-(2-Methylpropyl)-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-methyltetrahydropyran-4-ol; 4-Hydroxy-4-methyl-2-(2-methylpropyl)tetrahydropyran; Florosa; Rozanol	

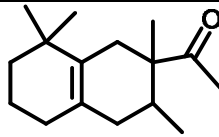
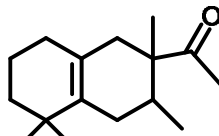
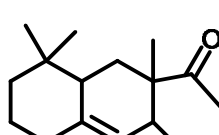
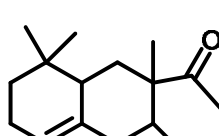
Current regulation: /

Clinical

data:

/

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES		54464-57-2
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9		54464-59-4
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9		68155-66-8
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2)		68155-67-9
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone (54464-59-4)		
1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-66-8)		
1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-67-9)		
54464-57-2: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone; 1',2',3',4',5',6',7',8'-Octahydro-2',3',8',8'-tetramethyl-2'-acetone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemon E; OTNE; Orbitone		

Current regulation: /

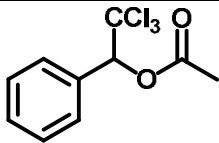
Clinical

data:

In the Frosch 2002 a study, 0.2% of 1855 consecutive patients reacted to the compound (brand name mentioned: „Iso E. Super“, 5% pet.) (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction both to 1% and 5% “Iso E Super ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15). The Larsen 2001 study yielded 1.7% positive reactions (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: According to CosIng: “Mixture of isomers: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-67-9); 1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-66-8)”, (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40504>, last accessed 2009-11-11).

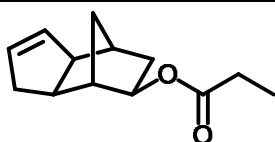
It is a “top 100” substance (IFRA, pers. comm. 2010)

TRICHLOROMETHYL PHENYL CARBINYL ACETATE	
CAS # 90-17-5	
EC # 201-972-0	
2,2,2-Trichloro-1-phenylethyl acetate	
Benzenemethanol, α-(trichloromethyl)-, acetate; Benzyl alcohol, α-(trichloromethyl)-, acetate (Trichloromethyl)phenylcarbinyl acetate; (±)-α-(Trichloromethyl)benzyl acetate; 2-Acetoxy-1,1,1-trichloro-2-phenylethane; Crystal rose; NSC 165582; Rosacetol; Rosephenone; Rosetone; Rosone; α-(Trichloromethyl)benzyl acetate	

Current regulation: /

Clinical data: /

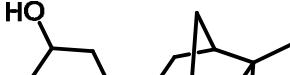
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

TRICYCLODECENYL PROPIONATE	
CAS # 17511-60-3	
EC # 241-514-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-6-yl propionate	
4,7-Methano-1H-inden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propionate; Cyclaprop; Florocyclene; Greenyl propionate; Tricyclo(5.2.1.02,6)dec-3-en-8-yl propionate.	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010).

3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL	

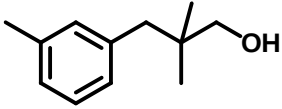
Opinion on fragrance allergens in cosmetic products

CAS # 3407-42-9	
EC # 222-294-1	
3-(5,5,6-Trimethyl-6-bicyclo[2.2.1]heptanyl)cyclohexan-1-ol	
3-(5,5,6-Trimethyl-2-norbornyl)-cyclohexanol; 3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexan-1-ol; 3-Hydroxy-1-(5-isocamphyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of “synthetic sandalwood oil”.

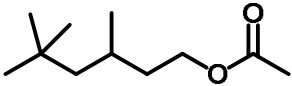
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

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).

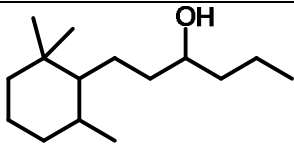
Additional information: /

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)

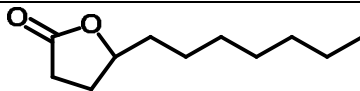
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethylcyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; Finotimber; Timberol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

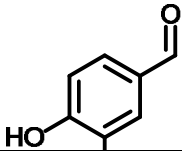
<i>gamma</i>-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ -lactone; (RS)- γ -Undecalactone; ($\pm$)- γ -Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 350120-1; Peach lactone; Peche Pure; Persicol; γ -(n-Heptyl)- γ -butyrolactone; γ -Heptyl- γ -butyrolactone; γ -Heptylbutyrolactone; γ -Undecalactone; γ -Undecanolactone; γ -Undecanolide; γ -n-Heptylbutyrolactone	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm. 2010)

VANILLIN	
CAS # 121-33-5	
EC # 204-465-2	

4-Hydroxy-3-methoxybenzaldehyde	
2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; 4-Formyl-2-methoxyphenol; 4-Hydroxy-5-methoxybenzaldehyde; 4-Hydroxy-m-anisaldehyde; H 0264; Lioxin; NSC 15351; NSC 403658; NSC 48383; Rhovanil; Vanillaldehyde; Vanillic aldehyde; Vanillum; m-Methoxy-p-hydroxybenzaldehyde; p-Hydroxy-m-methoxybenzaldehyde; p-Vanillin	

Current regulation: /

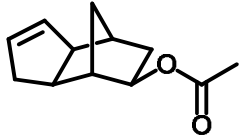
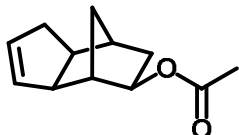
Clinical data:

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to vanillin was observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to vanillin 10 % pet. (22). The IVDK 2010 study, n=10, i.e., 0.19% (95% CI: 0.07 – 0.32%; percentages standardised for age and sex) of 4377 patients PTed reacted to the compound, tested 10% pet. (7). In n=102 patients with a positive reaction to MPR, 19 compounds of this natural mixture were tested, among these, vanillin, to which none reacted positively (208). In 21 patients with contact allergy to propolis, 2 also reacted to vanillin (10% pet.) (209).

A 13-year-old girl with recurrent (peri-)cheilitis after application of a vanilla lip salve tested strongly positive to this salve (as is), "Vanilla 10% pet." (unclear, whether natural extract or vanillin) and MPR (210). Trattner/David identified 1 / 641 consecutive patients with positive reaction to vanillin (31).

Additional information:

Naturally occurring in the fruit of *Vanilla planifolia* after a fermentation process, in styrax, clove oil, potatoes, wood, including Myroxylon pereirae resin, and other material (53). Nowadays, vanillin is synthesised from eugenol, guajakol and lignin residues from paper production, however, not fully achieving the subtle scent and taste of the natural material (53). It is a "top 100" substance and classified as R43 (IFRA, pers. comm. 2010).

VERDYL ACETATE	
CAS # 2500-83-6/ 5413-60-5	
EC # 219-700-4 / 226-501-6	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6)	2500-83-6
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat (5413-60-5)	
2500-83-6: 4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; NSC 142428; NSC 94573	
5413-60-5: 4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 8-Acetoxytricyclo[5.2.1.0 ^{2,6}]dec-3-ene; Greenyl acetate;	5413-60-5

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Herbaflorat; Jasmacyclene; NSC 6598	

Current regulation: /

Clinical data: /

Additional information:

In CosIng, both above CAS numbers are listed under “verdyl acetate” (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41289>, last accessed 2010-07-19).

In the CAS, there are 2 separate entries; moreover, there are 2 separate RIFM reviews:

- # 2500-83-6: Other names: Tricyclo[5.2.1.0^{2,6}]dec-4-en-8-yl acetate (REACH, EINECS, INCI Name according to CAS); 3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl Acetate; Tricyclodecen-4-yl 8-Acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available, stating that “no data is available” regarding the skin sensitising properties of the substance (211).
- # 5413-60-5: Other names: 3a,4,5,6,7,7a-hexahydro-4,7-methanoinden-6-yl acetate (REACH, EINECS, INCI Name according to CAS), 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 8-Acetoxytricyclo[5.2.1.0^{2,6}]dec-3-ene; Tricyclodecenyl acetate; Greenyl acetate; Herbaflorat; Jasmacyclene; NSC 6598; Verdyl acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available (212), citing 2 negative human maximisation tests and 1 negative HRIPT.

Natural extracts / essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic material. Moreover, several compounds cannot be synthesised at a competitive price, and the demand for perfumes based on natural materials is considerable (34).

The three main methods used to concentrate plant fragrance substances as essential oils comprise steam distillation, mechanical processes from the epicarp of Citrus fruits ("pressing") and dry distillation. An Essential oil is „obtained by steam distillation with addition of water in the still (hydrodistillation) or without addition of water in the still (directly by steam“)(213). Essential oil of fruit juice is „obtained by from a fruit juice during its concentration or during UHT (flash pasteurization) treatment“ (213). Cold pressed essential oil is „obtained by mechanical processes from the epicarp of the fruit of a Citrus, at ambient temperature“(213). Citrus peel oils, apart from distilled Citrus oils, are produced with various methods (214). The oil consists of a high volume of volatile terpenes, mostly monoterpenes but also contains small amounts of non-volatile compounds such as dyes, waxes and furocoumarines.

The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concrete: an extract „obtained from a fresh plant natural raw material by extraction with a solvent“¹⁸, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolute: „product, obtained by extraction with ethanol from a concrete, a floral pomade, a resinoid or a supercritical fluid extract. The ethanolic solution is generally cooled down and filtered in order to eliminate the «waxes»; the ethanol is then eliminated by distillation“¹⁹. Resinoids, used for their fixative properties, are „obtained from a dry plant natural raw material by extraction with a solvent“²⁰. The products are usually highly viscous and thus might sometimes be diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are extracts „of spice or aromatic herb“ by „treating a natural raw material with a solvent, then, after filtration if necessary, the solvent is eliminated“²¹.

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case report or clinical studies regarding essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g., masseurs,

¹⁸ ISO/DIS 9235

¹⁹ ISO/DIS 9235

²⁰ ISO/DIS 9235

²¹ ISO/DIS 9235

physiotherapists (215, 216), aromatherapists (217-221), beauticians doing massages (222); for further details, e.g., PT results with various essential oils, see original case reports. "Current Regulation" refers to the EU Cosmetics Directive only.

Catalogue of natural extracts / essential oils evaluated

ACORUS CALAMUS ROOT OIL

CAS 84775-39-3; EC 283-869-0

Calamus Oil; "Sweet Flag Oil"

(*Acorus calamus*, ext. = INCI name)

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "calamus" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to "calamus" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Acorus calamus* L. (sweet flag calamus). *Acorus Calamus* Root Oil is an essential oil obtained from the rhizomes of the calamus, *Acorus calamus* L., Araceae. It contains beta-asarone (up to 96%, depending on ploidy, and with this, origin (34)), calamene (about 4%), calamol (about 3%) alpha-asarone (about 1%), camphene (about 1%) and some beta-pinene and asaronaldehyde (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41330>, last accessed 2010-01-29. Use is restricted due to potential toxicity of beta-asarone (34).

CANANGA ODORATA and Ylang-ylang oil

Ylang-ylang and cananga oils are essential oils that are obtained from two subspecies of the cananga tree (34). In the INCI nomenclature, both are not differentiated.

CANANGA ODORATA FLOWER EXTRACT

CAS 83863-30-3; EC 281-092-1
(ylang-ylang, ext.) INCI name:
CANANGA ODORATA EXTRACT

CANANGA ODORATA FLOWER OIL

CAS 8006-81-3, 68606-83-7; EC / (oils,
ylang-ylang) INCI name: CANANGA
ODORATA OIL

Current regulation: ...

Clinical data:

Ylang-ylang oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma*

genuina)

In the Larsen 2002 c study, “synthetic ylang-ylang oil” caused 6.4% positive reactions in 218 patients with known contact allergy to fragrance ingredients (1). In a Japanese study, M. Sugawara et al. noted a significant decline of the proportion of patients reacting positively to “ylang-ylang oil 5% pet.” from 1971 to 1989, the overall number in patients with cosmetic dermatitis amounting to 176 of 1438 (12.2%, 95% CI: 10.6 – 14.0%) (223). In the Frosch 2002 b study, two fractions of Ylang-Ylang oil (I and II) were separately tested, each at 10% pet. Fraction I yielded 2.6%, fraction II 2.5% positive test reactions (no data on concomitant reactivity given) (17). The deGroot 2000 study, with 1825 consecutively tested patients, found 18 positive PT reactions to “ylang-ylang oil”, tested at 4% in pet. (12). The Sugiura 2000 study with 1483 patients with suspected cosmetic dermatitis observed 0.8% positive PT reactions with ylang-ylang oil (5% pet.) (14). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with ylang-ylang oil (2% pet.) 13.4% positive reactions (9). The Belsito 2006 study (20) yielded 0.6% positive reactions to ylang-ylang oil. The subsequent NACDG 2009 study identified 1.5% positive reactions in 4434 patients PTed with 2% “ylang-ylang oil” (21). The IVDK 2010c study found 2.5% positive reactions in 3175 consecutively tested patients, and 3.9% in 2155 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to ylang-ylang oil and 3 to “cananga oil” (48).

Cananga oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma macrophylla*. For Oil of cananga (*Cananga odorata* (Lam.) Hook. f. et Thomson, *forma macrophylla*) an ISO standard exists: ISO 3523:2002. Cananga oil is produced by steam distillation of the flowers of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *macrophylla* (*Annonaceae*). The composition resembles that of “ylang-ylang III”, but with a higher content of caryophyllene (30-40%). Cananga oil originates almost exclusively in Java; annual production about 50 t. The oil is used mainly in perfuming soaps where it is more stable than ylang-ylang oils due to its lower ester content (34).

Sugiura et al. (2000) found 1.1% positive reactions to “cananga oil”, tested 5% pet. (14). Cananga oil (2% pet.) mentioned in the same Portuguese study already cited (9) yielded 10.4% positive reactions. In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to cananga odorata oil tested at 2% concentration (13).

Studies with both oils

The Goossens 1997 study found 3 of 111 patients positive to “ylang-ylang oil 5% pet.”, and 4 to “cananga oil 15% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 1 positive reaction in 200 patients to “cananga” and 4 to “ylang-ylang” essential oil, both tested 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=10 (11.6%) positive reactions to “cananga” and n=8 (9.3%) to “ylang-ylang” essential oils, each tested at 2% pet. (27). Nakayama et al. found 1974 (after (29)) 11 “strong positive” and 15 “weak positive” reactions to “Cananga oil” and 9 and 16, resp., to “Ylang-ylang oil” (unknown test concentration) in 183 patients.

A number of case reports highlight the possibility of occupational contact and sensitisation, e.g. (222, 224).

Additional information:

Ylang-ylang oil

The composition of this essential oil is defined by a standard: ISO 3063:2004. Ylang-ylang oils are obtained by steam distillation of freshly picked blossoms of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *genuina* (*Annonaceae*). The oil is produced mainly in Madagascar and the Comoro islands. Four fractions are collected at progressively longer distillation times and are known as “extra”, “I”, “II” and “III”. The composition of the various oil fractions depends on the duration of distillation. The first fraction has the highest content of strongly odiferous constituents such as p-cresyl methyl ether (5-16%), methyl benzoate (4-9%), (-)-linalool (7-24%), benzyl acetate (5.5-17.5%), and geranyl acetate (2.5-14%). The other fractions contain increasing amounts of sesquiterpene hydrocarbons such as caryophyllene, germacrene-D, and (E,E)-alpha-farnesene (> 70% in “ylang-ylang III”). Components such as p-cresol, eugenol and isoeugenol are important for odour, although they are present only in low concentration (34). According to (30) the maximum observed concentration in ylang-ylang I and II are (in %): germacrene-D (28); (E,E)-alpha-farnesene (21); caryophyllene (17); linalool (I: 19.0; II: 9.5); benzyl benzoate (8.0); farnesol (4.0); benzyl salicylate (4.0); (E,E)-farnesyl acetate (3.5); geraniol (2.5); isoeugenol (0.8); benzyl alcohol (0.5); eugenol (0.5); p-cresyl methyl ether (I: 5.0; II 3.5); methyl benzoate (I: 5.5; II: 3.5); benzyl acetate (I: 10.0; II: 5.0); geranyl acetate (I: 15.0; II: 12.0).

CEDRUS ATLANTICA BARK OIL

CAS 92201-55-3; EC 295-985-9
(*Cedrus atlantica*, ext. = INCI) /
8000-27-9; EC / (Oils,
cedarwood) INCI name: CEDRUS
ATLANTICA OIL

Cedarwood oil

Current regulation: /

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=5 (0.7%) positive reactions to cedarwood oil 10% pet. (22). (The exact origin of “cedarwood oil” in this study is not clear.) The IVDK 2010 c study identified 0.8% positive reactions in 6223 patients tested in the context of a special series with a cedarwood oil tagged with CAS # 8000-27-9 (30).

Additional information:

Cedrus Atlantica Bark Oil is the volatile oil obtained from the bark of *Cedrus atlantica*, *Pinaceae*

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55309>, last accessed 2010-01-05). The main odiferous component is alpha-atlantone [32207-08-2] (39)

Nomenclature also used: *Cedrus atlantica* wood oil (*Cedrus atlantica* (Endl.) G.Manetti ex Carrière)²²

See also *Juniperus virginiana*.

²² ISO 4720:2009 nomenclature

CEDRUS DEODARA WOOD OILCAS 91771-47-0; EC 294-939-5 (*Cedrus deodara*, ext.)*Cedarwood oil*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “cedarwood” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “Himalayan cedarwood” essential oil 2% pet. (27). (The labelling in the latter report points to *Cedrus deodara* as source of “cedarwood oil” in these 2 Polish studies.)

Additional information:

Cedrus Deodara Wood Oil, Himalayan cedarwood oil (*Cedrus deodara* (Roxb. ex D.Don) G. Don)²³, is the volatile oil obtained by steam distillation of the stumps of the Deodar Cedar, *Cedrus deodara*, *Pinaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55311>, last accessed 2010-01-29).

Several other conifer species are called cedars, and the corresponding oils vary considerably in composition. These include Cedar leaf oil (*Thuja* oil) produced by steam distillation of fresh leaves and branch ends of *Thuja occidentalis* L. (*Cupressaceae*) from North America, containing a minimum of 60% thujone [8007-20-3] [90131-58-1] (34). Texas cedarwood oil is produced by steam distillation of chopped wood of *Juniperus mexicana* Schiede (*Cupressaceae*), containing alpha-cedrene (15-25%), thujopsene (25-35%), cedrol 20% minimum [8000-27-9] [91722-61-1] (34). Chinese cedarwood oil is similar to Texas cedarwood oil, obtained by steam distillation of *Cupressus funebris* Endl., *Cupressaceae* (*Chamaecyparis funebirs* (Endl.) France), which is a weeping cypress [8000-27-9] [85085-29-6] (34).

CINNAMOMUM CASSIA LEAF OIL

94961-46-6 [invalid] / 8007-80-5; EC / (Oils, cassia) INCI name: CINNAMONUM CASSIA OIL

*Cassia Oil; Cassia leaf Oil; Cinnamon Oil Chinense***CINNAMOMUM ZEYLANICUM BARK OIL**CAS 84649-98-9; EC 284-635-0 (*Cinnamomum zeylanicum*, ext. = INCI)*Cimmamon Bark Oil Ceylon; Cinnamon Oil Ceylon*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “cassia” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=24

²³ ISO 4720:2009 nomenclature

(27.9%) positive reactions to "cassia" essential oil 2% pet. (27).

A 32 year old Spanish physiotherapist developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: "cinnamon oil" (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Cinnamomum tsumu* Helms, syn. *Cinnamomum cassia* auct. and *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* J. Presl, respectively. Cassia oil (Chinese cinnamon oil) is obtained by steam distillation of the leaves, twigs, and bark of *Cinnamomum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamon bark oil (see below), cassia oil contains a considerable amount of 2-methoxycinnamal (3-15%), in addition to its main constituent, cinnamal (70-88%). Cassia oil is predominantly used in flavouring soft drinks, with an annual production of a few hundred tons (34). For Oil of cassia, Chinese type (*Cinnamomum aromaticum* Nees, syn. *Cinnamomum cassia* Nees ex Blume) an ISO standard exists: ISO 3216:1997

Cinnamomum Zeylanicum Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, *Cinnamomum zeylanicum*, *Lauraceae*. It contains mainly cinnamaldehyde (34), e.g. 50-60%, and lesser quantities of eugenol (4-8%), phellandrene

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75370>, last accessed 2009-11-16). For Oil of cinnamon leaf, Sri Lanka type (*Cinnamomum zeylanicum* Blume) an ISO standard exists: ISO 3524:2003

Cinnamomum Cassia Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamon, *Cinnamomum cassia* (L.), *Lauraceae*. It contains 80% eugenol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75368>, last accessed 2009-11-16). The cinnamon leaf oil produced by steam distillation of the leaves of *Cinnamomum zeylanicum* Blume (*C. verum* J.S. Presl) similarly has a content of 70-83% eugenol (34).

Considering the content of well-known allergenic compounds, the essential oil is considered an Established contact allergen in humans,

CITRUS AURANTIUM AMARA FLOWER OIL

CAS 8016-38-4, 68916-04-1; EC / (Oils, neroli) /

Neroli oil

CITRUS AURANTIUM AMARA PEEL OIL EXPRESSED

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

"Bitter Orange Oil"

INCI names: CITRUS AURANTIUM AMARA ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "neroli oil" (2% pet.) 6.6% positive reactions (9). The Rudzki 1976 study found 3 positive reactions in 200 patients to "bitter orange" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%)

positive reactions to “bitter orange” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.7% positive reactions in 6220 patients tested in the context of a special series (30)

Additional information:

ISO 4720:2009 nomenclature: *Citrus aurantium* L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso. For Oil of neroli (*Citrus aurantium* L. spp. *aurantium*, syn. *Citrus aurantium* L. spp. *amara* var. *pumilia*) an ISO standard exists: ISO 3517:2002. Citrus Aurantium Peel Oil Expressed is an essential oil expressed from the fresh epicarps of the Sour Orange, *Citrus aurantium*, Rutaceae. It contains D-limonene (about 90%), citral, decanaldehyde, methyl anthranilate, linalool, terpineol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41394>, last accessed 2010-01-29). The aldehyde content is lower and the ester content (e.g., linalyl and geranyl acetate) is higher than in sweet orange oil (34). It is predominantly used for flavouring alcoholic beverages. According to (30) the maximum observed concentration in neroli oil are (in %): linalool (44); limonene (18); β -pinene (17); linalyl acetate (15); *trans*- β -ocimene (8); geranyl acetate (5); *trans*-nerolidol (5); (*E,E*)-farnesol (4); myrcene (4); farnesol (4,0); geraniol (3,5); citral (0,3) (30).

CITRUS AURANTIUM AMARA LEAF OIL

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

Petitgrain oil Paraguay / ... bigarade

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Petitgrain bigarade” and “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “Petitgrain bigarade” and n=4 (4.6%) to “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* L. Pers. X *Citrus aurantium* L. ssp. *amara* var. *pumilia*. Petitgrain oils in general are steam distilled from the leaves of citrus trees. Citrus Aurantium Leaf Oil is an essential oil obtained from the leaves of the Sour Orange, *Citrus aurantium*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41392>, last accessed 2010-02-10). Petitgrain oil Paraguay is obtained from an acclimatised variety of the bitter orange tree. Main constituents are linalool (15-30%) and linalyl acetate (40-60%). A number of trace constituents contribute essentially to the odour (34). Petitgrain oil bigarade is derived from the same species of tree grown in France, Italy, Spain and North Africa (34). For Oil of bitter orange petitgrain, cultivated (*Citrus aurantium* L.) an ISO standard exists: ISO 8901:2003.

Considering the content of well-known allergenic compounds, the essential oil is regarded as an established contact allergen in humans

CITRUS BERGAMIA PEEL OIL EXPRESSED

CAS 89957-91-5, 8007-75-8; EC

	289-612-9 (<i>Bergamot, ext.</i>)
<i>Bergamot Oil, Bergamot Orange Oil</i>	INCI: CITRUS AURANTIUM BERGAMIA EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “Bergamot” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found no positive reaction to “Bergamot” essential oil 2% pet. (27). In 63 patients positive to the FM I, 2 had a positive PT reaction to bergamot oil, 2% pet., in the Santucci 1987 study (28). A case report from Zacher and Ippen describes 2 patients with allergic contact dermatitis due to bergamot oil (191), one a worker in a perfume factory, the other sensitised by non-occupational use of cosmetics.

Additional information:

ISO 4720:2009 nomenclature: *Citrus bergamia* (Risso et Poit.), syn. *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler. Citrus Bergamia Peel Oil Expressed is an essential oil expressed from the epicarps of the Bergamot, *Citrus bergamia* risso, Rutaceae. It contains 35-45% L-linalyl acetate, about 6% linalool, D-limonene, DL-limonene and bergaptene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=se_arch.details&id=41398, last accessed 2009-11-27). According to Surburg/Panten: linalyl acetate 22-36%, linalool 3-15%, geranial 0.25-0.5%, citral 1%, with a relatively low terpene content of 25-50% (34, 39). Bergaptene content by HPLC is 0.18-0.38% (34). Annual production from Italy, Brazil, Spain and Ivory Coast is 100 to 150 t. For Oil of bergamot [*Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler], Italian type an ISO standard exists: ISO 3520:1998.

CITRUS LIMONUM PEEL OIL EXPRESSED	CAS 84929-31-7, 8008-56-8; EC 284-515-8 (<i>Lemon, ext.</i>)
<i>Lemon oil</i>	INCI names: CITRUS MEDICA LIMONUM ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with “lemon oil” (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to “lemon oil” 2% pet. (22).

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lemon” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “lemon” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus limon* (L.) Burm. f. According to (30) the maximum observed concentration in lemon oil are (in %): limonene (80); β -pinene (16.5); γ -terpinene (12); citral (3.0); geranial (2.0); neral (1.2); β -bisabolene (0.9); geranyl

acetate (0.7); neryl acetate (0.6); linalool (0,3); geraniol (0,2) (30). An ISO standard exists for Oil of lemon [Citrus limon (L.) Burm. f.], obtained by expression: ISO 855:2003. The composition of lemon oil depends on the variety of lemon and the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

Parameter	Type	Mediterranean		Equatorial	
	American Origin				
	Coast	Desert	Italy	Spain	Ivory coast, Brazil
d_{20}^{20}	0.851–0.857	0.849–0.854	0.850–0.858	0.849–0.858	0.845–0.854
n_D^{20}	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4790
α_D^{20}	+57° to +65°6'	+67° to +78°	+57° to +66°	+57° to +66°	+57° to +70°
Composition by GC [area %]					
β -Pinene	9–14	10–13	10–16.5	10–16.5	7–16
Limonene	63–70	70–80	60–68	60–70	59–75
γ -Terpinene	8.3–9.5	6.5–8	8–12	8–12.8–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geraniol	1.0–2	0.5–0.9	0.8–2	0.6–2	0.5–2
Evaporation residue [weight %]					
	1.75–3.9		1.5–3.9	1.5–3.9	1.5–4
Carbonyl value					
	8–14	6.25–12	11–17	11–17	6–17
CD value	min. 0.2	min. 0.2	0.45–0.9	0.4–0.9	0.2–0.96

CITRUS PARADISI PEEL OIL

CAS 8016-20-4 ; EC /

Grapefruit oil, expressed

INCI: CITRUS GRANDIS OIL

Current regulation: II/358 R1

Clinical data: /

Additional information:

Citrus Paradisi Peel Oil is the volatile oil expressed from the peel of the Grapefruit, Citrus paradisi, Rutaceae
http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=55434

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

CITRUS SINENSIS (syn.: AURANTIUM DULCIS) CAS 97766-30-8, 8008-57-9, EC
 PEEL OIL EXPRESSED 307-891-8 (Orange, sweet,

(Sweet) Orange oil

Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 (Orange, sweet, ext.)

INCI names: CITRUS AURANTIUM DULCIS ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "orange oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to orange oil 2% pet. (22). The Rudzki 1976 study found 1 positive reaction in 200 patients to "sweet orange" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "sweet orange" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, neither positive nor irritant reaction to 1% and 5% "orange oil Bras." in pet., tested in 205 consecutive patients in Dortmund and Göttingen, were observed (15). The IVDK 2010 c study identified 0.2% positive reactions in 6246 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* (L.) Osbeck. For Oil of sweet orange (*Citrus sinensis* (L.) Osbeck), CAS 8008-57-9, obtained by mechanical treatment, an ISO norm exists: ISO 3140:2005. The oils have a high terpene hydrocarbon content (> 90%, mainly (+)-limonene. Important for aroma are aldehydes, mainly decanal and citral, and aliphatic and terpenoid esters. The sesquiterpene aldehydes alpha-sinensal [17909-77-2] and beta-sinensal [6066-88-8] contribute particularly to the special sweet aroma (34). According to (30) the maximum observed concentration in sweet orange oil are (in %): *limonene* (95.0); *linalool* (0.7); n-decanal (0.7); *citral* (0.3); alpha-sinensal (0.05); beta-sinensal (0.06) (30). Worldwide production is more than 30000 tons / year. Main uses comprise the flavouring of beverages and confectioneries and perfuming E.d.C, soaps and household products.

For the latter uses relevant here, both "Orange peel oil, sweet (*Citrus sinensis* (L.) Osbeck) (8008-57-9)", "Orange peel, sweet, extract (*Citrus sinensis* L. Osbeck) (8028-48-6)" and "Orange, sweet, Valencia, ext. (97766-30-8)" are among the top 100 used fragrance materials and classified as R43 (IFRA, pers. comm. 2010).

ORANGE OIL TERPENES (CAS # 68647-72-3) are a "top 100 mixture of substances and classified as R43 (IFRA, pers. comm.2010). Other names: ORANGE, SWEET, TERPENES (REACH); Terpenes and Terpenoids, sweet orange-oil (REACH). The CAS entry refers to a group of substances "Terpenes and Terpenoids, sweet orange-oil" (REACH).

CITRUS TANGERINA ...

Oil of tangerine

CAS 223748-44-5; EC /

[no info in CAS database]

Current regulation: /

Clinical data:

In a 17 year old girl, the perfume used for 3 months caused ACD due to the ingredient "oil of tangerine", with a strong positive PT reaction (to 2% or 10% in pet.; 50 controls

negative) (226).

Additional information:

Citrus Tangerina Peel Oil is the volatile oil expressed from the peel of the ripe fruit the Tangerine, *Citrus Tangerina*, *Rutaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55441>, last accessed 2010-01-29); (*Citrus tangerina* Tanaka).

CORIANDRUM SATIVUM HERB OIL

CAS 84775-50-8; EC 283-880-0
(Coriander, ext.)

Coriander oil

INCI: CORIANDRUM SATIVUM
EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “coriander” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “coriander” essential oil 2% pet. (27).

Additional information:

Coriander Sativum Herb Oil is an essential oil obtained from the herbs of the Coriander, *Coriandrum sativum* L., *Umbelliferae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39388>, last accessed 2010-01-29). The main component of coriander oil is linalool (by GC: 65-78%) and mono- and polyunsaturated fatty aldehydes contributing to the particular aroma. In contrast to the seed oil, coriander leaf oil contains these aldehydes as main constituents, e.g. 2-deccanal and 2-dodecanal (34). For Oil of coriander fruits (*Coriandrum sativum* L.) an ISO standard exists: ISO 3516:1997.

CYMBOPOGON OILS

Cymbopogon oils are produced from several aromatic grasses that belong to the genus *Cymbopogon* Speng. (*Poaceae*). The oils are obtained by steam distillation of the aerial parts of the plants (34).

The composition of the essential oil derived from *Cymbopogon flexuosus* (Nees ex Steudel) J.F. Watson is defined by a standard: ISO 4718:2004, as is the oil derived from *Cymbopogon citratus*: 3217:1974.

CYMBOPOGON CITRATUS LEAF OIL

Cymbopogon citratus (DC.) Stapf.²⁴

CAS 89998-14-1; EC 289-752-0
(*Cymbopogon citratus*, ext. =
INCI)

²⁴ ISO 4720:2009 nomenclature

Lemon Grass Oil; Indian Verbena Oil; Indian Melissa Oil

CYMBOPOGON SCHOENANTHUS OIL

Cymbopogon flexuosus (Nees ex Steudel) J.F. Watson²⁵

CAS 8007-02-1; EC 289-754-1 (oils, lemongrass) / 89998-16-3; EC 289-752-0 (Cymbopogon Schoenanthus, ext. = INCI)

Lemon Grass Oil

Current regulation: /

Clinical data:

The Frosch 2002 b study on 1606 consecutive patients reported 1.6% positive reactions to "lemongrass oil (East India), CAS 8007-02-1", PTed at 2% pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 3 positive reactions to lemongrass oil were observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=6 (0.8%) positive reactions to lemongrass oil 2% pet. (22). The IVDK 2010 c study identified 0.6% positive reactions in 2435 consecutively tested patients and 2.3% positive reactions in 8445 patients tested in the context of a special series (30).

Additional information:

Cymbopogon Citratus Leaf Oil is an essential oil obtained from the leaves of the Lemon Grass, *Cymbopogon citratus* (DC., ex Nees), *Poaceae*. It contains citral (75-85%), methylheptenone, citronellal, geraniol, limonene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39457>, last accessed 2009-11-12). According to Surburg/Panten, by GC: neral (31-40%), geranial (40-50%) (34).

Indian lemongrass oil is obtained by the so-called Indian variety of lemongrass, *Cymbopogon flexuosus* (Nees ex Steud.) Stapf. Content by GC: 25-35% neral, 35-47% geranial (34).

Cymbopogon Schoenanthus Oil is the volatile oil obtained by the steam distillation of fresh Lemon Grass, *Cymbopogon schoenanthus* (L.), *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75419>, last accessed 2009-11-12). According to (30) the maximum observed concentration in lemongrass oil are (in %): citral (85.0); geraniol (7.0); limonene (4.0); geranyl acetate (2.2); caryophyllene (1.6); trans-isocitral (1.4); 6-methyl 5-hepten-2-one (1.3); caryophyllene oxide (1.2); 4-nonanone (1); citronellol (0.8); eugenol (0.3); linalool (0.2) (also according to (227))

In a LLNA study by RIFM, the lemongrass oil as used was reported to contain 68.8% citral, 6.7% limonene, 6.1% geraniol, 2.2% geranyl acetate, 1.6% caryophyllene, 1.4% trans-isocitral, 1.3% 6-methyl 5-hepten-2-one, 1.2% caryophyllene oxide and 1% 4-nonanone, according to analyses of the supplier. The EC3 value was calculated to be 6.5% (227).

CYMBOPOGON MARTINI HERB EXTRACT

CAS 84649-81-0; EC 283-461-2 (Cymbopogon Martini, ext)

²⁵ ISO 4720:2009 nomenclature

INCI: CYMBOPOGON MARTINI OIL*Palmarosa oil*

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon martini* (Roxb.) Will. Watson var. *motia* and var. *sofia*. Cymbopogon Martini Herb Extract is an extract obtained from the herbs of the plant, *Cymbopogon martini*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39460>, last accessed 2009-11-24), namely, by steam distillation of wild or cultivated *Cymbopogon martini* (Roxb.) J.F. Wats., collected when in blossom (34). The main constituent is geraniol (72-94%) (34).

In a LLNA study by RIFM, the palmarosa oil as used was reported to contain 79.4% geraniol, 9.4% geranyl acetate and 1.9% caryophyllene, according to analyses of the supplier. The EC3 value was calculated to be 9.6% (227).

CYMBOPOGON NARDUS HERB OIL

CAS 89998-15-2; EC 289-753-6 (*Cymbopogon nardus*, ext. = INCI)

Citronella Oil (Sri Lanka)

CYMBOPOGON WINTERIANUS HERB OIL

CAS 91771-61-8; EC 294-954-7 (*Cymbopogon Winterianus*, ext. = INCI)

Citronella Oil (Java)

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 5 positive reactions in 200 patients to "citronella" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "citronella" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf. and *Cymbopogon winterianus* Jowitt, respectively. Cymbopogon Nardus Herb Oil is an essential oil obtained from the herbs of the plant, *Cymbopogon* (syn: *Andropogon*) *nardus* (L.), Gramineae. The Ceylon citronella oil contains geraniol (about 60%), citronellal (about 15%), camphene, limonene, linalool, borneol. According to Surburg/Panten, the Sri Lankan oil contains citronellal (3-6%), borneol (4-7%), citronellol (3-8.5%), geraniol 15-23%) and methyl isoeugenol (7.11%) (34).

The Java citronella oil contains 25-50% citronellal, 25-45% geraniol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=39469>, last accessed 2009-11-24). Cymbopogon Winterianus Herb Oil as a synonym for Java citronella oil is obtained from the herbs of the plant, *Cymbopogon winterianus*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=sea>

[rch.details&id=39472](#), last accessed 2009-11-24). This oil, produced in Taiwan and Java, contains citronellal (31-40%), geraniol (20-25%), citronellol (8.5-14%), geranyl acetate (2.5-5.5%), citronellyl acetate (2-4%) and many minor components. Annual worldwide production is currently at around 1000 t (34). For Oil of citronella, Sri Lankan type (*Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf.) an ISO standard exists: ISO 3849:2003, for Oil of citronella, Java type the ISO 3848:2001.

In a LLNA study by RIFM, the citronella oil as used was reported to contain 36.6% citronellal, 20.6% geraniol, 4.1% limonene, 3.7% geranyl acetate, 3.0% citronellyl acetate, 2.6% elemol, 2.2% beta-bourbonene, 1.9% delta-cadiene, 1.6% isopugenol I, 1.4% germacrene D and eugenol and linalol at < 1%, according to analyses of the supplier. The EC3 value was calculated as > 50 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

EUCALYPTUS SPP. LEAF OIL

CAS 92502-70-0; EC 296-357-7
(*Eucalyptus*, ext. = INCI)

Eucalyptus Oil

CAS 8000-48-4; EC / (Oils,
eucalyptus) INCI: *EUCALYPTUS*
GLOBULUS OIL

Current regulation: /

Clinical data:

In a study with 218 fragrance sensitive patients, 1.8% reacted positively to 10% eucalyptus oil (pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to "eucalyptus oil" was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to eucalyptus oil 2% pet. (22). The Rudzki 1976 study found 3 positive reactions in 200 patients to "eucalyptus" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "Eucalyptus" essential oil 2% pet. (27). The IVDK 2010 c study identified 0.2% positive reactions in 6680 patients tested in the context of a special series (30).

In a professional athlete, the use of an "analgesic and anti-inflammatory cream" over 2 years lead to ACD, which was attributed to eucalyptol (eucalyptus oil, 1% pet., 25 controls negative), the sole ingredient of the cream eliciting a positive PT reaction (228)

Additional information:

ISO 4720:2009 nomenclature: *Eucalyptus globulus* Labill. Eucalyptus oils are produced from plants belonging to the genus *Eucalyptus* (*Myrtaceae*), which includes about 500 species in Australia, the country of origin, alone. At present, few of the oils, which are used to characterise species, are commercially important (34). Some species are rich in 1,8-cineole (80-85% content). Other species contain less cineole, but 10-22% alpha-pinene. *E. citriodora* predominantly contains citronellal (min. 75% by GC), with some citronellol and isopulegol (5-10% each) (34). *E. dives* contains (-)-piperitone and 15-25% alpha-phellandrene (34). According to (30) the maximum observed concentration in eucalyptus oil are (in %): 1,8-cineole (58; 70-80 after rectification); α -pinene (22); limonene (8); para-cymene (5); trans-pinocarveol (5); aromadendrene (10); globulol (2.5) [the latter 2 components only traces after rectification] (30).

For Crude or rectified oils of *Eucalyptus globulus* (*Eucalyptus globulus* Labill.) an ISO standard exists: ISO 770:2002.

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL

CAS 8000-34-8; EC / (Oils, clove)

Clove oil

INCI: EUGENIA CARYOPHYLLUS OIL

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 19.3% of patients with known contact allergy to fragrance ingredients reacted positively to "clove bud oil" (10 % pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "clove oil" were observed (3). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with clove oil (2% pet.) 13.4% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.6% positive reactions 2% pet. (22). The Rudzki 1976 study found 2 positive reactions in 200 patients to "clove" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=12 (13.3%) positive reactions to "clove" essential oil 2% pet. (27). The IVDK 2010 c study identified 1.5% positive reactions 6893 patients tested in the context of a special series (30).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: cinnamon oil (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Syzygium aromaticum* (L.) Merr. & L. M. Perry syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison. Standards regarding the composition of clove oil are available: ISO 3141:1997, ISO 3142:1997, ISO 3143:1997. Clove oils are produced from the clove tree *Syzygium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Spreng.) Bullock ex S.G. Harrison]. The content of clove bud, clove leaf and clove stem oil has, with little variation, been determined by GC as 75-92% eugenol, 2-17% caryophyllene and 0.2-15% eugenyl acetate – the latter compound found in particularly high concentration in bud oil (34). According to another source, the following maximum content (%) has been observed regarding the constituents listed: eugenol (92,0);

caryophyllene (17); eugenyl acetate (15); isoeugenol (0.5) (30).

In a LLNA study by RIFM, the clove leaf oil as used was reported to contain 85.3% eugenol, 9.9% caryophyllene and 2.2% alpha humulene, according to analyses of the supplier. The EC3 value was calculated to be 7.1% (227).

EVERNIA FURFURACEA LICHEN EXTRACT

CAS 90028-67-4; EC 289-860-8
(*Evernia furfuracea*, ext. = INCI)

Tree moss extract

Current regulation: /

Clinical data:

The Larsen 1977 study in 20 “perfume-sensitive patients” yielded n=6 positive reactions with “treemoss abs. in benzyl benzoate, 5% petrolatum” (18). In the IVDK 2007 study, 2.7% (95% CI: 2.0 – 3.6%) of 1658 consecutive patients had a positive reaction to “tree moss absolute” (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to the allergen, tested at 2%, i.e., twice the commonly used concentration, and not in pet., but in diethylphthalate (6). The IVDK 2010 study, 6.02% (95% CI: 4.90 – 7.14%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7).

Additional information:

Syn.: *Pseudoevernia furfuracea* (L.) Zopf (53). The lichen grows on the bark of pine and fir trees. The extraction process with carbohydrate solvents yields a “concrete” (2-5% yield) which, in a next step eliminating waxy compounds, is extracted with warm alcohol and subsequent cooling, yielding an “absolute” (40-60% yield) (53).

EVERNIA PRUNASTRI

CAS 90028-68-5; EC 289-861-3
(*Evernia prunastri*, ext. = INCI)

Oak moss abs.

Current regulation: Annex III, part 1, n° 91

Clinical data:
In the “background information” section of the 1999 opinion, oak moss extract is classified as “most frequently reported allergen”; in consecutive PT patients, about 2.8% positive reactions had been reported (33). The German MAK commission has labelled oak moss extract as ‘sensitising to the skin’ (229).

Since the last SCCNFP-opinion of 1999, a “polymer based method” was developed to reduce the natural content of these two compounds from around 1 - several percent to < 75 ppm for atranol and < 25 ppm for chloratranol. However, PTing 14 subjects with previous positive PT reactions to the “oak moss” allergen preparation with the modified *Evernia prunastri* material still elicited positive reactions in 8/14 subjects; thus, the reduction in allergen content was deemed unsafe for the consumer (230). In a study of 885 consecutive eczema patients tested in Gentofte, Denmark, 3.2% had a positive or follicular patch test response to oak moss absolute. Two types of oak moss absolute were tested, one contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types of oak moss absolute

(231). The IVDK 2007 study yielded 2.2% (95% CI: 1.6 – 3.0%) positive reactions in 2063 consecutively tested patients (4). In the Groningen 2009 study, 1.9% (95% CI: 0.7 – 4.0%) had positive reactions to oak moss, tested at 2% pet., i.e., twice the commonly used concentration (6). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reaction (13) (test concentration 2% pet.). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.0% positive reactions (22). The IVDK 2010 study, 1.81% (95% CI: 1.07 – 2.56%) of 1213 consecutively tested patients reacted to the compound, while 5.59% (95% CI: 4.90 – 6.27%) of 4482 of patients tested in a more aimed manner, partly as breakdown testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with *E. prunastri* extract, yielding 2 positive reactions (48).

L. Kanerva et al. report on a 41 year old female hairdresser in whom oak moss abs. contained in a perming solution (concentration in the product unknown) was unequivocally identified as allergen causing (i) occupational hand dermatitis and (ii) scalp dermatitis after application to the own hair (232). Another case of occupational hand dermatitis in a grinding engineer was, at least partly, attributable to contact sensitisation to "oak moss resin" contained in a soluble oil (233).

Additional information:

Source: *Evernia prunastri* (Oak moss) (*Evernia prunastri* var. *prunastri* L. Ach). Oak moss is extracted as described above. Chloratranol and atranol are the degradation products of chloratranorin and atranorin, resp., which are recognised as the main sensitisers in *Evernia prunastri* extracts.

ILLICIIUM VERUM FRUIT OIL

CAS 84650-59-9, 8007-70-3; EC 283-518-1

"Anise Oil", Star anise oil

(Star anise, *Illicium verum*, ext. = INCI)

Current regulation: /

Clinical data:

In a study involving 100 consecutive patients, Rudzki and Grzywa found (i) a relatively high frequency of active sensitisation to star anise oil (n=5) tested with 0.5, 1 and 2% concentration (most likely in yellow petrolatum, as the other allergens in this series). Later patch testing with constituents of this essential oil (1%) in 3 patients yielded positive results to anethole in 3 cases, and to alpha-pinene and safrole in the 1 case tested to these substances. 34% of the consecutive patients reacted positively to star anise oil at 1%, which was considered as (marginally) non-irritating PT concentration (234).

Additional information:

ISO 4720:2009 nomenclature: *Illicium verum* Hook. f. *Illicium Verum* Fruit Oil is an essential oil distilled from the fruits of the Star Anise, *Illicium verum*, Illiciaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40297>, last accessed 2010-01-29). The main component is trans-anethole (86-93%), which can be purified from star anise oil. Main uses are alcoholic beverages, food flavouring and oral care products (34, 39). For Oil of star anise, Chinese type (*Illicium verum* Hook. f.) an ISO standard exists: ISO 11016:1999.

JASMINUM GRANDIFLORUM FLOWER EXTRACT	CAS 84776-64-7; EC 283-993-5 (Jasmine, <i>Jasminum grandiflorum</i> , ext. = INCI)
<i>Jasmine abs.</i>	
JASMINUM OFFICINALE FLOWER OIL	CAS 90045-94-6; EC 289-960-1 (Jasmine, <i>Jasminum officinale</i> , ext. = INCI)
JASMINUM OFFICINALE OIL	CAS 8022-96-6; EC / (Oils, jasmine) INCI: JASMINUM OFFICINALE OIL

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 1.2% of 1606 consecutive patients had a positive PT to "jasmine absolute", tested 5% in pet. (17). The deGroot 2000 study yielded 13 positive reactions to "jasmine, synthetic" in 1825 consecutively tested patients (12). In the early Larsen 1977 study, 18 of 20 "perfume sensitive patients" reacted to "Jasmin synthetic" 10% pet. (18), while 7 reacted to "Jasmin absolute" (10% pet.) – all of these also positive to the synthetic fragrance. The Sugiura 2000 study set in Nagoya, Japan, yielded 1% positive PT reactions in 1483 patients PTed for suspected cosmetic dermatitis, using 5% pet. as test concentration (14). The Larsen 2001 study in 178 patients with known contact allergy to fragrance ingredients found 16.9% positive reactions to jasmine absolute (10% pet.) (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to Jasmine officinale oil (Jasmine absolute, Egyptian), tested at 2% (13). In the NACDG 2009 study, 1.1% of 4447 patients tested with "Jasmine absolute 2% pet." were found PT-positive (21). The Belsito 2006 study (20) yielded 0.4% positive reactions to "jasmine absolute". The Goossens 1997 study found 5 of 111 patients positive to "jasmine absolute" (10% pet.) – all sensitised to other fragrance allergens (23). In 63 patients positive to the FM I, 13 had positive PT reactions to "jasmine absolute", 2% pet., and 12 to "jasmine synthetic", 2% pet. in the Santucci 1987 study – the amount of concomitant reactivity was not examined (28). Nakayama et al. found 1974 (after (29)) 19 "strong positive" and 25 "weak positive" reactions to "jasmin oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.5% positive reactions in 3668 consecutively tested patients and 1.2% positive reactions in 982 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with jasmine absolute, yielding 3 positive reactions, and with "Jasmine synthetic", also resulting in 3 positive reactions (48).

Additional information:

Jasminum Grandiflorum Flower Extract is an extract obtained from the flowers of the Spanish Jasmine, *Jasminum grandiflorum* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39752>, last accessed 2009-11-12).

Jasminum Officinale Flower Oil is an essential oil obtained by molecular distillation of the flowers from the Jasmine, *Jasminum officinale* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39754>, last accessed 2009-11-25).

Jasminum Officinale Oil is the volatile oil obtained from the flowers of the Jasmine, *Jasminum officinale* L., Oleaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=34776>, last accessed 2010-01-05); this latter extract is used by Almirall/Hermal/Trolab for the preparation of a PT allergen.

Jasmine absolute is obtained by solvent extraction, via concrete, from the flowers of *J. grandiflorum* (L.) Aiton from China and India. The main volatile compound is benzyl acetate, however, minor compounds such as indole [120-72-9], cis-jasmone [488-10-8] and methyl jasmonate [1211-29-6] contribute to the typical jasmine fragrance (34). Reported compounds include the following (maximum observed concentration given in parentheses): benzyl acetate (28); benzyl benzoate (24.0); phytol acetate (9); isophytol (8.5); phytol (7.4); linalool (7.0); eugenol (4.0); squalene (4); indole (3.5); benzyl alcohol (2.5); cis-jasmone (2.5); methyl linolenate (2.0); methyl palmitate (1.4); p-cresol (1.0); cis-3-hexenyl benzoate (1.0); benzyl salicylate (0.4); jasmin lactone (0.9); methyl jasmonate (0.7); isoeugenol (0.4) ((30), also according to (17))

JUNIPERUS VIRGINIANA OIL

CAS 8000-27-9; EC / (Oils, cedarwood) [this also refers to *Cedrus atlantica* ...] / 85085-41-2; EC 285-370-3 (Juniper, *Juniperus virginiana*, ext. = INCI)

JUNIPERUS VIRGINIANA WOOD OIL

CAS 85085-41-2; EC 285-370-3

Cedar Wood Oil (Virginian)

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 0.6% of 1606 consecutive patients had a positive PT to "cedarwood oil (Moroccan and Chinese 1:1)", tested 10% in pet. (17). After application of Penaten-baby™ oil as immersion oil for dermatoscopy a patient developed multiple patches of eczema at the application sites. Investigation revealed that the oil was kept in a bottle previously used for *Juniperus virginiana* oil, to which contact sensitisation was verified by patch testing (235).

Additional information:

ISO 4720:2009 nomenclature: *Juniperus virginiana* L.. *Juniperus Virginiana* Oil is the volatile oil obtained from the fruits and leaves of the Red Cedar, *Juniperus virginiana* L., Cupressaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=78070>, last accessed 2010-01-05)

Juniperus Virginiana Wood Oil is an essential oil obtained from the wood and twigs of the Red Cedar, *Juniperus virginiana* L., Cupressaceae. It contains chiefly (alpha and beta) cedrene and cedral (cedar camphor), cuparene, thujopsene, widdrol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39767>, last accessed 2009-11-12)(235). According to Surburg/Panten by GC: alpha-cedrene 22-35%, thujopsene 10-25%, cedrol 16-25% (34).

See also *Cedrus atlantica*. According to (30) the maximum observed concentration in cedar wood oil are (in %): α-cedrene (32); thujopsene (25); cedrol (25); β-cedrene (6); widdrol (5) and cuparene (traces) (30).

For Oil of cedarwood, Virginian (*Juniperus virginiana* L.) an ISO standard is available: ISO 4724:2004. For Oil of cedarwood, Texas (*Juniperus mexicana* Schiede) an ISO standard exists: ISO 4725:2004.

LAURUS NOBILIS OIL

CAS 8002-41-3; EC / (Oils, laurel)
 INCI: LAURUS NOBILIS OIL /
 8007-48-5; EC / (Oils, sweet
 bay)/ 84603-73-6; EC 283-272-5
 (Laurus nobilis, ext.) INCI:
 LAURUS NOBILIS EXTRACT

Laurel oil

Current regulation: Annex II, n° 359 (seed oil)

Clinical data:

In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to "laurel oil" 2% pet. (22).

After sensitisation by a one-time occlusive application a 36 year old Turkish patient developed widespread allergic contact dermatitis 3 days after massage with olive oil containing Laurus nobilis oil; sensitisation was proven by a strong positive reaction to the commercial test preparation and the massage oil previously used (236). Topical application of laurel oil for knee arthropathy led to an erythema exudativum multiforme-like rash on the legs of a 63 year old patient; interestingly, laurel oil yielded a "target like" strongly positive PT reaction in this case (237). In an earlier Turkish case with a similar history, the EEM-like appearance was lacking; however, a very intense, edematous reaction was noted (238). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "laurel oil" were observed (3). The IVDK 2010 c study identified 1.0% positive reactions in 6297 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Laurus nobilis* L. Laurel leaf oil is obtained by steam distillation of leaves from *Laurus nobilis* L. (Lauraceae), an evergreen cultivated primarily in the Mediterranean countries. The main components are 1,8-cineole (30-70%), linalool (about 10%) and eugenol (34). According to (30) the maximum observed concentration in laurel oil are (in %): 1,8-cineole (70); β -caryophyllene (11); linalool (11); limonene (5.0); eugenol (2.0); geraniol (0.3) (30).

LAVANDULA HYBRIDA HERB OIL

CAS 91722-69-9; EC 294-470-6
 (Lavender, *Lavandula hybrida*,
 ext. = INCI)

Lavandin Oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "lavandin" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "lavandin" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% lavandin oil in pet., tested in 205 consecutive patients in Dortmund and Göttingen, and just 1 irritant reaction to

the higher concentration, were observed (15).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. *Lavandula* *hybrida*, *Labiatae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39789>, last accessed 2010-01-29. Nomenclature according to Surburg/Panten: *Lavandula* x *intermedia* Lois, which is a hybrid of lavender and spike (see below) (34). The oils from the most important variants, abrial and grosso, contain linalool (24-38%), linalyl acetate (20-38%) as well as 1,8-cineole (4-11%), and camphor (6-11%) (34). A third variant is called super because of its high concentration of linalyl acetate (35-47%), more closely resembling lavender oil (34). For Oil of lavandin Grosso (*Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik.), French type an ISO standard exists: ISO 8902:2009, for Oil of lavandin Abrial (*Lavandula angustifolia* Miller x *Lavandula latifolia* Medikus), French type a different ISO standard: ISO 3054:2001.

It is a "top 100" substance (IFRA, pers. comm.2010)

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LAVANDULA OFFICINALIS FLOWER OIL

CAS 84776-65-8, 8000-28-0; EC 283-994-0 (*Lavender*, *Lavandula angustifolia angustifolia*, ext. = *INCI*)

Lavender oil

Current regulation: /

Clinical data:

In a large series from Nagoya, Japan, 1483 patients were tested with lavender oil 20% in pet., with overall 3.7% positive reactions from 1990 to 1998. However, within this period, a sharp increase was noted in 1997 and 1998, which as attributed to changed exposure by M. Sugiura et al. (14). On the individual level, relevance of positive reactions remained unclear in about half of the cases. The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lavender absolute" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Lavandula augustifolia oil" (Lavender absolute) 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "lavender oil 20% pet." – all of them sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "lavender" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "lavender" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 6 "strong positive" reactions to "Lavender oil" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with "lavender absolute", yielding 2 positive reactions (48).

R. Goiriz et al. report on a case of photo contact allergy (10 controls negative) in a 45 year old woman developing after application of a ketoprofen-containing topical gel ("Fastum") (239). A physiotherapist developed acute, recurrent dermatitis after use of "Difflam® gel", scented with lavender oil. Both the gel and lavender oil (2% pet.) tested positive; avoidance resulted in clearing (240). In a study on 218 patients with known

contact allergy to fragrance ingredients, Larsen (2002 c) found positive reactions to 10% lavender oil (pet.) in 2.8% of these (1). A case of vulvovaginitis with spread and affecting the dominant hand applying various tea tree and lavender oil creams was reported by S. Varma; the PT with 10% lavender oil abs. in pet. (50 controls negative) was positive (241). In two cases, facial "pillow dermatitis" due to lavender oil, applied to the pillows, developed, confirmed by positive PT to lavender abs. (2% pet.) (242).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. *Lavandula officinalis* Flower Oil is an essential oil obtained from the fresh flowering tops of the Lavender, *Lavandula officinalis* (syn: *L. vera*), *Labiatae*. It contains 30-40% esters calculated as linalyl acetate, linalool, pinene, limonene, geraniol, some eucalyptol (cineol) (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40370>, last accessed 2009-11-09). According to Surburg/Panten, lavender oil is obtained by steam distillation of freshly cut flowering tops of *Lavandula angustifolia* Mill. (Lamiaceae). Main constituents according to GC are linalyl acetate (25-45%), cis-ocimene (4-10%), trans-ocimene (1.5-6%), 1,8-cineole ($\leq 1\%$) camphor ($\leq 0.5\%$), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] ($\geq 2\%$) (34).

In addition to distillation, both *Lavandula officinalis* and Lavandin are also solvent extracted, yielding concretes and, after ethanol extraction, absolutes, which are said to have a longer-lasting odour (34).

For Oil of lavender (*Lavandula angustifolia* Mill.) an ISO standard exists: ISO 3515:2002.

LAVANDULA SPICA HERB OIL

CAS 97722-12-8; EC 307-762-6
(Lavender, *Lavandula spica*, ext.
= INCI

"Spike Oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "spike" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=8 (9.3%) positive reactions to "spike" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula latifolia* Medik. *Lavandula Spica* Herb Oil is an essential oil distilled from the flowering herbs of the Spikenard, *Lavandula spica* (syn: *Lavandula latifolia*), *Labiatae*. It contains eucalyptol (35%), camphor, linalool, borneol, terpineol, D-camphene and sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40372>, last accessed 2010-01-29). According to Surburg/Panten, Spanish spike lavender oil is steam distilled from the flowering tops of *Lavandula latifolia* Medik.. The main components are linalool (34-50%), 1,8-cineole (16-39%) and camphor (8-16%) (34). For Oil of spike lavender (*Lavandula latifolia* (L.f.) Medikus), Spanish type an ISO standard exists: ISO 4719:1999

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LITSEA CUBEBA FRUIT EXTRACT

CAS 90063-59-5, 68855-99-2; EC 290-018-7 (*Litsea cubeba*, ext.)
 INCI: LITSEA CUBEBA OIL

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 3 positive reaction in 200 patients to “*Litsea cubeba*” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to this essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Litsea cubeba* (Lour) Pers. *Litsea Cubeba* Fruit Extract is an extract obtained from the fruits of the plant, *Litsea cubeba*, Lauraceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40036>, last accessed 2009-11-24. The content by GC is: neral (25-33%), geranial (38-45%) – i.e. about ¾ citral, for which the extract had previously served as a raw material (34); direct use for perfuming is limited to household products (39). For Oil of *Litsea cubeba* (*Litsea cubeba* Pers.) an ISO standard exists: ISO 3214:2000.

In a LLNA study by RIFM, the “*Litsea cubeba* oil” as used was reported to contain 85.7% citral, 2.9% limonene, 1.7% linalool, 1.4% citronellal and < 1% caryophyllene and methyl heptanone, according to analyses of the supplier. The EC3 value was calculated as 8.4 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

MENTHA ARVENSIS LEAF OIL

CAS 68917-18-0 ; EC /

Cornmint oil

INCI: MENTHA ARVENSIS OIL

Current regulation: /

Clinical data: /

Additional information:

Mentha Arvensis Leaf Oil is the oil derived from the leaves of the Horse Mint, *Mentha arvensis* L., Labiatae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=57860)

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

MENTHA PIPERITA OIL

CAS 8006-90-4; EC / (Oils, peppermint) INCI: MENTHA

*PIPERITA OIL / 84082-70-2; EC
282-015-4 (Peppermint, ext.) INCI
names: MENTHA PIPERITA ...*

Peppermint oil

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% of 1606 consecutive patients reacted positively to "peppermint oil (American)", tested 2% in pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "peppermint oil" were observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to peppermint oil 2% pet. (22). Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 6 patients had positive (+ to +++) PT reactions to "peppermint oil" 1% pet. at D4, mostly combined with positive reactions to menthol (see above) and reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). The Rudzki 1976 study found 1 positive reaction in 200 patients to "Peppermint" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=6 (6.9%) positive reactions to "peppermint" essential oil 2% pet. (27). In 63 patients positive to the FM I, 3 had positive PT reactions to peppermint oil, 2% pet., in the Santucci 1987 study (28). The IVDK 2010 c study identified 0.6% positive reactions in 6546 patients tested in the context of a special series (30).

An unusual case of "baboon-like" allergic contact dermatitis of the vulva after drinking excessive amounts of a herbal tea containing, among other ingredients, peppermint. While the PT reaction to peppermint oil was only weak to doubtful, dramatic improvement after cessation and prompt relapse after repeat ingestion proved the diagnosis (243). Recurrent foot and lower leg dermatitis after the application of a "foot spray" (containing peppermint oil) was diagnosed as allergic contact dermatitis due to this ingredient in a 59 year old golf player (244). In another case, ACD after application of a transdermal system for the treatment of lumbar pain was attributed to CA to peppermint oil (2% pet.) and its main ingredient menthol (1% pet.) (155). In a patient with toothpaste-induced cheilitis, not only *M. piperita*, but also *M. arvensis*, but not *M. spicata* or *cardica* extracts (all tested 1% pet.), as well as natural and synthetic menthol caused positive PT reactions (245).

Additional information:

ISO 4720:2009 nomenclature: *Mentha x piperita* L. A standard by ISO exists for Oil of peppermint (*Mentha x piperita* L.): ISO 856:2006. A review by the Cosmetic Ingredient Review Expert Panel, Washington, DC on the "Final report on the safety assessment of *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water" is available (163), stating that "Peppermint Oil is used at a concentration of < or = 3% in rinse-off formulations and < or = 0.2% in leave-on formulations. Peppermint Oil is composed primarily of menthol and menthone. Other possible constituents include pulegone, menthofuran, and limone. According to Surburg/Panten: (-)-menthol (34-46%), (-)-menthone (15-27%), (-)-menthyl acetate (2.5-7%) and menthofuran [17957-94-7] (0.5-6%) (34). According to (30) the maximum observed concentration in peppermint oil are (in %): (-)-menthol (49); (-)-menthone (28); (-)-menthyl acetate (8); mentofuran (8); isomenthone (8); neo menthol (6); pulegone (3.5); limonene (3.0); linalool (0.4) (30). Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. ... Repeated

intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits” concluding that “with the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations”.

MENTHA SPICATA HERB OIL

CAS 84696-51-5, 8008-79-5; EC 283-656-2 (Spearment, ext.)

Spearment oil

INCI: MENTHA VIRIDIS EXTRACT

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to “spearment oil (American)”, tested 2% in pet. (17). The CAS # quoted (8008-79-5) refers, according to CosIng, to MENTHA VIRIDIS LEAF OIL, the volatile oil obtained from the dried tops and leaves of the Garden Mint, *Mentha viridis* L., Labiatae. The Larsen 2001 study diagnosed 5.0% positive reactions in 178 patients with known contact allergy to fragrance ingredients, using this oil at 5% pet. test concentration (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to “*Mentha viridis* oil” 5% (13). PT results with toothpaste ingredients were positive in 7 patients, of whom 4 had strong positive reactions to spearment (246).

Additional information:

ISO 4720:2009 nomenclature: *Mentha spicata* L. *Mentha Spicata* Oil is an essential oil obtained from the herbs of the Spearment, *Mentha spicata* L., Labiatae (syn: *Mentha viridis* L., Labiatae). It contains carvone (more than 50%), limonene, pinene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40394>, last accessed 2009-11-11). According to Surburg/Panten, the content is limonene (9-16.5%), (-)-carvone (60-70%), menthone (0-0.2%) and viridiflorol (0-0.5%) (34). Exposure by toothpastes, and subsequent contact allergic reaction of the lips or the oral mucosa, have been reported (e.g., (247, 248)). L-Carvone is a component of the oil from *Mentha spicata* (spearment) (53) and had been tested with positive results in “toothpaste cases”, even at a concentration as low as 0.067% (68).

For Oil of spearment -- Part 1: Native type (*Mentha spicata* L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearment -- Part 2: Chinese type (80 % and 60 %) (*Mentha viridis* L. var. *crispa* Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearment -- Part 3: Indian type (*Mentha spicata* L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearment -- Part 4: Scotch variety (*Mentha x gracilis* Sole): ISO 3033-4:2005.

MYROXYLON PEREIRAE RESIN

CAS 8007-00-9; EC 232-352-8 (Balsams, Peru)

Balsam of Peru

INCI: MYROXYLON PEREIRAE / Balsams, Peru

Current regulation: Annex III, part1, n° 154

Clinical data:

This natural mixture has been employed as screening agent in Baseline series worldwide for many decades. Hence, a wealth of data is available; table 3.2 – 1 summarises results of the past 10 years.

Additional information:

ISO 4720:2009 nomenclature: *Myroxylon pereirae* (Royle) Klotzsch, syn. *Myroxylon balsamum* var. *pereirae* (Royle) Harms. *Myroxylon pereirae* resin (MPR, Balsamum peruvianum) is harvested from the balsam of Peru tree, *Myroxylon balsamicum* (L.) HARMS var. *pereirae* (ROYLE) HARMS, synonymous *Myroxylon pereirae* (ROYLE) KLOTZSCH (249) after thermal stress, almost exclusively in El Salvador. Main constituents of the pleasantly, vanilla-like smelling dark brown liquid are benzyl esters of cinnamic and benzoic acid (35 – 75%), up to 30% cinnamic acid, up to about 10% benzoic acid, approximately 5% alpha- and beta-nerolidol, benzyl alcohol and mostly less than 1% cinnamyl alcohol, benzyl ferulate and -isoferulate, cinnamic acid amyl ester, coniferyl alcohol, coniferyl benzoate, eugenol, isoeugenol, farnesol, vanillin, and several trace constituents (250-253). The composition of MPR varies with the origin and other factors; moreover, MPR is sometimes blended with other natural mixtures such as turpentine, styrax or colophonium (249).

MPR can be used to improve taste or smell in gargling solutions, cosmetic products such as soaps, shampoo or lipsticks, as well as sweets, tobacco and beverages (249, 254). According to EU legislation and IFRA guidelines MPR should not be used in products intended for skin contact; however, extracts and distillates of MPR may be used in a concentration of < 0,4% (IFRA-Guidelines, www.ifraorg.org (255)). E. Temesvári et al. report on the interesting case of severe ACD with subsequent hypopigmentation after a “temporary henna tattoo”, which was, unexpectedly, not due to p-phenylene diamine, but to the oil used to disperse the pigment, which presumably contained allergens also included in the FM I and MPR, both of which were extreme positive on a later PT (256).

In addition to delayed type hypersensitivity reactions, MPR (and some of his constituents such as benzoic acid (257)) are capable of eliciting (non-immunological) urticarial immediate reactions (258-260). In one case, the immediate reaction to MPR (and to FM I) at the test site spread systemically in terms of a generalised urticaria, while no delayed type reactions were observed to the PT (261). Generally, there is apparently no association of immediate reactions to MPR (and cinnamal or cinnamyl alcohol) and contact sensitisation to these compounds (262). In animal experiments the sensitising potency of MPR was clearly established (250), with coniferyl benzoate identified as single compound with the most marked potency (252). However, due to the limited chemical stability of this compound it is unclear whether other, more stable compounds are, in fact, more important allergens, such as cinnamic acid and (iso-) ferulic acid esters or oxidised constituents of the resin fraction (263).

Table 3.2.2 – 1: Results with contact allergy to fragrance ingredients screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Myroxylon pereirae resin** (Balsam of Peru) 1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the reviewers.

Country	Population	Years	No. tested	Crude % positive (95% CI) §
Tel Aviv, Israel (264) #	Consecutive patients	1999-2000	943	6.6 % (5.1 – 8.4) §
South Korea (13)	Consecutive patients	04/2002 – 06/2003	422	7.3% (5.1 – 10.3%) §

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Tel Aviv, Israel (265)	Consecutive patients	1998-2004	2156	3.6 (2.9 – 4.5) § %
Manipal, India (266)	Dermatitis patients	1989-1998	1780	n=17
Tehran, Iran (267)	Consecutive patients	2002-2004	250	2.4 (0.9 – 5.2) § %
Sevilla, Spain (268)	Consecutive patients	2002-2004	863	5.8 (4.3 – 7.6) § %
Ankara, Turkey (269)	Consecutive patients	1992-2004	1038	2.1 (1.3 – 3.2) § %
Vienna, Austria (22)	Consecutive patients of one clinic	1997-2000	2660	5.4% (4.6 – 6.3%) §
Czech Republic (270)	Consecutive patients	1997-2001	12058	7.3% (6.8 – 7.8) §
Copenhagen, Denmark (271)	Consecutive patients	1985-2007	16173	3.9 (3.6 – 4.2) § %
Sweden (272)	Consecutive patients	2000	3790	6.5%
9 European countries (273) §	Consecutive patients	2002-2003	9672	6.1 %
Germany, 3 Swiss + 1 Austrian Dept. (7)	Consecutive patients	2005-2008	36919	8.0% (7.7 – 8.3%)
10 depts. From 7 EU countries (274) *	Consecutive patients	1996-2000	26210	6.0 %
USA (Canada) (20)	Probably consecutive patients	2003	1603	6.6%
NACDG 2009 (21)	Consecutive patients	2005-2006	4449	11.9%

§ Calculated by reviewers, where possible (if actual numbers were given)

Probably included in (265)

\$ > 5-fold difference between departments

* About 4-fold difference between departments

NARCISSUS SPP. EXTRACT / OIL

CAS: diverse

Narcissus abs.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 1.3% positive reactions to “narcissus absolute” (2% pet.) were observed in 1606 consecutive (17). The extract used by the PT allergen provider Almirall/Hermal/Trolab has the CAS number 90064-25-8. The IVDK 2010 c study identified 0.5% positive reactions in 2445 consecutively tested patients and 0.6% positive reactions in 809 patients tested in the context of a special series (30).

Additional information:

Commonly used: *Narcissus poeticus* L. According to (30) the maximum observed concentration in Narcissus abs. are (in %): α -terpineol (23.7); trans-Isoeugenol methyl ether (20); benzyl benzoate (20); coumarin (5.7); benzyl alcohol (4.0); Δ^3 -carene (3.4); cinnamyl alcohol (2.5); phenylethyl alcohol (2.2); ethyl palmitate (2.2); phenylpropyl acetate (1.7); 1,8-cineole (1.5); caryophyllene (1.0); benzyl acetate (0.7); isoeugenol (0.5); farnesol (0.3) (also according to (17)) (30).

OCIMUM BASILICUM HERB OIL

CAS 84775-71-3; EC 283-900-8 (*Ocimum basilicum*, ext. = INCI)

Basil Oil (sweet)

Current regulation: /

Clinical data:

/

Additional information:

ISO 4720:2009 nomenclature: *Ocimum basilicum* L. For Oil of basil, methyl chavicol type (*Ocimum basilicum* L.) an ISO standard exists: ISO 11043:1998. *Ocimum Basilicum* Herb Oil is an essential oil obtained from the herbs of the Sweet Basil, *Ocimum basilicum* L., *Labiatae*. (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40474>, last accessed 2009-11-24). The chemical composition varies greatly with the origin (34):

- Basil oil of the methylchavicol type (Réunion type) is extracted from flowering tops or whole plants from Réunion, Comores, Madagascar, but also other countries such as Egypt. Mainly used for seasoning food. Content by GC: methylchavicol 75-87%, linalool 0.5-3%
- Basil oil, linalool type is produced mainly in the Mediterranean area. Content by GC: Linalool 45-62%, methylchavicol trace to 30%, eugenol 2-15%
- Indian Basil oil is produced exclusively in India. Content by GC: methylchavicol trace to 70%, linalool 25%.

In a LLNA study by RIFM, the basil oil as used was reported to contain 51% linalool, 10.4% eugenol, 7.7% cineol, 3.7% bergamotene, 2.7% germacrene D, 2.7% cadinol and 1.3% cadinene, according to analyses of the supplier. The EC3 value was calculated to be < 2.5% (227).

PELARGONIUM GRAVEOLENS FLOWER OIL

CAS 90082-51-2; EC 290-140-0 (*Pelargonium graveolens*, ext. = INCI) / 8000-46-2; EC / (Oils, geranium) INCI: GERANIUM

Geranium Oil Bourbon

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "geranium oil Bourbon" (2% pet.) 7.4% positive reactions (9). In the Larsen 2001 study, 8.4% positive reactions were observed in 178 patients with known contact allergy to fragrance ingredients ("geranium oil Bourbon", 10% pet.) (19). The Goossens 1997 study found 3 of 111 patients positive to "geranium oil 20% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 3 positive reactions in 200 patients to "geranium" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "geranium" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 3 "strong positive" reactions to "Geranium oil" (unknown test concentration) in 183 patients, Trattner/David 1 / 641 consecutive patients positive to "Geranium oil" (31). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to geranium oil bourbon (48).

Additional information:

ISO 4720:2009 nomenclature: *Pelargonium x ssp.* For Oil of geranium (*Pelargonium X ssp.*) an ISO standard exists: ISO 4731:2006 *Pelargonium Graveolens* Flower Oil is the volatile oil obtained from the flowers of the Bourbon Geranium, *Pelargonium graveolens* L. Hér. Ex Aiton, *P. roseum* Willdenow (and other nondefined hybrids that have developed in different regions of the world) Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=57527>, last accessed 2009-11-16)(34). The Bourbon type (Réunion, Madagascar) is more valuable than the North African and Chinese products, and differs in characteristic components: (-)-6,9-guaiadiene [36577-33-0] 5-9% in the Bourbon type, and 10-epi-gamma-eudesmol [15051-81-7] 3-6% in the African type, in addition to the main components (-)-citronellol, isomenthone, formates and tiglates. Chinese oil is similar to Bourbon oil, however, it contains more citronellol (32-43%) and lower amounts of linalool (2-4.5%) and geraniol (5-12%) (34).

In a LLNA study by RIFM, the geranium oil as used was reported to contain 41.1% citronellol, 9.8% 2,6-guiadine, 6.2% isomethone, 4.9% geraniol, 2.2% cis-rose oxide, 2.1% linalool, 1.5% geranyl formate, 1.3% phenyl ethyl tiglate, 1.0% trans-rose oxide, and geranyl tiglate and alpha-pinene at < 1%, according to analyses of the supplier. The EC3 value was calculated to be > 50% (227).

PELARGONIUM ROSEUM LEAF OIL

CAS 90082-55-6; EC 290-144-2
(*Pelargonium roseum*, ext. =
INCI)

Geranium Oil; Rose Geranium Oil

Current regulation: /

Clinical data:

In the Sugiura 2000 study, among 1483 patients with suspected cosmetic dermatitis, 2.1% positive PT reactions to "geranium oil" (tested 20% in pet.) were observed (14).

Additional information:

Pelargonium Roseum Leaf Oil is an essential oil obtained from the leaves of the plant, Pelargonium roseum, Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40565>, last accessed 2009-11-16).

PIMENTA RACEMOSA LEAF/FRUIT OIL

CAS 85085-61-6; EC 285-385-5

Bay oil (34)

Current regulation: /

Clinical data:
/

Additional information:

ISO 4720:2009 nomenclature: *Pimenta racemosa* (Mill.) J.W. Moore. For Oil of bay [*Pimenta racemosa* (Mill.) J.W. Moore] an ISO standard exists: ISO 3045:2004 Pimenta Racemosa Leaf/Fruit Oil is an essential oil obtained from the fruits of the plant, Pimenta racemosa, Myrtaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41014>, last accessed 2010-02-10).

Steam distillation of the leaves of Pimenta racemosa (Mill.) J.W. Moore (Myrtaceae) yields bay oil, which consists of myrcene (20-30%), eugenol (42-56%) and chavicol (8-13%) (34).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

Pinus mugo leaf and twig oil and extract

CAS 90082-72-7, 8000-26-8; EC 290-163-6

Dwarf pine needle oil
(German: Latschenkiefernöl)

Current regulation: Annex III, part 1, 109

Clinical data:

In the Frosch 2002 b study, 0.7% positive reactions to dwarf pine needle oil (2% pet.) were observed in 1606 consecutive (17). The Rudzki 1976 study found 4 positive reactions in 200 patients to "Pine needle" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "pine needle" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Pinus mugo* Turra syn. *Pinus montana* Mill.) Pinus Mugo Twig Oil is an essential obtained from the twigs of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09). Pinus Mugo Twig Leaf Extract is an extract obtained from the twigs leaves of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09).

[h.details&id=41473&back=1](#), last accessed 2010-03-09).

Dwarf pine needle oil is obtained from *Pinus mugo* Turra subsp. *mugo* and subsp. *pumilio* (Haenke) Franco (34). For Oil of dwarf pine (*Pinus mugo* Turra) an ISO standard exists: ISO 21093:2003. American pine oils contain almost no 3-carene or camphene (34).

PINUS PUMILA TWIG LEAF EXTRACT / OIL

CAS 97676-05-6; EC 307-681-6
(Pine, *Pinus pumila*, ext. = INCI)

Dwarf pine needle oil

Current regulation: Annex III, part 1, 114

Clinical data: /

Additional information:

Pinus Pumila Twig Leaf Extract obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41483&back=1>, last accessed 2009-11-12), *Pinus Pumila* Twig Leaf Oil is the essential oil obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41484&back=1>, last accessed 2009-11-12). Main constituents are alpha-pinene (60-70%) and beta-pinene (20-25%). (34) Occurrence from Siberia to Japan, classified as Endangered Species

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

POGOSTEMON CABLIN OIL

CAS 8014-09-3; EC / (Oils, patchouli) / 84238-39-1; EC 282-493-4 (Patchouli, ext.)

Patchouli oil

INCI: POGOSTEMON CABLIN / Patchouli, ext.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% positive reactions to patchouli oil (10% pet.) in 1606 consecutive were observed (17). Nakayama et al. found 1974 (after (29)) 3 “strong positive” and 8 “weak positive” reactions to “Patchouli oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 0.6% positive reactions in 2446 consecutively tested patients and 1.4% positive reactions in 828 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Pogostemon cablin* (Blanco) Benth. syn. *Mentha cablin* Blanco. An ISO standard is available for Oil of patchouli (*Pogostemon cablin* (Blanco) Benth.): ISO 3757:2002. *Pogostemon Cablin* Leaf Oil is an essential oil obtained from the fermented leaves of the Patchouli, *Pogostemon cablin* (syn: *Pogostemon patchouli*),

Labiatae (Lamiaceae (34)). It contains patchouli alcohol, beta-patchoulene, azulene, eugenol, sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40927>, last accessed 2009-11-12). Although the sesquiterpene alcohol (-)-patchoulol [5986-55-0] is the main component of patchouli oil (27-35%), the compound largely contributing to the characteristic odour is norpatchoulol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-β-patchoulene [514-51-2] (1.8-3.5%) and (-)-seychellene [20085-93-2] (1-3%) (34). According to (30) the maximum observed concentration in patchouli oil are (in %): (-)-patchoulol (35); (+)-alpha-lulnesene (21); (-)-alpha-guajene (16); β-pinene (6); (-)-β-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulol (1) (30).

It is a "top 100" substance (IFRA, pers. comm.2010).

ROSE FLOWER OIL (ROSA SPP.)

CAS 8007-01-0; EC / (Oils, rose)

ROSA ALBA FLOWER EXTRACT

CAS 93334-48-6; EC 297-122-1
(Rose, *Rosa alba*, ext. = INCI)

ROSA CANINA FLOWER OIL

CAS 84696-47-9; EC 283-652-0
(Rose, *Rosa canina*, ext.) INCI:
ROSA CANINA

ROSA CENTIFOLIA FLOWER OIL

CAS 84604-12-6, EC 283-289-8
(Rose, *Rosa centifolia*, ext.) INCI:
ROSA CENTIFOLIA / Rose, *Rosa centifolia*, ext.

ROSA DAMASCENA FLOWER OIL

CAS 90106-38-0; EC 290-260-3
(Rose, *Rosa Damascena*, ext. = INCI)

ROSA GALLICA FLOWER OIL

CAS 84604-13-7; EC 283-290-3
(Rose, *Rosa Gallica*, ext.) INCI:
ROSA GALLICA

ROSA MOSCHATA OIL

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ROSA RUGOSA FLOWER OIL

CAS 92347-25-6; EC 296-213-3
(Rose, *Rosa rugosa*, ext.)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "rose oil Bulgaria" (2% pet.), yielding 0.4% positive reactions (14); Trattner/David found 2 / 641 consecutive patients positive to "Rose oil (Bulgarian)" (31). The Bulgarian rose oil usually corresponds to *Rosa Damascena* Flower Oil (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-16). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "rose Bulgarian oil" (2% pet.) 4.5% positive reactions (9). One case of contact allergy to "Bulgarian rose oil (2 % pet.)" – and geraniol – in a 48-year-old female with ACD after application of "Eau de Rochas" E.d.C. was diagnosed, among 326 patients with suspected contact allergy to fragrance ingredients had tested negative (275). However, other rose oils are also used (and capable of eliciting ACD) as illustrated by the case of a 27 year old woman who developed ACD after using "Rose Absolute Eau ® eau de

parfum", a "non-scented" body lotion and a number of other topicals. PTing revealed a number of (previously) relevant reaction, including "Rose centifolia" (5% alc.) and "Rose oil Bulgarian" (2% pet.) essential oil preparations (276). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Rose oil Bulgarian", tested at 2% concentration (13). Nakayama et al. found 1974 (after (29)) 4 "strong positive" reactions to "Rose oil Bulgarian" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with rose oil absolute, yielding 6 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Rosa x damascena* Mill. and *Rosa sertata* X *Rosa rugosa*. Rose Flower Oil is the volatile oil obtained from the flowers of *Rosa* spp. , rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59362>, last accessed 2009-11-16). "Rose oil, meaning either rose otto (attar of rose, attar of roses) or rose absolute, is the essential oil extracted from the petals of various types of rose. Rose ottos are extracted through steam distillation, while rose absolutes are obtained through solvent extraction or supercritical carbon dioxide extraction, with the absolute being used more commonly in perfumery" (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-17). There are several more specifically named flower extracts used for masking or perfuming:

- Rosa Alba Flower Extract is an extract obtained from the flowers of the Rose, *Rosa alba* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40969>, last accessed 2009-11-16).
- Rosa Canina Flower Oil is the volatile oil obtained from the flowers of the Hip Rose, *Rosa canina* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59263>, last accessed 2009-11-16).
- Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of the Cabbage Rose, *Rosa centifolia* (L.), Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79757>, last accessed 2009-11-16).
- Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of the Damask Rose, *Rosa damascena*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79760>, last accessed 2009-11-16).
- Rosa Gallica Flower Oil is the volatile oil obtained from the flowers of the French Rose, *Rosa gallica* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59346>, last accessed 2009-11-16).
- Rosa Moschata Oil is the oil obtained from the Musk Rose, *Rosa moschata*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79761>, last accessed 2009-11-16).
- Rosa Rugosa Flower Oil is the volatile oil obtained from the flowers of the Rose, *Rosa rubiginosa* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=83588>, last accessed 2009-11-16).

Apparently, the *Rosa Damascena* and the *Rosa centifolia* are the species most commonly used for extraction of essential rose oils, the former mostly grown in Bulgaria, Turkey, Russia, India and China, the latter more commonly in Morocco, France and Egypt (276). Main constituents by GC are: citronellol (20-49%), geraniol (6-23%), nerol (3-12%) and phenylethyl alcohol (up to 3.5%) (34).

For Oil of rose (*Rosa x damascena* Miller) an ISO standard exists: ISO 9842:2003.

ROSMARINUS OFFICINALIS FLOWER OILCAS 84604-14-8; EC 283-291-9
(Rosemary, ext.)

"Rosemary Oil"

INCI: ROSMARINUM OFFICINALIS
/ Rosemary, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "rosemary" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "rosemary" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Rosmarinus officinalis* L. Rosmarinus Officinalis Flower Oil is an essential oil obtained from the leaves and fresh flowering tops of the Rosemary, *Rosmarinus officinalis* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40978>, last accessed 2010-01-29). Major constituents are: 1,8-cineole (17-55%), alpha-pinene (9-26%), camphor (5-22%) and verbenone [18309-32-5] as traces in North African oils, but between 0.7 and 2.5% in Spanish oils (34). For Oil of rosemary (*Rosmarinus officinalis* L.) an ISO standard exists: ISO 1342:2000.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SALVIA spp. HERB OIL

Sage oil

SALVIA OFFICINALIS LAVANDULIFOLIA HERB OILCAS 97952-71-1; EC 308-365-0
(Sage, *Salvia officinalis* *lavandulifolia*, ext. = INCI)**SALVIA LAVANDULIFOLIA HERB OIL**CAS 90106-49-3; EC 290-272-9
(Sage, *Salvia lavandulifolia*, ext. = INCI)**SALVIA SCLAREA FLOWER OIL**CAS 84775-83-7; EC 283-911-8
(Sage, *Salvia sclarea*, ext.) INCI: SALVIA SCLAREA / Sage, *Salvia sclarea*, ext.**SALVIA HISPANICA HERB OIL**CAS 93384-40-8; EC 297-250-8
(Sage, *Salvia hispanica*, ext. = INCI)

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "Clary sage", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "clary sage" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Salvia officinalis* L. *Salvia Officinalis Lavandulifolia* Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia officinalis* L. spp. *lavandulifolia*, Lamiaceae, Syn. Dalmatian sage (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41084>, last accessed 2010-01-29).

Salvia Lavandulifolia Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia lavandulifolia*, Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40987>, last accessed 2010-01-29).

Salvia Sclarea Flower Oil is an essential oil obtained from the flowers and foliage of the Clary Sage, *Salvia sclarea* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41086>, last accessed 2010-01-29).

Salvia Hispanica Herb Oil is an essential oil obtained from the herbs of the Spanish Sage, *Salvia hispanica* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40985>, last accessed 2010-01-29).

Clary sage oil is obtained by steam distillation of flowering tops and foliage of cultivated *Salvia sclarea* L. (Lamiaceae). Main constituents are linalyl acetate (56-78%) and linalool (6.5-24%) (34). Dalmatian sage oil is steam distilled from partially dried leaves of *S. officinalis* L. (Lamiaceae). The content by GC is: alpha-thujone (18-43%), beta-thujone (3-8.5%), 1,8-cineole (5.5-13%), camphor (3-8.5%) as main constituents (34). Spanish sage oil does not contain thujone, but mainly camphor (15-36%) and 1,8-cineole (11-30%), and is used mainly in pharmaceutical preparations and technical perfumery (34). For Oil of sage, Spanish (*Salvia lavandulifolia* Vahl) an ISO standard exists: ISO 3526:2005, for Oil of Dalmatian sage (*Salvia officinalis* L.): ISO 9909:1997.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SANTALUM ALBUM WOOD OIL

CAS 84787-70-2; EC 284-111-1
(Sandalwood, ext.) INCI:
SANTALUM ALBUM / Sandalwood,
ext.

Sandalwood oil ([East] India)

SANTALUM ALBUM OIL

CAS 8006-87-9; EC / (Oils,
sandalwood)

Sandalwood oil ([East] India)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "sandalwood oil" (2% pet.), yielding 0.8% positive reactions (14). In the Frosch 2002 b study, "sandalwood oil (East India)" is mentioned with a CAS # 8015-65-4, which, however, is attributed to AMYRIS BALSAMIFERA BARK OIL, see above. Assuming that this CAS # is erroneous, study results are considered to be valid for *S. album* wood oil, tested at 2% and 10% concentration, yielding 0.4% and 0.9% positive reactions,

respectively (17). Out of 6 of 15 patients with a positive reaction to the higher concentration no clinical relevance was found, compared to 2 of 7 patients positive to the lower concentration (17). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "sandalwood oil" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 10 of 422 consecutive patients, i.e., 2.4%, had positive reactions to "Santalum album oil" 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "sandalwood oil 10% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "sandalwood", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "sandalwood" essential oil 2% pet. (27). In 63 patients positive to the FM I, 1 had a positive PT reaction to sandalwood oil, 2% pet., in the Santucci 1987 study (28). Nakayama et al. found 1974 (after (29)) 6 "strong positive" and 8 "weak positive" reactions to "Sandalwood oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.3% positive reactions in 3671 consecutively tested patients and 1.8% positive reactions in 1002 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with sandalwood oil, yielding 2 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Santalum album* L. *Santalum Album* Oil is the volatile oil obtained from the heartwood of the Sandalwood, *Santalum album* L., Santalaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=80209>, last accessed 2009-11-26).

Santalum Album Wood Oil is an essential oil obtained from the wood of the Sandalwood, *Santalum album* L., Santalaceae. It contains 75% santalol isomers (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41092>, last accessed 2009-11-12), typically up to 55% .alpha.-santalol and up to 24% .beta.-santalol (30). East Indian sandalwood oil consists almost exclusively of closely related sesquiterpenoids; by far the main constituents are the alcohols alpha-santalol [115-71-9] (41-55%) and cis-beta-santalol [77-42-9] (16-24%), the latter being mainly responsible for the specific odour (34, 39).

An ISO standard regarding the composition of "*Santalum album* oil" is available: ISO 3518:2002. "Sandalwoods" are labelled as *Amyris balsamifera*, *Eremophila mitchelli*, *Fusanus acuminatus* (= *Santalum acuminatum*), *Santalum album*, *S. austrocaledonicum*, *S. latifolium*, *S. spicatum* and *S. yasi*. The majority of currently available trade oils, reportedly from *S. album*, contained approximately 50-70% santalols (Z-alpha and Z-beta), as analysed with gas chromatography-mass spectrometry (GC-MS) (277). A review on the toxicological properties of "*Santalum album* oil" is available (278).

AMYRIS BALSAMIFERA BARK OIL (*Sandalwood oil (Caribbean)*), CAS 8015-65-4; EC / (Oils, amyris) / 90320-49-3; EC 90320-49-3 (*Amyris balsamifera*, ext. = INCI name) is used as a cheap substitute for East Indian Sandalwood in perfumes and cosmetics. Originally cultivated primarily in Haiti where it was known as 'candle wood' and used as a torch by locals due to the tree's high oil content (<http://www.amphora-retail.com/sandalwood-amyris-essential-10ml-p-107.html>, last accessed 2009-11-12). The major components are sesquiterpenoids such as valerianol, elemol, β -eudesmol and epi-gamma-eudesmol (39). For Oil of amyris (*Amyris balsamifera* L.) an ISO standard exists: ISO 3525:2008. *Amyris Balsamifera* Bark Oil is the volatile oil distilled from the bark of the tree, *Amyris balsamifera*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=74455>, last accessed 2009-11-12).

SANTALUM SPICATA WOOD OILCAS 8024-35-9; EC 296-618-5
(Sandalwood oil, Western
Australia)*Sandalwood oil (Australia)*

Current regulation: /

Clinical data:

In clinical studies, mostly *S. album* wood oil had been used (see above); in a number of studies this is not clear.

Additional information:

ISO 4720:2009 nomenclature: *Santalum spicatum* (R.Br.) A. DC, syn. *Eucarya spicata* (R.Br.) Sprag & Summ. For Oil of Australian sandalwood (*Santalum spicatum* (R.Br.) A.DC.) an ISO standard exists: ISO 22769:2009. Santalum Spicata Wood Oil is an essential oil obtained from the wood of the Australian Sandalwood, *Santalum spicata*, Santalaceae. It contains 75% santalols and 10% farnesol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41093>, last accessed 2009-11-12). This oil also contains santalols as main constituents but differs somewhat in the remaining composition. Today, it makes up a considerable part of the sandalwood oil market (34).

Considering the content of well-known allergenic compounds (santalols), this essential oil is regarded as established contact allergen in humans.

TAGETES PATULA FLOWER OILCAS 91722-29-1; EC 294-431-3
(*Tagetes patula*, ext. = INCI)*"Marigold Oil; Tagetes Oil"*

Current regulation: /

Clinical data:

In an aromatherapist, an essential oil solvent-extracted from *Tagetes patula*, patch tested at 1.5% in grapeseed oil (vehicle negative, 7 controls negative to essential oils) resulted in a +++ reaction, in accordance with a work-related bilateral hand dermatitis (217).

Additional information:

Tagetes Patula Flower Oil is an essential oil obtained by hydrodistillation of the flowers of the *Tagetes*, *Tagetes patula* L., *Compositae*. It contains mainly D-limonene, ocimene, 2,6-dimethyloct-7-en-4-one (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41506>, last accessed 2010-01-28). According to Surburg/Panten, tagetes oil is steam distilled from the flowering plants of *Tagetes minuta* L. (*T. glandulifera* Schrank., *Asteraceae*). Main components comprise cis-ocimene, dihydrotagetone, tagetone, and cis- and trans-ocimenone (34, 39).

THYMUS spp. HERB OIL

THYMUS VULGARIS HERB OIL

CAS 84929-51-1, 8007-46-3; EC 284-535-7 (Thyme, Thymus vulgaris, ext.)

"Thyme oil"

INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "thyme" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "thyme" essential oil 2% pet. (27). In 63 patients positive to the FM I, none had a positive PT reaction to thymol, 1% pet., in the Santucci 1987 study (28).

Additional information:

ISO 4720:2009 nomenclature: *Thymus vulgaris* L. *Thymus vulgaris* Herb Oil is an essential oil obtained from the herbs of the Thyme, *Thymus vulgaris* L., Lamiaceae. It contains 20-40% thymol and carvacrol, cymene, pinene, linalool, bornyl acetate (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41133>, last accessed 2010-01-29).

Other species are used for extraction, e.g., *Thymus Mastichina* (CAS 84837-14-9), *Thymus Serpillum* (CAS 84776-98-7), *Thymus Zygis* (CAS 85085-75-2), according to CosIng. The main constituent is thymol (37-56%) (34). For Oil of thyme containing thymol, Spanish type [*Thymus zygis* (Loefl.) L.] an ISO standard exists: ISO 14715:2010, for Oil of Spanish wild marjoram (*Thymus mastichina* L.): ISO 4728:2003.

TURPENTINE (oil)

CAS 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / -

Current regulation: III/124 ; III/125 ; III/126

Clinical data:

Oil of turpentine has been patch tested in a number of baseline series, i.e., in consecutive patients, although not included in the European Baseline series.

In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190)

Table 3.2.2 – 2: Overview of results with **Oil of turpentine** in patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS.

Country	Population	Years	No. tested	Crude % positive (95% CI) §

Opinion on fragrance allergens in cosmetic products

Lisbon, Portugal (189); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 % (1.9 – 2.8) §
Birmingham, UK (190)	Potters with occup. dermatitis hand	6 months; prior to 1996	24	14 / 24 pos. to “Indonesian turpentine”
Austria/Germany (IVDK) (279)	Consecutive patients	1992-1995	27658	0.47 % (0.39 – 0.55) §
Austria/Germany (IVDK) (280)	Consecutive patients	1996-2002	59478	Annual prevalences 1.6 to 4.4 %
Augsburg/Germany (281)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (273)	Consecutive patients	2002/03	3767	1.6 %
Austria/Germany/Switzerland (IVDK) (7)	Consecutive patients	2005-2008	37163	1.8 %

Additional information:

ISO 4720:2009 nomenclature: *Pinus pinaster* Aiton and *Pinus massoniana* Lamb. Turpentine, oil: Any of the volatile predominately terpenic fractions or distillates resulting from the solvent extraction of, gum collection from, or pulping of softwoods. Turpentine is a mixture of terpene hydrocarbons obtained from various species of *Pinus* http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=41521

The composition of oil of turpentine varies with its origin, in particular, the content of .delta.-3-carene, one of its main allergenic compounds (189, 279). Similarly, the peroxide degree may vary. The main constituents are .alpha.-pinene (50-72%), .beta.-pinene (6-15%), carenes (< 0.1-17%), camphene (up to 1%), dipentene (0.5-5%), along with a number of other substances (279).

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

Verbena absolute (Lippia citriodora Kunth.)

CAS 8024-12-2, 84961-67-1; EC /)

Current regulation: Annex III, part 1, n° 206

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Aloysia citriodora* Palau syn. *Lippia citriodora* Kunth syn. *Aloysia triphylla* (L' Hér.) Kuntze. An older RIFM review is available citing several positive human maximisation studies both with “Verbena absolute” and “Verbena oil” (128).

VETIVERIA ZIZANOIDES ROOT OIL

CAS 8016-96-4; EC / (Oils, vetiver) / 84238-29-9; EC 282-490-8 (Vetiveria zizanioides, ext. = INCI)

"Vetiver oil; khas khas oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "vetiver" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=9 (10.4%) positive reactions to "vetiver" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Vetiveria zizanioides* (L.) Nash. *Vetiveria Zizanioides* Root Oil is an essential oil distilled from the dried roots of the grass *Vetiveria zizanioides* (L.) Nash *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41293>, last accessed 2010-01-29). Vetiver oil has a high sesquiterpene content. The ketones alpha-vetivone [15764-04-2] (6-12%) and beta-vetivone [18444-79-6] (4-10%), which usually form more than 10% of the oil, as well as khusimol [16223-63-5] (24-36%) and isovelencenol [22387-74-2] (12-24%) are the main constituents (in Bourbon oil, i.e., from Réunion) (34). For Oil of vetiver (*Vetiveria zizanioides* (L.) Nash) an ISO standard exists: ISO 4716:2002.

Acknowledgement: We thank Erich Schmidt for critically reviewing the nomenclature of natural extracts and for providing ISO terminology.

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Annex II - Animal Data

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Substance		Vehicle (AOO=acetone:olive oil; DEP=diethyl phthalate; DMF=dimethyl formamide; DMSO=dimethyl sulphoxide; EtOH=ethanol; MEK=methyl ethyl ketone)	Conc. in vehicle (%, generally w/v)	No. animals per dose group	EC3 value *				Comment (deviation from OECD 429 etc)	Reference
INCI name (other name)	CAS no.				%	µg/cm ²	M	lowest for the substance (%)		
<i>Allyl phenoxyacetate</i>	7493-74-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	3.1	775	0.16	3.1		RIFM, 2007a
Amyl cinnamal	122-40-7	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	7.6	1900	0.38	7.6		RIFM, 2006a
Amyl cinnamal	122-40-7	4:1 AOO	-	4	10.6	2650	0.52		Elahi gives ref to Basketter et al 1999, but no data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment	Elahi et al., 2004

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Aptula gives ref to Kimber et al 2003, but no LLNA data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment; original reference is not given.	Aptula et al., 2007
Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Original ref not given.	Roberts et al., 2007
Amylcinnamyl alcohol	101-85-9	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	> 25	>6250	>1.22	> 25	Should have been tested at higher concentrations	RIFM, 2004a
Anise alcohol	105-13-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.9	1475	0.43	5.9		RIFM, 2005a
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref	Roberts et al., 2007
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Benzyl alcohol	100-51-6	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>4.62	> 50	Should have been tested at higher concentrations	RIFM, 2005b

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Benzyl benzoate	120-51-4	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>2.36	> 50	Should have been tested at higher concentrations	RIFM, 2005c
Benzyl cinnamate	103-41-3	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	18.4	4600	0.77	18.4		RIFM, 2005d
Benzyl salicylate	118-58-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.9	725	0.13	2.9		RIFM, 2005e
<i>p</i> -tert-Butyl-dihydrocinnamaldehyde	18127-01-0	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	4.3	1075	0.23	4.3		RIFM, 2007b
Butylphenyl methylpropional (BMHCA)	80-54-6	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	2.9	725	0.14	2.9		RIFM, 2001a
Butylphenyl methylpropional (BMHCA)	80-54-6	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	4.1	1025	0.20			RIFM, 2001b
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	13.9	3475	0.68			RIFM, 2001c
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 DEP:EtOH	0.3, 1.0, 3.0, 10.0, 30.0	4	8.8	2200	0.43			RIFM, 2001d
Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	16.8	4200	0.82			RIFM, 2001e

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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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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)

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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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)

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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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)

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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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)

Coumarin	91-64-5	DMF	10, 25, 50	4	>50	>12500	>3.42	>50	Should have been tested at higher concentrations	Vocanson et al., 2006
<i>Dibenzyl ether</i>	103-50-4	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	6.3	1575	0.32	6.3		RIFM, 2007e
Eugenol	97-53-0	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	5.3	1325	0.32	5.3		RIFM, 2001f
Eugenol	97-53-0	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	10.5	2625	0.64			RIFM, 2001g
Eugenol	97-53-0	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	10.7	2675	0.65			RIFM, 2001h
Eugenol	97-53-0	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	15.1	3775	0.92			RIFM, 2001i
Eugenol	97-53-0	4:1 AOO	2.5, 5.0, 10.0, 25.0, 50.0	-	11.9	2975	0.72			Basketter et al., 1999
Eugenol	97-53-0	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	5.0, 10.0, 20	4	> 20	>5000	N/a	> 20	Should have been tested at higher concentrations	RIFM, 2004k
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	10.0, 25.0	4	> 25	>6250	N/a		Too few concentrations tested	RIFM, 2004d

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Evernia prunastri extract <i>Oakmoss</i>	90028-68-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.88	970	N/a	3.88		RIFM, 2004j
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	5.5	1375	0.25		Should also have been tested at lower concentrations	RIFM, 2004d
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	4.1	1025	0.18	4.1	Should also have been tested at lower concentrations	RIFM, 2004d
Geraniol	106-24-1	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	5.6	1400	0.36	5.6		RIFM, 2001j
Geraniol	106-24-1	3:1 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	11.4	2850	0.74			RIFM, 2003t
Geraniol	106-24-1	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	11.8	2950	0.76			RIFM, 2001k
Geraniol	106-24-1	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	20.4	5100	1.32			RIFM, 2001l
Geraniol	106-24-1	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	25.8	6450	1.67			RIFM, 2001m
Geraniol	106-24-1	-	-	-	26	6500	1.69			Roberts et al., 2007
<i>trans-2-Hexenal</i>	6728-26-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5, 10	4	2.6	650	0.26	2.6		RIFM, 2005g
<i>trans-2-Hexenal</i>	6728-26-3	-	-	-	5.5	1375	0.56			Roberts et al., 2007

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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)

Hexyl cinnamal	101-86-0	generally 4:1 AOO		-O162	5.3-14.7	1325-3675	0.25-0.68	5.3	"numerous accounts in the literature"
2-Hexylidene cyclopentanone	17373-89-6	1:3 EtOH:DEP	0.1, 0.5, 1.0, 2.5, 5.0	5	2.4	600	0.14	2.4	RIFM, 2008a
Hexyl salicylate	6259-76-3	1:3 EtOH:DEP	0.05, 0.25, 0.5, 1.0, 2.5	4	0.18	45	0.008	0.18	RIFM, 2006b
Hydroxycitronellal	107-75-5	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.3	4825	1.12	19.3	RIFM, 2001n
Hydroxycitronellal	107-75-5	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.7	4925	1.14		RIFM, 2001o
Hydroxycitronellal	107-75-5	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	22.2	5550	1.29		RIFM, 2001p
Hydroxycitronellal	107-75-5	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	26.4	6600	1.53		RIFM, 2001q
Hydroxycitronellal	107-75-5	AOO	25, 50, 100	-	-	-	-	EC3 value not given	Ashby et al., 1995
Hydroxycitronellal	107-75-5	4:1 AOO	2.5, 5, 10, 25, 50	4	33.0	8250	1.92		Basketter et al., 2001
Hydroxycitronellal	107-75-5	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Hydroxycitronellal	107-75-5	-	-	-	25.25	6313	1.47		Estrada et al., 2003

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Hydroxycitronellal	107-75-5	4:1 AOO	10, 25	-	23	5750	1.34		Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	4:1 AOO	1.0, 2.5, 5, 10, 25, 50	4	17.1	4275	0.81	17.1		RIFM, 2001r
<i>p</i> -Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	70% EtOH	10.0, 25.0, 50.0, 100.0	4	9.5	2375	0.46	9.5	Should also have been tested at lower concentrations	RIFM, 2001w
<i>Isocyclocitral</i>	1335-66-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	7.3	1825	0.48	7.3		RIFM, 2006c
<i>Isocyclogeraniol</i>	68527-77-5	1:3 EtOH:DEP	5.0, 10.0, 25.0, 50.0	4	> 25	>6250	>1.62	> 25	Should have been tested at higher concentrations	RIFM, 2005h
Isoeugenol	97-54-1	4:1 AOO	0.5, 5.0	6	0.54	145	0.033	0.54	Too few concentrations tested	RIFM, 2001s
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.6	150	0.037			RIFM, 2002a
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.76	191	0.046			RIFM, 2002b
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.79	199	0.048			RIFM, 2002c

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Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.19	296	0.072	RIFM, 2001t
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.28	320	0.078	RIFM, 2004e
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	6	1.54	385	0.094	RIFM, 2001u
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.95	488	0.119	RIFM, 2001v
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0		3.3	825	0.20	Basketter et al., 1999
Isoeugenol	97-54-1	-	-	-	-	-	-	No data in the ref (poster abstract) Basketter et al., 2002
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.3	325	0.079	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.3	825	0.20	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.8	450	0.11	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.1	775	0.19	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.6	400	0.097	Loveless et al., 1996
Isoeugenol	97-54-1	AOO	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	MEK	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	1.4	350	0.085	Wright et al., 2001

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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)

Isoeugenol	97-54-1	propylene glycol	0.5, 1.0, 2.5, 5.0, 10.0	4	2.5	625	0.15			Wright et al., 2001
Isoeugenol	97-54-1	DMSO	0.5, 1.0, 2.5, 5.0, 10.0	4	0.9	225	0.055			Wright et al., 2001
Isoeugenol	97-54-1	90:10 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	1.8	450	0.11			Wright et al., 2001
Isoeugenol	97-54-1	50:50 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	4.9	1225	0.30			Wright et al., 2001
<i>Jasmine absolute (Grandiflorum)</i>	8022-96-6	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	5.9	1475	N/a	5.9		RIFM, 2006d
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	35.4	9100	N/a	35.4		RIFM, 2006e
<i>d-Limonene**</i>	5989-27-5	EtOH	10.0, 20.0, 50.0, 75.0, 100.0	4	< 10	< 250	<0.73	< 10	Should also have been tested at lower concentrations	RIFM, 2004l
<i>d-Limonene**</i>	5989-27-5	3:1 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	22.0	5500	1.61			RIFM, 2004m
<i>d-Limonene**</i>	5989-27-5	1:3 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	38.0	9500	2.79			RIFM, 2004n
<i>d-Limonene**</i>	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
<i>d-Limonene**</i>	5989-27-5	4:1 AOO	25, 50, 100	4	68.5	17125	5.03			Warbrick et al., 2001

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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)

Linalool**	78-70-6	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
<i>Menthadiene-7-methyl formate</i>	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
<i>4-Methoxy-α-methyl benzenpropanal</i>	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
<i>α-Methyl cinnamic aldehyde</i>	101-39- 3	-	-	-	4.5	1125	0.31	4.5		Roberts et al., 2007
<i>Methylenedioxyphenyl methylpropanal</i>	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
<i>6-Methyl-3,5-heptadien-2- one</i>	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
<i>α-iso-Methylionone</i>	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
<i>Methyl octine carbonate</i>	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 <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	9.3	2325	0.62			RIFM, 2008b
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.95	987	N/a	3.95		RIFM, 2004h
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.5	625	N/a	2.5		RIFM, 2004i
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	0.5, 1.0, 2.5	4	>2.5	>625	N/a			RIFM, 2004i
Phenylacetaldehyde	122-78-1	4:1 AOO	2.5, 5, 10, 25, 50	4	3.0	750	0.25	3.0		Basketter et al., 2001

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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 <i>Majantol</i>	103694-68-4	4:1 AOO	3.0, 10.0, 30.0	4	~30	~7500	~1.68	30	Should have been tested at higher concentrations	RIFM, 2002d
Vanillin	121-33-5	4:1 AOO	2.5, 5, 10, 25, 50	4	>50.0	>1250	>3.3	>50.0		Basketter et al., 2001

* source of EC3 value value: % given in the RIFM report or references; µg/cm2 given in the RIFM report and RIFM poster; M calculated by SCCS working group

**material with low levels of oxidation according to RIFM, 2009

- = no data given; A216

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Annex III - Tabular summary of dose-elicitation studies in sensitised patients**Contents**

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Chloroatranol

Chloroatranol (allergen in oak moss absolute: <i>Evernia prunastri</i>) (1)	
Design	blinded, randomised with regard to doses and controlled
Test subjects	13 patients previously identified as sensitized to chloroatranol and oak moss absolute
Controls	10 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	200 ppm to 0.0063 ppm (10 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction
ROAT	volar aspect of forearms
area	3 x 3 cm ²
applications/day	two
dose	chloroatranol in ethanol: Step 1: 5 ppm Step 2: 25 ppm
dose/application/cm ²	step 1: 0.025 µg step2: 0.125 µg
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step
Results	
PT ED10% (95% CI)	0.013 (0.002-0.03) ppm =0.0004 µg/cm ²
PT ED50% (95% CI)	0.15 (0.077-0.295) ppm =0.0045 µg/cm ²
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (5 ppm)	12/13 (92%)
Step 2 (25 ppm)	13/13 (100%)
Controls	Negative
Other information	None relevant

In a subsequent study chloroatranol and atranol, both ingredients in *Evernia prunastri*, were tested in equimolar concentrations in serial dilution in 10 eczema patients with known sensitization to chloroatranol and oak moss. A positive response was defined as any degree of reaction. Ethanol was included as the control and gave no response. No use tests were done and no control subjects included.

Results: All patients reacted to the highest concentrations of the two substances. For both substances there was a significant dose-dependence and the estimated difference in elicitation potency of chloroatranol relative to atranol was 217%. The dose-response curve is seen in figure 1 below (2).

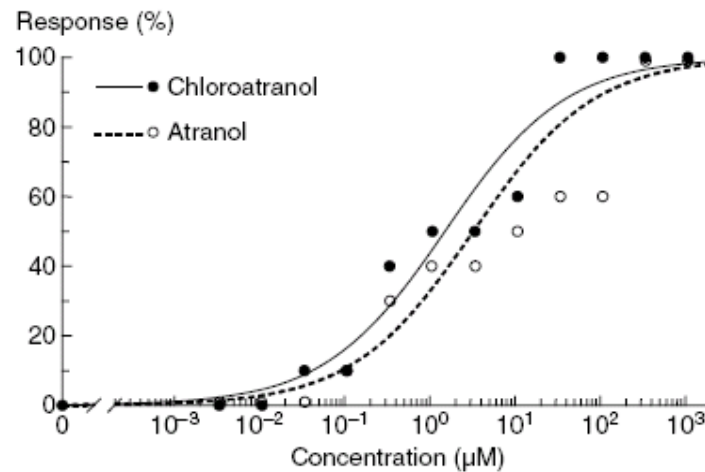


Fig. 1. Observed response rates and fitted parallel logistic dose-response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

Cinnamal

Cinnamal (3)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to cinnamal and additional 4 with a doubtful response
Controls	20 healthy controls
Substance	Purity: >98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (7 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of responses
ROAT	outer aspect of upper arm
area	5 x 5 cm ²
applications/day	two with atomizer pump
dose	Step 1: 0.02% Step 2: 0.1% Step 3: 0.8%
dose/application/cm ²	Not given
control substance	ethanol
definition of positive	The response was classified as positive no matter the degree of reaction.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.24% = 96 µg/cm ² (calculated from the data in the paper)
PT no effect level(observed)	0.01 % in pet. = 0.4 µg/cm ²
ROAT	Cumulative responses
Step 1 (0.02%)	0/18
Step 2 (0.1%)	8/18 (44 %)
Step 3 (0.8%)	13/18 (72 %)
Controls	No eczema reactions were seen
Other information	2 patients and 2 controls developed immediate reactions to the cinnamal solution

Cinnamal (4)	
Design	blinded, randomised doses and controlled
Test subjects	17 patients with a positive patch test to cinnamal (8 patients in part 1 and 9 in part two)
Controls	20 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2 % to 0.00006 % (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting a + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (89-700 mg per application of solution) average cases: 263 mg/application controls: only range given
dose	Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32% Part two: Step 1: 0.01% Step 2: 0.032% Step 3: 0.1%
dose/application/cm ²	Part two estimated: step one: 0.26 µg; step two: 0.84 µg; 2.63 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	0.002%
ROAT	Cumulative responses
Step 1 (0.01)	2/9 (22%)
Step 2 (0.032)	6/9 (67%)
Step 3 (0.1)	8/9 (88%)
Controls	No reactions were seen
Other information	Only reactions seen to the cinnamal-containing deodorants at ROAT, difference to matrix axilla ($p<0.001$) and all control

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	persons negative ($p < 0.001$)

Hydroxycitronellal

Hydroxycitronellal (5)	
Design	blinded, randomised doses and controlled
Test subjects	7 patients with a positive patch test to hydroxycitronellal
Controls	7 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	4% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (172-591 per application of solution) average cases: 294 mg/application controls: only range given
dose	Step 1: 0.032% Step 2: 0.1% Step: 0.32%
dose/application/cm ²	Estimated: step 1: 0.94 µg; step 2: 2.94 µg; step 3: 9.40 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	<0.00012 %
ROAT	Cumulative responses
Step 1 (0.032)	4/7 (57%)
Step 2 (0.1)	5/7 (71%)
Step 3 (0.32)	7/7 (100%)
Controls	No reactions were seen
Other information	Reactions were only seen to the hydroxycitronellal-containing deodorant at ROAT, difference to matrix treated axilla ($p<0.001$) and all control persons negative ($p<0.001$)

Hydroxycitronellal (6)	
Design	double blinded, randomised
Test subjects	13 patients with a positive patch test to hydroxycitronellal
Controls	/
Substance	purity: unknown
Patch test	confirmatory
-dilution steps	
-control/vehicle	
-definition of threshold	
ROAT	finger immersion in fragrance solution in 10% ethanol
area	/
applications/day	Once per day for 10 min
dose	Step 1: 10 ppm Step 2: 250 ppm
dose/application/cm ²	Not applicable
control substance	10% alcohol
definition of positive	clinical grading scale and laser doppler comparison between active and control
period	two weeks with each concentration: maximum four weeks
Results	
PT ED10% (95% CI)	Not relevant
PT ED50% (95% CI)	Not relevant
PT no effect level(observed)	Not relevant
ROAT	Cumulative responses
Step 1 (10 ppm)	1/13
Step 2 (250 ppm)	5/13
Vehicle control	4/13
Other information	No difference between active substance and control application was found.

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (7)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to HICC
Controls	7 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006%
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	volar aspect of lower arm
area	3 x 3 cm ²
applications/day	two with droplet bottle (theoretical:30 mg per application of solution)
dose	Step 1: 0.5% Step 2: 3%
µg/application/cm ²	Step 1: 15.3 (3.4-22.2) Step 2: 126.2 (40.5-226.2)
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step; total maximum 4 weeks
Results	
PT ED10% (95% CI)	0.9 µg/cm ² 29 (7-69) ppm
PT ED50% (95% CI)	20 µg/cm ² 662 (350-1250)ppm
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (0.5%)	11/18 (61%)
Step 2 (3%)	16/18 (89%)
Controls	No reactions were seen
Other information	Difference between test and control group statistically significant

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (8)	
Design	blinded, randomised and controlled
Test subjects	15 patients with a positive patch test to HICC
Controls	10 healthy controls
Substance	Purity: > 98.8%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006% (5 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	Axilla
area	76 cm ² (template)
applications/day	two with roll on deodorant
dose	Step 1: 200 ppm Step 2: 600 ppm Step 3: 1800 ppm
dose/application/cm ²	median 0.79 µg HICC
control substance	deodorant matrix
definition of positive	spotty erythema involving at least 25% of the exposed area and infiltration represented by at least one papule.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	0.75 µg/cm ² 25 ppm (0.69-120)
PT ED50% (95% CI)	18.3 µg/cm ² 610 ppm (120-2800)
PT no effect level (observed)	< 0.0006%
ROAT	Cumulative responses
Step 1 (200 ppm)	9/14* (64%)
Step 2 (600 ppm)	12/14* (86%)
Step 3 (1800 ppm)	14/14* (100%)
Controls	No reactions were seen
Other information	*14 patients completed the use test study Difference between HICC deodorant and matrix deodorant in cases ($p=0.0001$). Difference between controls and patients ($p=0.004$).

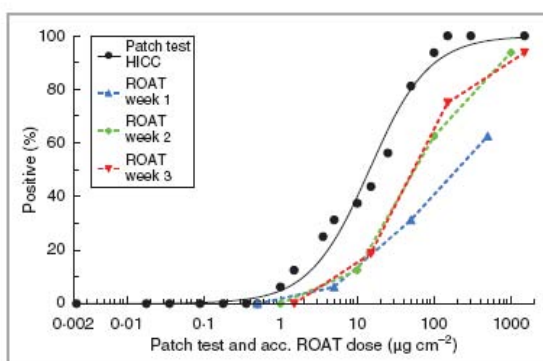
Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (9)	
Design	blinded, randomised and controlled
Test subjects	17 patients with a positive patch test to HICC
Controls	15 healthy controls
Substance	IFF lot SM/8059062
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	1500 to 0.0022 µg/cm ² HICC (19 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of forearms
area	3 x 3 cm (5 areas)
applications/day	two with micropipette (20 µl per application)
dose	Simultaneous application to 5 areas, four doses each and vehicle
µg /application/cm ²	Dose 1:0.0357 Dose 2: 0.357 Dose 3: 3.57 Dose 4: 35.7
control substance	ethanol
definition of positive	at least 5 points on a clinical scale, corresponding to erythema in 25% of test area and at least 1 papule
period	Three weeks. All concentrations applied simultaneously (randomised)
Results	
PT ED10% (95% CI)	0.662 µg/ cm ² (0.052-2.35)
PT ED50% (95% CI)	11.1 µg/ cm ² (3.41- 33.1)
PT no effect level(observed)	<0.0022 µg/ cm ²
ROAT	Cumulative responses
Dose 1 (0.0357)	0/16*
Dose 2 (0.357)	3/16 (19%)
Dose 3 (3.57)	12/16 (75%)
Dose 4 (35.7)	15/16 (94%)
Controls	No reactions were seen
Other information	*16 patients completed the use test study The evaporation rate of HICC was calculated to 72% over a 24-h period. ED10% ROAT: 0.064 µg/cm ² (more info see below)

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Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 1 week	Total accumulated dose after 1 week ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 2 weeks	Total accumulated dose after 2 weeks ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 3 weeks	Total accumulated dose after 3 weeks ($\mu\text{g HICC cm}^{-2}$)
35.7	14	500	28	1000	42	1500
3.57	14	50	28	100	42	150
0.357	14	5	28	10	42	15
0.0357	14	0.5	28	1	42	1.5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

Fig 3. The fitted dose-response curve for the patch test ($n = 16$) and the 1-week, the 2-week 3-week accumulated ROAT doses.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

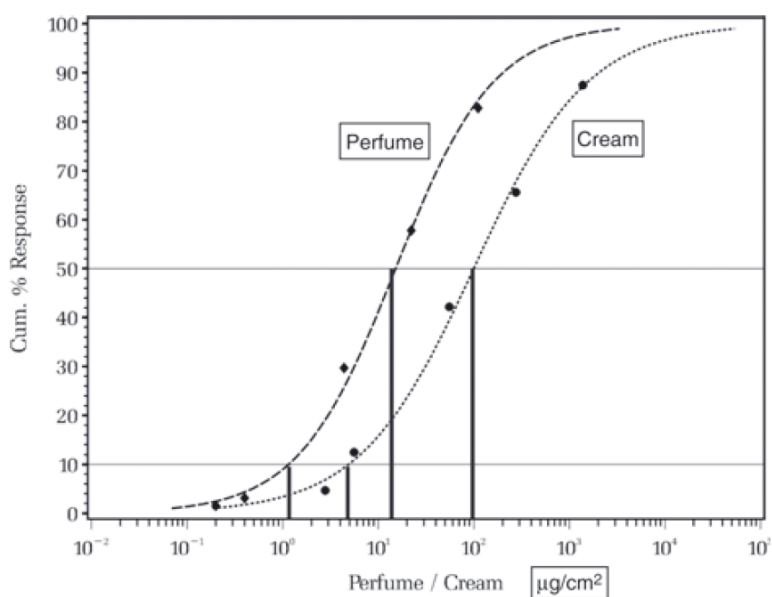
In a study by the German Contact Dermatitis Group, 64 persons previously diagnosed with HICC contact allergy were exposed to increasing doses of HICC in 2 different formulations, a hydrophilic cream and an ethanol solution, to mimic everyday exposures, following a standardised ROAT protocol (10). The concentration of HICC tolerated by 90% of the sensitised was estimated as 1.2 µg/cm² for perfume and 4.9 µg/cm² for cream. The dose-response curve is shown in Fig. 4.3 – 1 below.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (10)			
Design	randomised and vehicle controlled		
Test subjects	67 patients with a previous positive patch test to HICC		
Controls	None		
Substance	Provided by International Flavor & Fragrances Inc, Hilversum, NL		
Patch test			
-dilution steps	2.5% and 5%		
-control/vehicle	petrolatum		
-definition of threshold	lowest concentration giving a positive skin reaction in a continuous line to next higher concentration.		
ROAT	Volar forearms (both sides)		
area	3 x 3 cm (4 areas: one test and one control each for alcoholic solution and cream, respectively)		
applications/day	two		
dose	2.8 µg/cm ² in cream	0.2 µg/cm ² in ethanol	
	5.6 µg/cm ² in cream	0.4 µg/cm ² in ethanol	
	55.6 µg/cm ² in cream	4.4 µg/cm ² in ethanol	
	277.8 µg/cm ² in cream	22.2 µg/cm ² in ethanol	
	1388.9 µg/cm ² in cream	111.1 µg/cm ² in ethanol	
µg /application/cm ²	See above		
control substance	Ethanol 96% and glyceryl stearate 15% in water, resp.		
definition of positive	(spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number		
period	Two weeks for each step until positive reaction or end of study, whichever occurred first		
Results			
PT ED10% (95% CI)	Not calculable; 52 of 60 Patients patch tested positive to 2.5% HICC, 57 / 60 to 5% HICC		
PT ED50% (95% CI)	Not calculable		
PT no effect level (observed)	Not calculable		
ROAT	Cumulative responses:		
	Cream preparation: 2.8 µg/cm ² : 4.7%	Ethanol preparation: 0.2 µg/cm ² : 1.6%	

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	5.6 $\mu\text{g}/\text{cm}^2$: 12.5% 55.6 $\mu\text{g}/\text{cm}^2$: 42.2% 277.8 $\mu\text{g}/\text{cm}^2$: 65.6% 1388.9 $\mu\text{g}/\text{cm}^2$: 87.5%	0.4 $\mu\text{g}/\text{cm}^2$: 3.1% 4.4 $\mu\text{g}/\text{cm}^2$: 29.7% 22.2 $\mu\text{g}/\text{cm}^2$: 57.8% 111.1 $\mu\text{g}/\text{cm}^2$: 82.8%
Controls	No reactions to vehicle in the patients included into analysis	
Other information	See figure below. Three patients were excluded from the study, so results are based on 64 patients.	

Figure 4.3 – 1: Dose-response curve of 64 patients sensitised to HICC, according to a previous PT, regarding two preparations: perfume and cream, the rhomboid and dot symbol, respectively, indicating the observed response. The curve was fitted by a logistic function (10).



Isoeugenol

Isoeugenol (11)	
Design	blinded, randomised doses and controlled
Test subjects	20 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (8 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line
ROAT	outer aspect of upper arms
area	5 x 5 cm (2 areas: one test and one control)
applications/day	two with roll-on
dose	0.2% in ethanol
μg /application/ cm^2	Doses measured to 0.14 -0.13 mg/application the first 14 days = 5.6 $\mu\text{g}/\text{cm}^2$
control substance	ethanol
definition of positive	any degree of reaction
period	Two weeks at upper arm and if negative another two weeks including application to base of neck
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.08% 32 $\mu\text{g}/\text{cm}^2$
PT no effect level (observed)	< 0.01% = 0.4 $\mu\text{g}/\text{cm}^2$
ROAT	
Dose: 0.2%	12/19 (63%)
Controls	No reactions were seen
Other information	

Isoeugenol (12)	
Design	blinded, randomised
Test subjects	27 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of lower arm
area	3 x 3 cm (2 areas)
applications/day	two with droplet bottle (30 mg per application)
dose	0.05% in ethanol and 0.2%
µg /application/cm ²	Doses were calculated as mean 2.2 µg/cm ² (low conc.) and 9 µg/cm ² (high conc.)
control substance	ethanol
definition of positive	clear visible erythema
period	28 days
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	< 0.0005% (5 ppm)
ROAT	Cumulative responses
Dose 1: 0.05%	10/24 (42%)
Dose 2: 0.2%	16/24 (67%)
Controls	No reactions were seen
Other information	Response to the low concentration in the ROAT appeared after median 15 days and to the high concentration after median 7 days.

Isoeugenol (13)	
Design	blinded, randomised and controlled
Test subjects	13 patients with a positive patch test to isoeugenol and 4 in part 1 (pre-test)
Controls	10 healthy controls (dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (w/v) (16 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting at least + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll-on deodorant (117-586 mg per application of solution) average cases: 266 mg/application controls: only range given
dose	Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2% Part 2: Step1:0.0063% Step 2:0.02% Step 3: 0.063%
dose/application/cm ²	Part 2: Step 1: 0.167 Step 2: 0.53 Step 3: 1.67 µg/application/cm ² (calculated based on data)
control substance	deodorant matrix
definition of positive	eczematous response covering 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	<0.0005% (0.15 µg/cm ²)
ROAT	
Step 1 (0.0063%)	3/13 (23%)
Step 2 (0.02%)	9/13 (69%)
Step 3 (0.063%)	10/13 (77%)
Controls	No reactions were seen
Other information	Deodorants containing cinnamal were responsible for all reactions in cinnamal sensitized individuals ($p<0.001$) and all control persons were negative ($p<0.001$)

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FOREWORD

INTRODUCTION

LINALYL ACETATE

CAS N°: 115-95-7

SIDS Initial Assessment Report

For

SIAM 14

Paris, France, 26-28 March 2002

1. Chemical Name: Linalyl Acetate

2. CAS Number: 115-95-7

3. Sponsor Country: Switzerland

National SIDS Contact Point in Sponsor Country:
Dr. Georg Karlaganis
Swiss Agency for the Environment, Forests and Landscape
CH-3003 Berne, Switzerland
e-mail: georg.karlaganis@buwal.admin.ch

4. Shared Partnership with:

5. Roles/Responsibilities of the Partners:

- Name of industry sponsor /consortium
- Process used

6. Sponsorship History

- How was the chemical or category brought into the OECD HPV Chemicals Programme ?
This substance is evaluated under the OECD HPV programme and is submitted for first discussion at SIAM 14.

7. Review Process Prior to the SIAM:

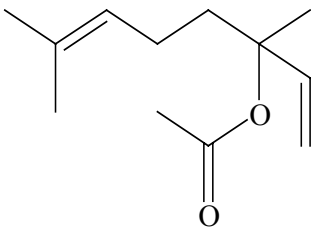
8. Quality check process:

9. Date of Submission: May 2002

10. Date of last Update:

11. Comments: No testing (X) Testing ()

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	115-95-7
Chemical Name	Linalyl acetate
Structural Formula	
RECOMMENDATIONS The chemical is currently of low priority for further work.	
SUMMARY CONCLUSIONS OF THE SIAR Human Health Linalyl acetate is of very low acute toxicity to mammals, the acute oral LD₅₀ is >13,360 mg/kg, while the inhalation LC₅₀ is >2740 mg/m³. Linalyl acetate has no or a very low potential to irritate the human skin. No information on possible eye irritation is available. Based on the use of linalyl acetate in cosmetics (constituent of perfumes) it is doubted that the substance has significant sensitising properties. Linalyl acetate is an ester that is expected to be hydrolysed to linalool and acetic acid in the gastro-intestinal tract. The main effects of the hydrolysis product, linalool (72.9%), in a 28-day oral rat study were increased liver and kidney weight, thickened liver lobes and pale areas on the kidneys and in females only hepatocellular cytoplasmic vacuolisation. Other findings were related to local irritation of the gastro-intestinal tract. Based on the effects on liver and kidney a NOAEL of 160 mg/kg bw/d was derived for linalool (equivalent to 148 mg/kg bw/d linalyl acetate). In this study no effects on male and female gonads were found. Linalool (72.9%) was tested in a reproduction screening test (non-OECD). The NOAEL for maternal toxicity based on clinical signs and effects on body weight and food consumption was 500 mg/kg bw/d for linalool (equivalent to 464 mg/kg bw/d linalyl acetate). The NOAEL on reproduction toxicity and developmental toxicity is 500 mg/kg bw/d (equivalent to 464 mg/kg bw/d linalyl acetate) based on the decreased litter size at birth and pup morbidity/mortality thereafter. Linalyl acetate does not induce gene mutations or chromosomal effects <i>in vitro</i>. Environment Linalyl acetate is a liquid with a vapour pressure of 0.61 Pa (at 25°C), a water solubility of 30 mg/L and a Log K_{ow} of 3.9 (measured). It has a calculated half-life for photo-oxidation of 1.1 hours. Linalyl acetate will partition primarily to water (Mackay level III modelling). In a hydrolysis study linalyl acetate was found to disappear from the test medium within 2.4 hours at pH 4, 7 and 9 (at 50°C) (t_{1/2} < 24 hours at 20°C). Hydrolysis products are linalool and acetic acid. Linalyl acetate is readily biodegradable. Based on the log K_{ow} a BCF of 412 was calculated. Linalyl acetate has potential for sorption to soil (predicted log K_{oc} 2.9), however, in view of the rapid hydrolysis of the substance it is unlikely that significant uptake or sorption may occur.	

Linalyl acetate is toxic to fish and daphnia. All values are based on measurements of the parent compound, which is expected to hydrolyse very quickly. The 96-hour LC_{50} in fish is 11 mg/L. The 48-hour EC_{50} for daphnia is 6.2 mg/L. In a test with algae (*Scenedesmus subspicatus*, 72-hours exposure), a reduction of biomass was seen at 1.2 mg/L and above. The 72-hour E_bC_{50} was 4.2 mg/L, the E_rC_{50} was 16 mg/L).

The EC_{50} for the inhibition of micro-organisms is 415 mg/L (ethanol was used as dispersant).

Exposure

The market for linalyl acetate is 1000-5000 tonnes. It is used as an intermediate and can be found in consumer products as soaps, cleaning products, cosmetics (perfume), oil paint and extracts. Linalyl acetate is a food additive. Foodstuffs contain between 1.9 (soft drinks) and 13 ppm (chewing gum) linalyl acetate.

There is a potential for occupational exposure. Consumers may be exposed orally and dermally. There is potential exposure to the aquatic compartment arising from the production/formulation and consumer use of this substance.

NATURE OF FURTHER WORK RECOMMENDED

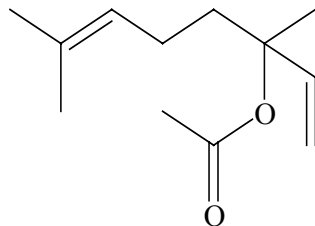
No further work recommended, because this molecule hydrolyzes rapidly.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 115-95-7
EINECS Number 204-116-4
Chemical Name 3,7-dimethyl-1,6-octadien-3-yl acetate
IUPAC Name: 1,5-dimethyl-1-vinylhex-4-enyl acetate
Molecular Formula: $C_{12}H_{20}O_2$
Structural Formula*:



Molecular Weight: 196.29
Synonyms: Linalyl acetate, linalool acetate, acetic acid linalool ester

* The compound is a chiral molecule. No data on differences between the enantiomers are available.

1.2 Purity/Impurities/Additives

Purity >96%

1.3 Physico-Chemical properties

Linalyl acetate (CAS no. 115-95-7) is a liquid having the following physical-chemical properties and characteristics, which have been obtained from various reference sources including company substance documentation and handbook data.

Table 1 Summary of physico-chemical properties

Property	Value
Physical state	Liquid
Melting point	<0°C
Boiling point	220°C at 1013 hPa
Relative density	0.895 (20°C)
Vapour pressure	0.61 Pa (20°C)
Water solubility	30 mg/L (room temperature) (ref. 34)
Partition coefficient n-octanol/water (log value)	log K _{ow} 3.9 (ref. 3)
Solubility in organic solvents	Soluble in ethanol and diethyl ether
Flash point	84-85°C

The value for water solubility could not be checked for validity due to the limited information available. However, the result of the test is supported by model calculations with the Syracuse programme (21 mg/L) and the maximum concentrations (~27 mg/L) in the flow-through fish test (ref. 13).

2 GENERAL INFORMATION ON EXPOSURE

Estimated Production or Import Volume

Yearly around 1000-5000 tonnes of linalyl acetate are produced. The following information has been gathered by different authorities in the world. Within the USA the quantity produced, imported and exported is 49 tonnes/year. In Canada 3 to 39 tonnes are imported (data from 1986). In Norway the substance is registered as a component in 37 products with a total quantity of < 0.1 tonne. It is expected that the total quantity used or produced in Norway is less than 1 tonne. The Danish product register indicates that the total quantity of in industrial products was < 1 tonne.

Uses and Exposure

The substance is used as an intermediate and found in soaps, cleaning products and cosmetics (perfume) (ref. 1). It is a component of oil paint, is used in extracts and as a substitute for petitgrain oil (information from USA). In view of the reported use, both workers and consumers can be exposed.

Linalyl acetate is a food additive. Foodstuffs contain between 1.9 (soft drinks) and 13 ppm (chewing gum) linalyl acetate (ref.1).

The Danish product register indicates that linalyl acetate is a constituent of several industrial products (Odour agents, Cleaning/washing agents, Cosmetics, Absorbents and Adsorbents, Process regulators, Welding and soldering agents, Non-agricultural pesticides and preservatives, Surface treatment). The total amount per product of the substance was 2% or less, except in odour agents (<20%)(The Danish Product Register, 26-02-2002).

The Norwegian Product register indicates that linalyl acetate is used in car polish (wax), ordinary cleaning products and detergents (for textile). The content in the products is between 0 and 1% (The Norwegian Product Register, 27-12-2001).

2.1 Environmental Exposure and Fate

Linalyl acetate is not very soluble in water, has a vapour pressure of 0.61 Pa and a calculated Henry's Law constant of $1.74\text{E-}03 \text{ atm.m}^3/\text{mol}$ (Epiwin vs 3.10).

For Switzerland an emission limit of 150 mg/m^3 is set (mass flux $\geq 3 \text{ kg/h}$) (ref. 1).

Linalyl acetate is classified as weakly hazardous for water (Germany, ref. 1).

2.1.1 Sources of Potential Release to the Environment

No data on emissions to the environment are available. It can not be excluded that during the production process linalyl acetate is released to the environment. However, in an investigation in rivers of south-west Germany to establish the amount of terpenes, no linalyl acetate was found in the surface water except in one spot (ref. 14). The only conclusion that can be drawn from this report is that it cannot be excluded that linalyl acetate may reach the aquatic environment, since disposal of cosmetic products or chemical processes can lead to emissions to water.

2.1.2 Photodegradation

The atmospheric oxidation potential (AOP, reaction with hydroxyl radicals) predicted from the Epiwin program (vs 3.10, AOP Program v1.90) indicates that linalyl acetate is photo-reactive with a

degradation half-life of about 1.1 hours. A summary of the Epiwin calculations is attached as Appendix A.

2.1.3 Stability in Water

In a hydrolysis study performed according to EEC-Directive 92/69, C7, linalyl acetate was found to disappear from the test medium at 50 °C within 2.4 hours at pH 4, 7 and 9 ($t_{1/2} < 24$ hours) (ref. 24). The hydrolysis products were not identified, but it is expected that an ester like linalyl acetate will be hydrolysed to the corresponding acid and alcohol. For linalyl acetate hydrolysis products would be linalool (CAS 78-70-6, see also corresponding Initial Assessment Report) and acetic acid. In a HCl/citrate buffer (pH 3) linalyl acetate was rapidly hydrolysed, yielding linalool, α -terpinol and geraniol (51, 22 and 8%, respectively after 25 hours) (ref. 17). Other degradation products were present at levels below 5%. 8.8% was recovered as the parent compound. The half-life of linalyl acetate at this low pH is about 7 hours.

2.1.4 Transport between Environmental Compartments

Information available on the transport between environmental compartments has been assessed with the EQC model. Since the substance is soluble in water to some extent, the substance is considered to be a Type I substance. For this assessment the following defaults have been chosen as worst case estimation (in view of the Epiwin, biodegradation and hydrolysis data):

$T_{1/2}$ air: 1 hours, $t_{1/2}$ water 24 hours, $t_{1/2}$ soil 360 hours and $t_{1/2}$ sediment 1440 hours, and using the following physical chemical parameters: water solubility 30 mg/l, vapour pressure 0.6 Pa, Log Kow 3.9, melting point 0°C.

As information on possible emission route is not available, emissions have been considered only to water. The level III fugacity model has been considered and attached as Appendix B. Based on the results of the model it is predicted that 0.3% of the substance will end up in air, 0% in soil, 95% in water and 5% in sediment (see appendix B).

Calculations with the Epiwin model (emission to water only) gave 90% in water, 0.14% in air and 10% in sediment (see appendix A).

From the calculated log K_{ow} of 3.9 the log K_{oc} was determined to be 2.9 (EU Technical Guidance Document QSAR, chapter 4 section 4.3, average outcome of two QSARs on esters¹) indicating a potential for sorption to soil. This sorption was not observed in the modelling results described in the previous paragraph. In fact no sorption is expected in view of the hydrolytic instability of linalyl acetate (see 2.1.3).

The distribution in a sewage treatment plant has been estimated using the SimpleTreat model to be 80.4% degraded, 1.4% to air, 11.4% to water, 6.8% to sludge, based on ready biodegradability, log Kow = 3.9, water solubility = 30 mg/L and vapour pressure = 0.61 Pa.

Conclusion:

Based on the relevant physical-chemical, linalyl acetate will partition primarily to water (Mackay level III modelling shows >95% in water). In the sewage treatment plant linalyl acetate will degrade (80%) and be present in the effluent (11%).

¹ $\text{LogKoc} = 0.47 \text{ logKow} + 1.09$

$\text{LogKoc} = 0.49 \text{ logKow} + 1.05$

2.1.5 Biodegradation

Linalyl acetate is considered to be readily biodegradable (ref. 7) in a MITI-test after incubation for 28 days at 20 °C. The compound turned out to degrade for more than 75%.

Conclusion:

Linalyl acetate is considered to be readily biodegradable.

2.1.6 Bioaccumulation

The calculated bioconcentration factor is 412 (EU Technical Guidance Document QSAR, chapter 4 section 4.5.2.1).

Conclusion:

Based on Log K_{ow} 3.9 a BCF of 412 is calculated. Linalyl acetate is expected to bioaccumulate. However, in view of the rapid hydrolysis of the substance it is unlikely that significant uptake may occur.

2.2 Human Exposure

Linalyl acetate is not only used in closed systems (USA, Canada, Denmark). So worker exposure may be possible. Use by consumers is expected (USA, Canada, Denmark).

In the USA it is expected that about 155,491 employees at 11,043 facilities are potentially exposed to linalyl acetate. This will be the case in the manufacturing and applying of oil paints and in the manufacturing of perfume.

For the exposure due to the use in oil paints, area sampling was requested by representatives of artists (painters). 12 artists were interviewed and some of them complained about the odour of the paint and slight dermatitis. No relationship with the use of paint containing linalyl acetate was established (no area samples were taken). Measures to reduce the risks were advised (ref. 29 and data from USA).

Consumers using oil paint and perfume are potentially exposed to linalyl acetate (information from USA).

For occupational exposure no limit values are known.

Linalyl acetate is generally recognised as safe for human consumption (GRAS). The ADI (accepted daily intake) is 0.25 mg/day (ref. 1, 18).

Conclusion:

Workers may be exposed during production. But the main exposure is expected with consumers; both oral and dermal exposure may be possible.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Linalyl acetate is hydrolysed in gastric and pancreatic fluids with mean half-lives of 5.5 and 52.6 minutes respectively (ref. 34). As expected hydrolysis occurs more rapidly at the low pH of gastric fluids. The reaction products are linalool and acetic acid (ester hydrolysis). This is supported by the findings of the hydrolysis study (ref. 24) at pH 4, 7 and 9. Therefore it is expected that linalool is the substance that will enter the systemic circulation after oral uptake of linalyl acetate.

Linalool is probably converted to geraniol and its metabolites, 1,5-dimethyl-hexadiene-1,6-dicarboxylic acid and 7-carboxy-5-methylocto-6-enoic acid (ref. 10). The information in this publication was limited to the above mentioned. No indication of the species under investigation was provided.

Conclusion

Linalyl acetate will be hydrolysed to linalool and acetic acid in the gastro-intestinal tract.

3.1.2 Acute Toxicity

Studies in Animals

Oral

The information on acute oral toxicity is limited (ref. 31). The results are presented in Table 3.1. Observed effects included depression, coma and wet posterior.

Table 3.1 Acute oral toxicity

Species	LD ₅₀ (mg/kg bw)
Rat	14550
Mouse	13360

Dermal

No data available.

Inhalation

Inhalation exposure of Swiss mice to 2.74 mg linalyl acetate/L air during 90 minutes led to reduced motor activity compared to untreated controls. The effect was more severe in mice of aged 6-8 weeks (up to 100% reduction) than in mice of 6 months (up to 81% reduction). A relationship with dose was suspected, based on the (not reported) results of a separate test with a double dose in old mice (ref. 16).

Other

No data available

Human experience

No data available.

Conclusion:

Linalyl acetate is of very low acute toxicity to mammals, the acute oral LD₅₀ is >13,360 mg/kg, while the inhalation LC₅₀ is >2740 mg/m³.

3.1.3 IrritationSkin Irritation*Studies in Animals*

Linalyl acetate (100%) appeared to be severely irritating to rabbit skin and moderately irritating to the skin of the guinea pig. In a test with miniature swines application of 0.05 g linalyl acetate under a patch for 48 hours, no irritation was observed (ref. 11).

Studies in Humans

Application of linalyl acetate in acetone (33%) to the back of male volunteers without known allergies during 48 hours under occlusion did not induce signs of irritation up to 120 hours after removal of the patch (ref. 11).

Conclusion

The data available indicate that linalyl acetate has no or a very low potential to irritate the human skin. A significant species difference was reported. It has to be noted that the concentration tested in humans was lower than the concentration used in animal testing. No guideline studies are available.

Eye Irritation

No data available.

3.1.4 Skin Sensitisation

In ref. 27 linalyl acetate is identified as one of the constituents of lavender oil that may cause allergic reactions. No testing results were available for linalyl acetate, but lavender oil was positive in a human patch test in a hairdresser (ref. 28).

Conclusion

Limited information is available. Based on the use of linalyl acetate in cosmetics (constituent of perfumes) it is doubted that the substance has significant sensitising properties. The substance is not listed by the Scientific Committee on Cosmetic and Non-food Products as a compound of concern regarding sensitisation.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Oral

Since no reliable studies with linalyl acetate are available and the compound is expected to be hydrolysed to linalool in the gastro-intestinal tract, repeated dose studies on the hydrolysis product linalool are presented in this section.

CrI:CD/BR rats received 160, 400 or 1000 mg/kg bw linalool during 28 days. One male and one female of the high dose group were found dead. Total protein/albumin were increased in males at 400 mg/kg bw and in both sexes at 1000 mg/kg bw. Calcium was increased at 1000 mg/kg bw in males only. Serum glucose levels were decreased in males at 400 and 1000 mg/kg bw. Liver weight was increased dose related and significantly at 400 and 1000 mg/kg bw. Kidney weight was increased in males at 400 mg/kg bw (relative kidney weight) and in all animals at 1000 mg/kg (absolute). Macroscopically this was accompanied by thickened liver lobes and pale areas on the kidneys. All treated female groups showed hepatocellular cytoplasmic vacuolisation while the high-dose males had an increase in degenerative lesions in the renal cortex. Thickening of the stomach mucosa with concomitant lesions in the nonglandular part of the stomach, with some erosion, subacute inflammation and acanthosis were reported in middle- and high-dose animals (ref. 23).

The NOAEL derived was 160 mg/kg bw based on effects in liver and kidney.

In a study in rats to establish effects of linalool on drug metabolising enzymes, increased liver weight was reported on day 64. Rats received 500 mg/kg bw linalool by gavage during 64 days. Microsomal protein was increased from day 20 onwards. Cytochrome P450 and cytochrome b5 showed biphasic response (initial depression followed by an increase).

4-Methylumbelliferone glucuronyl transferase increased to 150% by day 64. Alcohol dehydrogenase was initially depressed by 33% on day 3, then increased by 36% on day 7, before regaining normal values by day 14 and thereafter (ref. 21).

The observed effects of linalool are interpreted to represent a physiological adaptation to exposure and not toxicity in a strict sense.

Inhalation

No data available

Conclusion

The 28-day rat study was selected to be the key study. Based on the data present it can be concluded that effects of linalool are limited to the liver and to the kidney. A NOAEL of 160 mg/kg bw could be derived.

Observations in man

It is reported that repeated skin exposure to Bergamot oil (45% linalyl acetate) leads to nervous and digestive symptoms. Sensitive persons may develop photodermatitis, eczema and pigment changes (ref. 9).

3.1.6 Mutagenicity

In vitro Studies

An Ames test was performed with linalyl acetate in Salmonella strains TA97, TA98, TA100, TA1535 and TA102 with and without metabolic activation. Linalyl acetate was found to be not mutagenic in this assay (ref. 12).

In a chromosome aberration test with human lymphocytes linalyl acetate did not induce aberrations, both with and without S9-mix (ref. 19).

In vivo Studies

No *in vivo* data on mutagenicity with linalyl acetate were available.

Conclusion

Linalyl acetate does not induce gene mutations or chromosomal effects *in vitro*.

3.1.7 Carcinogenicity

Linalyl acetate was tested in a carcinogenicity assay in mice (A/He). Animals received 3 times per week an injection (intra peritoneal (i.p.)) with linalyl acetate at 200 or 1000 mg/kg (total exposure 8 weeks, total 24 injections). Total observation time was 24 weeks. Control animals were included in the study design. Full histopathology was performed on the lungs only. Liver, kidney, spleen, thymus, intestine and salivary and endocrine glands were investigated macroscopically. No increase in tumour bearing animals was reported (ref. 8).

In Swiss mice (ICR/Ha) dermal coapplication of linalyl acetate (3 mg/ in acetone) with benzo[a]pyrene did slightly increase the number of skin papillomas and carcinomas compared to benzo[a]pyrene controls (ref.27).

Conclusion

Due to the limited information and shortcomings of the studies (duration of exposure is considered to be short and the intraperitoneal route seems to be not the most suitable route of exposure for linalyl acetate), no conclusions can be drawn from these studies.

3.1.8 Toxicity for Reproduction

Studies in Animals

An essential oil of coriander containing 72.9% of natural linalool was administered to 10 female rats/treatment from day 7 before mating with untreated males until day 4 after parturition. The dosage levels were 250, 500 and 1000 mg/kg bw. No effects on female fertility became apparent. All treated females showed an increased body weight gain and food consumption during gestation compared to control animals. In the high dose group a decrease of body weight and food consumption was seen during the pre-mating period. Salivation was apparent in females at 500 and 1000 mg/kg. One or two females at the high dose group developed ataxia and/or decreased motor activity. At 1000 mg/kg bw females delivered significantly less live pups than in the other dose groups. During the lactation period pup morbidity and mortality was increased (ref. 22). The NOAEL for maternal toxicity based on clinical signs and effects on body weight and food consumption was 500 mg/kg bw (equivalent to 464 mg/kg bw linalyl acetate). The NOAEL on reproduction toxicity and developmental toxicity is 500 mg/kg bw (equivalent to 464 mg/kg bw linalyl acetate), based on the decreased litter size at birth and morbidity/mortality thereafter.

From the 28-day repeated dose toxicity study with the same essential oil of coriander (ref. 23), no remarkable effects on the primary reproductive organs in both females (ovaries and uteri) and males (testes and epididymides) were noted in any animal from any dosage group up to 1000 mg/kg bw, both macroscopically at dissection and also microscopically during histopathology of every single (10 male, 10 female) high-dose animal.

Observations in Humans

No data available.

Conclusion

Based on the available data it can be concluded that linalyl acetate has a NOAEL for reproduction/developmental toxicity of 500 mg/kg bw (equivalent to 464 mg/kg bw linalyl acetate). At 1000 mg/kg bw maternal toxicity was observed.

3.2 Initial Assessment for Human Health

Linalyl acetate is of very low acute toxicity to mammals, the acute oral LD₅₀ is >13,360 mg/kg, while the inhalation LC₅₀ is >2740 mg/m³. Linalyl acetate has no or a very low potential to irritate the human skin. No information on possible eye irritation is available. Based on the use of linalyl acetate in cosmetics (constituent of perfumes) it is doubted that the substance has significant sensitising properties.

Linalyl acetate is an ester that is expected to be hydrolysed to linalool and acetic acid in the gastro-intestinal tract. The main effects of the hydrolysis product, linalool (72.9%), in a 28-day oral rat study were increased liver and kidney weight, thickened liver lobes and pale areas on the kidneys and in females only hepatocellular cytoplasmic vacuolisation. Other findings were related to local irritation of the gastro-intestinal tract. Based on the effects on liver and kidney a NOAEL of 160 mg/kg bw/d was derived for linalool (equivalent to 148 mg/kg bw/d linalyl acetate). In this study no effects on male and female gonads were found.

Linalool (72.9%) was tested in a reproduction screening test (non-OECD). The NOAEL for maternal toxicity based on clinical signs and effects on body weight and food consumption was 500 mg/kg bw/d for linalool (equivalent to 464 mg/kg bw/d linalyl acetate). The NOAEL on reproduction toxicity and developmental toxicity is 500 mg/kg bw/d (equivalent to 464 mg/kg bw/d linalyl acetate) based on the decreased litter size at birth and pup morbidity/mortality thereafter.

Linalyl acetate does not induce gene mutations or chromosomal effects *in vitro*.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Data are available on the acute toxicity of linalyl acetate to fish, daphnids and algae.

Fish and Invertebrates

In a 96-hour flow-through test with carps (*Cyprinus carpio*), fish were exposed to 10, 18, 32, 56 and 100 mg/L (nominal concentrations). Mean measured concentrations were 7.9, 12.3, 20.1, 27.3 and 27.2 mg/L. At the highest concentrations (32 mg/L and above) all fish died and a precipitate was observed. At all concentrations hypoactive swimming behaviour, loss of equilibrium and/or immobility was observed. A 96-hour LC₅₀ of 11 mg/L was derived based on measured concentrations (ref.13).

A calculation with ECOSAR yields a 96-hour LC₅₀ value of 2.86 mg/L

For *Daphnia magna* a 48-hour EC₅₀ was calculated by the reviewer with the Spearman-Kärber method. The EC₅₀ derived, based on nominal concentrations in a static test, was 15 mg/L (mean measured 6.2 mg/L). In accordance with the expectation of hydrolysis, the measured concentration after 48 hours was below the detection limit (except in the highest concentration). As the EC₅₀ for linalool is reported to be 59 mg/L (SIAR on linalool), the toxicity observed can not be attributed to the hydrolysis product only. The EC₅₀ value based on mean measured concentrations is not very reliable, since this mean concentration overestimates exposure to linalyl acetate (t_{1/2} hydrolysis <24 hours). However, the alternative of using the nominal concentrations to derive an EC₅₀ is even less realistic (ref. 5).

A calculation with ECOSAR yields a 48-hour EC₅₀ value of 2.91 mg/L

Conclusions:

Linalyl acetate is moderately toxic to fish and toxic to daphnia. All values are based on measurements of the parent compound, which is expected to hydrolyse very quickly. The ECOSAR prediction provides an indication for a possible higher toxicity than found in the described tests. The 96-hour LC₅₀ in fish is 11 mg/L. The 48-hour EC₅₀ for daphnia is 6.2 mg/L (based on mean measured concentrations).

Algae

In a test with algae (*Scenedesmus subspicatus*, 72-hours exposure), a reduction of biomass was seen at nominal concentrations of 4.4 mg/L and above. The 72-hour E_bC₅₀ was 16 mg/L and the 72-hour E_rC₅₀ was 62 mg/L, both based on nominal concentrations. Analyses showed test concentrations below the limit of detection for all treatments after 72 hours, which is again expected in view of hydrolytic instability of the compound. The EC₅₀ for linalool is >1000 mg/L (SIAR linalool). Based on the considerations mentioned regarding the daphnia study (see above), the EC₅₀ were based on mean measured concentrations, E_bC₅₀ 4.2 mg/L and E_rC₅₀ 16 mg/L (ref.6).

The NOEC for effects on algal growth based on the area under the growth curve is <4.4 mg/L (measured concentration <1.2 mg/L) (ref. 6).

A calculation with ECOSAR yields a 96-hour EC₅₀ value of 0.244 mg/L

Conclusions:

Based on the information available linalyl acetate is toxic to algae (72-hour EC_{50} 4.2 mg/L). The ECOSAR prediction provides an indication for a possible higher toxicity than found in the described test.

Microorganisms

In a test with activated sludge (according to OECD guideline 209) the EC_{50} for the inhibition of micro-organisms was determined to be 415 mg/L. The substance was dissolved in ethanol, which explains that the EC_{50} value is above water solubility of the EC_{50} (ref. 4).

Conclusion:

Linalyl acetate is of low toxicity towards aquatic micro-organisms.

Determination of PNEC aqua

Data are available from short term tests at 3 trophic levels. Based on the lowest value (calculated for algae E_bC_{50} 4.2 mg/L) and applying an assessment factor of 100 in accordance with the OECD guidance the resultant $PNEC_{aqua}$ is 0.042 mg/L. The same assessment factor would be recommended using the EU TGD as no significant difference in sensitivity between species tested appears to be present.

Conclusions:

Linalylacetate is of moderate hazard to the aquatic environment with a $PNEC_{aqua}$ of 0.042 mg/L.

4.2 Terrestrial and Sediment Effects

No data available.

4.3 Other Environmental Effects

Based on the very low $\log K_{ow}$ of 3.9, linalyl acetate may accumulate (BCF of 412). However, in view of the rapid hydrolysis of the substance it is unlikely that significant uptake may occur.

4.4 Initial Assessment for the Environment

Linalyl acetate is a liquid with a vapour pressure of 0.61 Pa (at 25°C), a water solubility of 30 mg/L and a $\log K_{ow}$ of 3.9 (measured). It has a calculated half-life for photo-oxidation of 1.1 hours.

Linalyl acetate will partition primarily to water (Mackay level III modelling). In a hydrolysis study linalyl acetate was found to disappear from the test medium within 2.4 hours at pH 4, 7 and 9 (at 50°C) ($t_{1/2} < 24$ hours at 20°C). Hydrolysis products are linalool and acetic acid.

Linalyl acetate is readily biodegradable. Based on the $\log K_{ow}$ a BCF of 412 was calculated. Linalyl acetate has potential for sorption to soil (predicted $\log K_{oc}$ 2.9), however, in view of the rapid hydrolysis of the substance it is unlikely that significant uptake or sorption may occur.

Linalyl acetate is toxic to fish and daphnia. All values are based on measurements of the parent compound, which is expected to hydrolyse very quickly. The 96-hour LC_{50} in fish is 11 mg/L. The 48-hour EC_{50} for daphnia is 6.2 mg/L. In a test with algae (*Scenedesmus subspicatus*, 72-hours exposure), a reduction of biomass was seen at 1.2 mg/L and above. The 72-hour E_bC_{50} was 4.2 mg/L, the E_rC_{50} was 16 mg/L).

The EC₅₀ for the inhibition of micro-organisms is 415 mg/L (ethanol was used as dispersant).

5 RECOMMENDATIONS

The compound is currently of low priority for further work.

Data on all SIDS endpoints are available and are in general of good quality.

Search criteria

Only original literature was included in this SIAR, except for internal review reports by the manufacturer of linalyl acetate (ref. 1, 2, and 18). The studies in these rather old reports are not expected to be identified as a key study in view of their age. In general good quality studies were available for all SIDS endpoints.

Key studies are studies with the highest reliability/adequacy. If several studies showed comparable reliability/adequacy, the study with the lowest LC/LD/EC₅₀ or NOEC/ NOAEL was indicated as the key study.

The following sources were investigated:

Company substance information: older test reports, safety datasheets, information about production and use, specifications etc.

Handbooks and original literature cited therein: CRC Handbook of Chemistry and Physics, Merck Index, Patty Industrial Hygiene and Toxicology, Sax & Lewis Dangerous Properties of Industrial Materials, Ullmann Enzyklopädie der technischen Chemie.

Databases: RTECS, DIMDI.

Also some calculated values are given obtained using Syracuse prediction software (Epiwin) or as indicated in the text.

In addition Medline and Toxline were searched under the CAS number 115-95-7 and the name linalyl acetate in January 2002.

6 REFERENCES

	Author	Title	Performing laboratory / source	Year
1	-	Linalyl acetate MSDS	Roche	1996
2	-	Internal report	Roche	1979
3	Schmiedel U.	Determination of the partition coefficient of linalylacetate in n octanol/water	RCC UmweltChemie AG	
4	Calame R, Ronchi W.	Linalyl acetate activated sludge, respiration inhibition test	Givaudan	1989
5	Grade R.	Report on the acute toxicity of linalylacetat on daphnia (<i>Daphnia magna</i> Strauss 1820)	Ciba-Geigy Ltd.	1993
6	Grade R.	Report on the growth inhibition test of linalylacetat to green algae (<i>Scenedesmus subspicatus</i>)	Ciba-Geigy Ltd.	1994
7	Rudio J.	Linalyl acetate synthetic determination of the ready biodegradability	Givaudan	1991
8	Stoner G., Shimkin M., Kniazeff A., et al	Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A Mice	Cancer Research 33, 3069-3085	1973
9	Gosselin R., Hodge H., Smith R., et al.	Clinical toxicology of commercial products Acute poisoning	Oil of Bergamot, 154	1981
10	FAO	Toxicological evaluation of some flavouring substances and non-nutrtive sweetening agents	FAO Nutrition Meetings Report Series No. 44A	1967
11	Motoyoshi K., Toyoshima Y, Sato M., et al.	Comparative studies on the irritancy of oils and synthetic perfumes to the skin of rabbit, guinea pig, rat, miniature swine and man	Cosm. Toil. 94: 41-48	1979
12	Kirchner S.	Mutagenicity evaluation of Ro-8282/000 in the Ames test	Roche	1998
13	Bogers M.	96-hour acute toxicity study with linalylacetate (flow-through)	NOTOX	1998
14	Jüttner F.	Flavour compounds in weakly polluted rivers as a means to differentiate pollution sources	Wat. Sci. Tech. 25 (2): 155-164	1992

15	Weidenhamer J., Macias F., Fischer N., et al.	Just how insoluble are monoterpenes?	J. Chem. Ecology 19 (8):1799-1807	1993
16	Buchbauer G., Jirovetz L., Jäger W.	Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation	Z. Naturforsch. 46c: 1067-1072	1991
17	Clark B., Chamblee T., Iacobucci G.	Micellar-induced selectivity and rate enhancement in the acid-catalyzed cyclization and rearrangement of monoterpenes. The solvolysis of linalyl and geranyl acetates	J. Org. Chem. 54 (5):1032-1036	1989
18	Bauer D.	Occupational and environmental health hazard review: linalylacetate (Ro 02-8282)	Roche	1981
19	Bertens A.	Evaluation of the ability of linalylacetate to induce chromosome aberrations in cultured peripheral human lymphocytes	NOTOX	2000
20	Lewis RJ Sr.		Sax's Dangerous properties of industrial materials, Ninth edition: 2051.	1996
21	Parke DV	Effect of linalool on hepatic drug-metabolizing enzymes in the rat.	Biochem Soc Trans 2: 615-618.	1974
22	Hoberman A.,	Reproductive and developmental toxicity screening test of B10 administered orally via gavage to Crl:CD(SD)BR female rats	Lorillard Inc.	1989
23	Serota D.	28-day oral toxicity study in rats, compound B10.	Lorillard Inc.	1990
24	Brekelmans M.	Determination of the hydrolysis of linalyl acetate as a function of pH	F. Hoffmann-La Roche Ltd.	2001
25	Ghelardini C., et al,	Local anaesthetic activity of the essential oil of <i>Lavandula angustifolia</i>		
26	Ceschel G., et al.	<i>In vitro</i> permeation through porcine buccal mucosa of Salvia desoleana Atzei & Picci essential oil from topical formulations	Intern. J. Pharmaceutics 195: 171-177	2000
27	Van Duuren B., et al.	Cocarcinogenesis studies on mouse skin and inhibition of tumor induction	J. Nat. Cancer Inst. 46(5): 1039-1044	1971

28	Brandão F.	Occupational allergy to lavender oil	Contact Dermatitis 15(4):249-250	1986
29	NIOSH	Health Hazard Evaluation Report	HETA 83-006-1379	1983
30		The Merck Index 12 th Ed vs 12.3		2000
31	Jenner P., et al.	Food flavourings and compounds of related structure I. acute oral toxicity	Fd Cosmet. Toxicol. 2:327-343	1964
32	Ishidate M., et al.	Primary mutagenicity screening of food additives currently used in Japan*	Fd Cosmet. Toxicol. 22:623-636	1984
33	Florin I., et al.	Screening of tobacco smoke constituents for mutagenicity using the Ames' test*	Toxicology 15:219-232	1980
34	Hall	Unpublished report from McCormick & Co. Inc. to the flavor and extracts manufacturers' association of the United States	McCormick & Co. Inc. to the flavor and extracts manufacturers' association of the United States, Washington DC, US	1979
35	Oda et al.	Mutagenicity of food flavores in bacteria **	Shokuhin Eisi Hen, 9, 177-181	1978
36	Heck et al.	An evaluation of food flavoring ingredients in a genetic toxicity screening battery	Toxicologist 9, 257	1989
37	Oser	Unpublished report**		1967

* Reference was not included because it did not provide information on linalyl acetate.

** Reference was not retrievable.

APPENDIX A**Results of the Epiwin model vs 3.10**

SMILES : O=C(OC(C=C)(CCC=C(C)C)C)C

CHEM : 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate

CAS NUM: 000115-95-7

MOL FOR: C12 H20 O2

MOL WT : 196.29

----- EPI SUMMARY (v3.10) -----

Physical Property Inputs:

Water Solubility (mg/L): 30

Vapor Pressure (mm Hg) : -----

Henry LC (atm-m3/mole) : -----

Log Kow (octanol-water): 3.90

Boiling Point (deg C) : -----

Melting Point (deg C) : -----

Log Octanol-Water Partition Coef (SRC):

Log Kow (KOWWIN v1.66 estimate) = 4.39

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPWIN v1.40):

Boiling Pt (deg C): 228.95 (Adapted Stein & Brown method)

Melting Pt (deg C): -2.09 (Mean or Weighted MP)

VP(mm Hg,25 deg C): 0.131 (Mean VP of Antoine & Grain methods)

BP (exp database): 220 deg C

VP (exp database): 1.11E-01 mm Hg at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.40):

Water Solubility at 25 deg C (mg/L): 21.35

log Kow used: 3.90 (user entered)

no-melting pt equation used

ECOSAR Class Program (ECOSAR v0.99g):

Class(es) found:

Esters

Henrys Law Constant (25 deg C) [HENRYWIN v3.10]:

Bond Method : 1.74E-003 atm-m3/mole

Group Method: Incomplete

Henrys LC [VP/WSol estimate using EPI values]: 1.128E-003 atm-m3/mole

Probability of Rapid Biodegradation (BIOWIN v4.00):

Linear Model : 0.6443

Non-Linear Model : 0.9294

Expert Survey Biodegradation Results:

Ultimate Survey Model: 2.6935 (weeks-months)

Primary Survey Model : 3.6401 (days-weeks)

Readily Biodegradable Probability (MITI Model):

Linear Model : 0.6648

Non-Linear Model : 0.6802

Atmospheric Oxidation (25 deg C) [AopWin v1.90]:

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant = 116.2433 E-12 cm³/molecule-secHalf-Life = 0.092 Days (12-hr day; 1.5E6 OH/cm³)

Half-Life = 1.104 Hrs

Ozone Reaction:

OVERALL Ozone Rate Constant = 43.174999 E-17 cm³/molecule-secHalf-Life = 0.027 Days (at 7E11 mol/cm³)

Half-Life = 38.222 Min

Reaction With Nitrate Radicals May Be Important!

Soil Adsorption Coefficient (PCKOCWIN v1.66):

Koc : 517.9

Log Koc: 2.714

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v1.67]:

Total Kb for pH > 8 at 25 deg C : 4.610E-002 L/mol-sec

Kb Half-Life at pH 8: 174.013 days

Kb Half-Life at pH 7: 4.764 years

BCF Estimate from Log Kow (BCFWIN v2.14):

Log BCF = 2.303 (BCF = 200.9)

log Kow used: 3.90 (user entered)

Volatilization from Water:

Henry LC: 0.00174 atm-m³/mole (estimated by Bond SAR Method)

Half-Life from Model River: 1.901 hours

Half-Life from Model Lake : 138.2 hours (5.759 days)

Removal In Wastewater Treatment:

Total removal: 53.44 percent

Total biodegradation: 0.20 percent

Total sludge adsorption: 21.28 percent

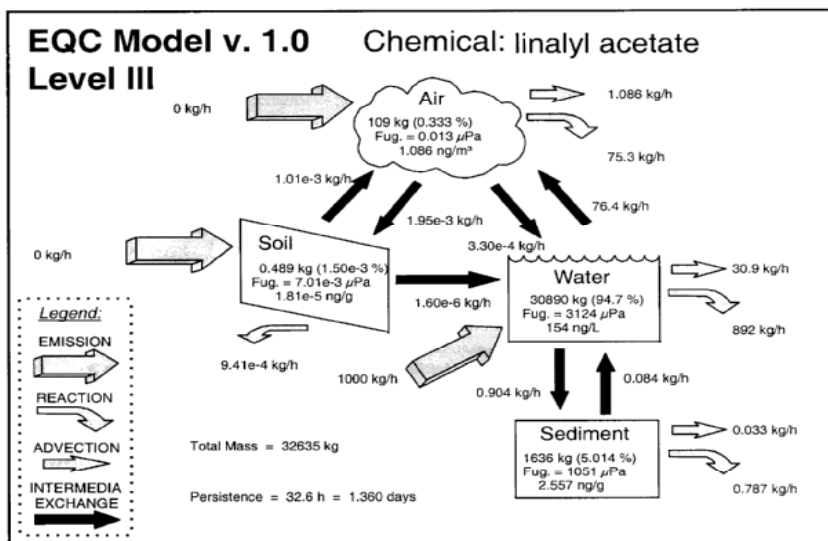
Total to Air: 31.97 percent

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.139	0.494	0
Water	89.9	900	1000
Soil	0.00313	900	0
Sediment	9.97	3.6e+003	0
Persistence Time: 280 hr			

APPENDIX B

EQC model



AM

**SIDS Dossier on the HPV Chemical
Linalyl acetate**

CAS no. 115-95-7

Sponsor Country: Switzerland

Date of submission to OECD:

List of Abbreviations

^a	Absolute to body weight
-	Absent
+	Present
a.i.	Active ingredient
ALP	Alkaline phosphatase
BCF	Bioconcentration factor
CFU	Colony forming units
d	Decrease
dc	Decrease (significant)
DOC	Dissolved organic carbon
DR	Dose related
F	Female
FID	Flame ionisation detection
GC	Gas chromatography
i	Increase
ic	Increase (significant)
LC	Liquid chromatography
LOD	Limit of detection
M	Male
MCV	Mean corpuscular volume
MS	Mass spectrometry
N/A	Not applicable
QC	Quality control
^r	Relative to body weight
TLC	Thin layer chromatography
UV	Ultra violet
WHC	Water holding capacity

1.01. Chemical identity

CAS No. : 115-95-7

OECD name : Linalyl acetate

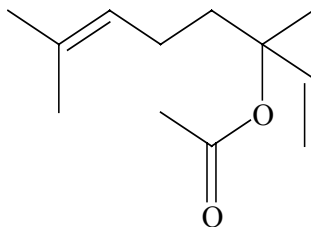
Chemical/IUPAC name : 1,5-dimethyl-1-vinylhex-4-enyl acetate

EINECS number : 2041164

Molecular formula : C₁₂H₂₀O₂

Molecular weight : 196.29

Structural formula :



1.02. OECD information

Sponsor country : Switzerland

Lead organisation : Dr. Georg Karlaganis
Swiss Agency for the Environment, Forests and Landscape
CH-3003 Berne, Switzerland
e-mail: georg.karlaganis@buwal.admin.ch

Name of responder (leader of consortium) : This substance is evaluated under the OECD HPV programme

1.1. General substance information

Type of substance : Organic

Physical state : Liquid

Purity : >96%

1.2. Impurities

Dihydrolinalool, dehydrolinalool, tetrahydrolinalylacetate, linalool

1.3. Additives

1.4. Synonyms

Linalyl acetate, linalool acetate, acetic acid linalool ester

1.5. Quantity

Yearly around 1000-5000 tonnes of linalyl acetate are produced.

1.6. Use pattern

The substance is used as an intermediate and found in soaps, cleaning products and cosmetics (perfume) It is a component of oil paint, is used in extracts and as a substitute for petitgrain oil. Linalyl acetate is a food additive.

1.7. Sources of exposure

There is a potential for occupational exposure during production/formulation, used not only in closed systems.

Consumers using oil paint and perfume may be exposed orally and dermally.

There is potential exposure to the aquatic compartment arising from the production/formulation and consumer use (cosmetics) of this substance.

1.8. Additional information

2.1. Melting Point

Title Safety Data Sheet Linalyl Acetate
Date of report 2000
GLP No data
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result <0 °C
Reliability 4 Secondary literature

Title Internal report
Date of report 1979
GLP No
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result <0 °C
Reliability 4 Secondary literature

2.2. Boiling Point

Title The Merck Index
Date of report 2000
GLP No data
Reference 30
Test substance Linalyl acetate
Test method Not indicated
Result 220 °C
Reliability 2 Handbook data

Title Safety Data Sheet Linalyl Acetate
Date of report 2000
GLP No data
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result 220 °C
Reliability 4 Secondary literature

Title Internal report
Date of report 1979
GLP No
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result 219.7 °C at normal atmospheric pressure
Reliability 4 Secondary literature

Title Sax's Dangerous properties of industrial materials, Ninth edition
Date of report 1996
GLP No data.
Reference 20
Test substance Linalyl acetate, purity not indicated
Test method Not indicated
Result 108-110 °C
Reliability 4 Handbook data

2.3. Density (relative density)

Title Safety Data Sheet Linalyl Acetate
Date of report 2000
GLP No data
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result 0.895 g/cm³ at 20°C
Reliability 4 Secondary literature

Title Internal report
Date of report 1979
GLP No
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result 0.910 g/cm³ at 20°C
0.842 g/cm³ at 100°C
Reliability 4 Secondary literature

Title Sax's Dangerous properties of industrial materials, Ninth edition
Date of report 1996
GLP No data.
Reference 20
Test substance Linalyl acetate, purity not indicated
Test method Not indicated
Result 0.898-0.914
Reliability 4 Handbook data

2.4. Vapour Pressure

Title Safety Data Sheet Linalyl Acetate
Date of report 2000
GLP No data
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result 0.006087 hPa at 20°C
322.227 hPa at 100°C
Reliability 4 Secondary literature

Title Internal report
Date of report 1979
GLP No
Reference 1
Test substance Linalyl acetate
Test method Not indicated
Result 0.006087 hPa at 20°C
322.227 hPa at 100°C
Reliability 4 Secondary literature

2.5. Partition Coefficient

Title	Determination of the partition coefficient of linalylacetat in n-octanol/water
Date of report	July 17, 1995.
GLP	Yes.
Reference	3.
Test substance	Linalylacetat, purity 97.0%.
Guidelines	92/69/EEC, A.8; OECD 107.
Procedure	Octanol and water were mutually saturated with each other. 25 mL of a stock solution of linalylacetat in octanol (corresponding to 44 mg of linalylacetat) was transferred to a glass vessel. Additionally 15 mL octanol and 40 mL water, 25 mL octanol and 25 mL water or no octanol and 50 mL water was added. The vessels were sealed and subsequently shaken for 5 min at 25 rpm, 20-25°C. The mixtures were centrifuged (2000 rpm, 5 min), each phase was sampled in duplicate and analysed by GC-FID after dilution with acetone (octanol-phase) or extraction with n-pentane (water phase) using calibration curves.
Findings	QCs for analytical method in water phase fortified at 1.0, 2.5 and 3.5 mg/L were 103-111% of nominal (note 1), method validation with known amount of test substance 98-104%.

<i>treatment</i>	<i>A1</i>	<i>A2</i>	<i>B1</i>	<i>B2</i>	<i>C1</i>	<i>C2</i>
amount of test substance [mg]	44	44	44	44	44	44
volume of octanol phase [mL]	40	40	50	50	25	25
volume of water phase [mL]	40	40	25	25	50	50
concentration in octanol phase [mg/L]	1164	1181	919	958	1981	2014
concentration in aqueous phase [mg/L]	0.14	0.14	0.11	0.06	0.21	0.23
recovery [%]	106	108	105	109	113	115
P _{ow}	8281	8622	8096	16814*	9416	8922
log(P _{ow})	3.9	3.9	3.9	4.2	4.0	4.0
average P _{ow}	8452		8096		9169	
average P _{ow} ± RSD [%]	8668 ± 6.1%					
¹⁰ log(P _{ow})	3.9					

* Outlier, not included in calculation

Conclusion $^{10}\log(P_{ow})$ 3.9.

- Rev. note**
- For the analytical method in the water phase QCs were included at high fortification rates (1.0-3.5 mg/L) in relation to the concentrations measured in n-pentane during the study (0.26-1.02 mg/L). Since calibration curves were drawn using duplicate samples of concentrations ranging between 0.1 and 10 mg/L, the method is still acceptable.
 - Temperature was reported to be within the range 20-25°C and pH was not reported. A constant temperature and pH are necessary for a stable system. Since the $\log(P_{ow})$ values were within a range of ± 0.3 , the stability of the system is guaranteed. The results of the replicate C2 were not included in the calculation of the $\log(P_{ow})$ because they are not in accordance with the other 5 values found. Probably the concentration in the water phase was incorrect.

Reliability 1

Critical study for SIDS endpoint

2.6.1. Water Solubility and Dissociation Constant

Title	Report on the hydrolysis of linalylacetate and ethylformate by artificial gastrointestinal juices
Date of report	April 1979.
GLP	No.
Reference	34.
Test substance	Linalyl acetate, purity not indicated.
Test method	Not specified.
Procedure	Water solubility was measured at room temperature.
Results	Water solubility 30 mg/L Little amounts of linalool were present (determined by GC)
Rev. note	No further details were available.
Reliability	4.

Title	Epiwin vs 3.10
Date of report	2002
GLP	
Reference	Epiwin vs 3.10
Test substance	Linalyl acetate
Test method	Calculation based on Kow of 3.9
Result	21.35 mg/L at 25 °C
Reliability	4

Title	Safety Data Sheet Linalyl Acetate
Date of report	2000
GLP	No data
Reference	1
Test substance	Linalyl acetate
Test method	Not indicated
Result	< 1 g/L at 20 °C
Reliability	4 Secondary literature

Title	Just how insoluble are monoterpenes?
Date of report	1993.
GLP	No.
Reference	15.
Test substance	Linalyl acetate, purity not indicated.
Test method	Not specified.
Procedure	A saturated aqueous solution was prepared by adding an excess linalyl acetate to 1.5 mL water in test vials. The vials were sealed and sonicated for 30 min in a water bath (25-30°C). The vials were equilibrated for three days at ambient temperatures. Analyses were performed by GC-FID and quantification was based on peak area.
Conclusion	Water solubility linalyl acetate <10 mg/L.
Rev. note	- Four important remarks should be made about the test, partly due to the limited description of the test available in the report: - Nothing was said about temperature control during the equilibration (OECD 30°C). The test vials were reported to be equilibrated at ambient temperatures. Because temperature is an important factor in the water solubility of the test substance and therefore should be kept constant, the study reliability is lowered. - The pH is not reported. Therefore it can not be excluded that esters were hydrolysed in water. - The purity of the test substance was not specified and impurities could have a disturbing effect on the water solubility. - Sonication and equilibration times should be long enough to be sure the measured water concentration equals the solubility of the test substance. Since no variations were made in sonication and equilibration times, the measured concentrations were not proved to be the actual maximum water solubility concentration. <i>Minor remark.</i> Number of replicates not indicated.
Reliability	3 Secondary literature (note 1).

2.6.2. Solubility in organic solvents

Title	Safety Data Sheet Linalyl Acetate
Date of report	2000
GLP	No data
Reference	1
Test substance	Linalyl acetate
Test method	Not indicated
Result	Soluble in ethanol and diethyl ether
Reliability	4 Secondary literature

2.7 Flash Point

Title	Safety Data Sheet Linalyl Acetate
Date of report	2000
GLP	No data
Reference	1
Test substance	Linalyl acetate
Test method	Not indicated
Result	84 °C
Reliability	4 Secondary literature

Title	Sax's Dangerous properties of industrial materials, Ninth edition
Date of report	1996
GLP	No data.
Reference	20
Test substance	Linalyl acetate, purity not indicated
Test method	Not indicated
Result	185 °F (~85 °C)
Reliability	4 Handbook data

3.1. Stability

A Photodegradation

Reference	Epiwin vs 3.10
Test substance	Linalyl acetate
Test method	Calculation with AOPWIN vs1.9, based on: SMILES : O=C(OC(C=C)(CCC=C(C)C)C)C CHEM : 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate MOL FOR: C12 H20 O2 MOL WT : 196.29
Result	----- SUMMARY (AOP v1.90): HYDROXYL RADICALS ----- Hydrogen Abstraction = 3.0433 E-12 cm³/molecule-sec Reaction with N, S and -OH = 0.0000 E-12 cm³/molecule-sec Addition to Triple Bonds = 0.0000 E-12 cm³/molecule-sec Addition to Olefinic Bonds = 113.2000 E-12 cm³/molecule-sec Addition to Aromatic Rings = 0.0000 E-12 cm³/molecule-sec Addition to Fused Rings = 0.0000 E-12 cm³/molecule-sec OVERALL OH Rate Constant = 116.2433 E-12 cm³/molecule-sec HALF-LIFE = 0.092 Days (12-hr day; 1.5E6 OH/cm³) HALF-LIFE = 1.104 Hrs ----- SUMMARY (AOP v1.90): OZONE REACTION ----- OVERALL OZONE Rate Constant = 43.174999 E-17 cm³/molecule-sec HALF-LIFE = 0.027 Days (at 7E11 mol/cm³) HALF-LIFE = 38.222 Min
Reliability	4

B Stability in Water

Title	Determination of the hydrolysis of linalylacetate as a function of pH.																											
Date of report	2001 (finalisation date).																											
GLP	Yes.																											
Reference	24.																											
Test substance	Linalyl acetate, purity 97.6%..																											
Test method	EEC-Directive 92/69, C.7.																											
Procedure	Test system: A sterile 0.05 M acetate buffer pH 4, a sterile 0.05 M phosphate buffer pH 7 and a sterile 0.05 M borate buffer pH 9. Method: 400 µL of a linalyl acetate solution (1080 mg/L acetonitrile) was added to 50 mL of each of the buffer solutions under a nitrogen atmosphere. The test solutions were incubated in duplicate at 50°C in the dark. The concentration of linalyl acetate in n-hexane extract was analysed by GC-MS immediately after preparation and after 2.4 hours of incubation. The pH was measured at room temperature at the beginning and end of the test.																											
Results	<table border="1"> <thead> <tr> <th>pH</th><th>Measured pH</th><th>Sampling time (hours)</th><th>Rel. concentration test substance (%)</th></tr> </thead> <tbody> <tr> <td rowspan="2">4</td><td>4.0</td><td>0</td><td>100</td></tr> <tr> <td>4.0</td><td>2.4</td><td>2</td></tr> <tr> <td rowspan="2">7</td><td>7.0</td><td>0</td><td>100</td></tr> <tr> <td>7.0</td><td>2.4</td><td>3</td></tr> <tr> <td rowspan="2">9</td><td>9.0</td><td>0</td><td>100</td></tr> <tr> <td>9.0</td><td>2.4</td><td>4</td></tr> </tbody> </table>	pH	Measured pH	Sampling time (hours)	Rel. concentration test substance (%)	4	4.0	0	100	4.0	2.4	2	7	7.0	0	100	7.0	2.4	3	9	9.0	0	100	9.0	2.4	4		
pH	Measured pH	Sampling time (hours)	Rel. concentration test substance (%)																									
4	4.0	0	100																									
	4.0	2.4	2																									
7	7.0	0	100																									
	7.0	2.4	3																									
9	9.0	0	100																									
	9.0	2.4	4																									
Conclusion	At each pH, a decrease in concentration > 50% was observed after 2.4 hours (half-life time at 25°C < 1 day). Hence it was concluded that linalyl acetate is hydrolytically unstable in aqueous solutions buffered at pH 4, pH 7 and pH 9.																											
Rev. note	1. The hydrolysis products, which are linalool and acetic acid, were not identified.																											
Reliability	1.																											

Critical study for SIDS endpoint

C Stability in Soil

3.2. Monitoring Data

Title	Flavour compounds in weakly polluted rivers as a means to differentiate pollution sources
Date of report	1992.
GLP	No.
Reference	14.
Test substance	Linalyl acetate, purity not relevant.
Test method	Not specified.
Test system	The occurrence of different Volatile Organic Compounds (VOCs) in eighth rivers and brooks of different origin (industry, agriculture, wood preserving, etc.) in South-West-Germany was investigated. 10 L samples were taken from the middle of the river. The mixture of VOCs from the sampled water was brought onto Tenax cartridges and subsequently a GC-MS analyses was performed. Reference standards were used to compare retention times and mass spectra.
Results	28 different mono- and sesquiterpenes were identified in the different rivers or brooks. Linalyl acetate was identified only in Stockacher Aach.
Conclusion	Linalyl acetate was found in one river.
Rev. note	No individual data were available to check the occurrence of linalyl acetate. The amount of linalyl acetate in Stockacher Aach was also not clear. The only conclusion that can be drawn from this study is that linalyl acetate can reach the aquatic environment.
Reliability	4 Secondary literature.

3.3 Transport and Distribution between Environmental Compartments

Title	Epiwin vs 3.10
Date of report	2002
GLP	
Reference	Epiwin vs 3.10
Test substance	Linalyl acetate
Test method	Calculation with Epiwin vs 3.10, based on: Chem Name : 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate Molecular Wt: 196.29 Henry's LC : 0.00174 atm-m3/mole (Henrywin program) Vapor Press : 0.131 mm Hg (Mpbpwin program) Log Kow : 3.9 (user-entered) Soil Koc : 3.26e+003 (calc by model)
	Emission to water only

Result	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)		
Air	0.139	0.494	0		
Water	89.9	900	1000		
Soil	0.00313	900	0		
Sediment	9.97	3.6e+003	0		
	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	4.84e-013	545	3.89	54.5	0.389
Water	1.11e-008	194	252	19.4	25.2
Soil	5.5e-014	0.00675	0	0.000675	0
Sediment	7.82e-009	5.37	0.558	0.537	0.0558

Persistence Time: 280 hr
Reaction Time: 376 hr
Advection Time: 1.09e+003 hr
Percent Reacted: 74.4
Percent Advected: 25.6

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):

Air: 0.4944
Water: 900
Soil: 900
Sediment: 3600
Biowin estimate: 2.693 (weeks-months)

Advection Times (hr):

Air: 100
Water: 1000
Sediment: 5e+004

Reliability 4

Title EQC v 1.0

Date of report 2002

GLP

Reference EQC v 1.0

Test substance Linalyl acetate

Test method Calculation:

Since the substance is soluble in water to some extent, the substance is considered to be a Type I substance.

Defaults:

T_{1/2} air: 1 hours, t_{1/2} water 24 hours, t_{1/2} soil 360 hours and t_{1/2} sediment 1440 hours

Physical chemical parameters:

Water solubility 21 mg/l, vapour pressure 0.6 Pa, Log Kow 3.9, melting point 0°C.

Emissions have been considered to water only.

Result Air: 0.3%

Soil: 0%

Water: 95%

Sediment: 5%

Reliability 4

3.4. Biodegradation

Title	Linalyl acetate synthetic Determination of the ready biodegradability.		
Date of report	February 25, 1991.		
GLP	No.		
Reference	7.		
Test substance	Linalyl acetate, purity 96.0%.		
Test method	OECD 301C.		
Test system	Treatment	Activated sludge (30 mg/L): prepared from soil of river bank, sludge of a municipal treatment plant and sludge of an industrial sewage plant.	
		- 2 flasks Treated (basal culture medium + activated sludge + linalyl acetate (100-102 mg/L));	
		- 1 flask Positive Control (basal culture medium + activated sludge + Aniline (92 mg/L));	
		- 1 flask Blank Control (basal culture medium + activated sludge).	
	Procedure	Incubation was performed for 28 days at 20°C. The oxygen consumption was determined daily with a volumetric respirometer.	
Results	Table below	Biodegradation values for linalyl acetate and positive control. Values are corrected for blank control values.	

Treatment	Mean % biodegradation [% of ThOD] on day:							
	1	4	7	10	14	18	22	28
Linalylacetate	4	33	54	62	68	71	73	75
Positive control	0	9.4	72	74	77	78	80	84

Conclusion	Ready biodegradability.			
Rev. note	1. Only limited information is available on the test. No information about the composition of the medium, pH during the test, light regime and shaking during the study. All of these are important factors in the degradation of the test substance. 2. In a curve showing the oxygen consumption over 28 days, beside a sludge control (30 mg/L) also a blank control (100 mg/L) is included. This is probably due to a mistake, because in the report no information is given about an abiotic control included in this study.			
Reliability	2 Limited information (note 1).			
Critical study for SIDS endpoint				

Title	Micellar-induced selectivity and rate enhancement in the acid-catalyzed cyclization and rearrangement of monoterpenes. The solvolysis of linalyl and geranyl acetates		
Date of report	1989.		
GLP	No.		
Reference	17.		
Test substance	Linalyl acetate, purity 96%.		
Test method	Not specified.		
Procedure	90 mL of a buffer with pH 3.1 (36 mM HCl; 15 mM sodiumcitrate) with or without 38 mM SDS was deaerated with Argon and treated with 5.4 mg linalyl acetate (final conc. 0.30 mM). Additionally a test was included without SDS, but with a deaerated mixture of buffer/methanol (44/1) treated with 5.4 mg linalyl acetate (final conc. 0.30 mM). Each sample was stirred, deaerated again, sonicated for 15 minutes and subsequently stirred at 25°C. Samples (10 mL) were taken at approximately 7, 25, 50 and 240 h and analysed by extraction/concentration/GC-MS (analysis in duplicate).		
Results	The half-life of linalyl acetate was ~7 h in all tests. For product distribution after 25 h: see table below.		

Products	Concentration product, mole %		
	HCl/citrate buffer*	HCl/citrate buffer/2% MeOH	SDS/HCl/citrate buffer
linalylacetate	8.8	9.0	7.1
linalool	51	46	54
a-terpineol	22	19	8.7
neryl acetate	2.4	2.3	0.90
geranyl acetate	4.2	4.1	1.6
nerol	2.8	2.6	4.2
geraniol	7.7	7.2	16
Total	99	91	92

* Mean of duplicate test.

Conclusion	$T_{1/2}$: ~7 h (pH 3); with SDS product ratios of acyclic/cyclic alcohols were higher during the first 50 h.
Rev. note	This test is not a standard investigation according to the OECD guidelines. The extreme conditions (pH 3) made it impossible to deduce a reliable DT_{50} -value under normal circumstances in the aquatic environment. Further no validation of the analysis method was reported.
Reliability	2

4.1. Acute Toxicity to fish

Title	96-hour acute toxicity study in carp with linalylacetate (flow-through)		
Date of report	December 23, 1998.		
GLP	Yes.		
Reference	13.		
Test substance	Linalyl acetate, purity 97.6%.		
Guideline	EEC 92/69, C.1; OECD 203.		
Stat. method	Maximum likelihood estimation method (Probit analysis; Finney, 1971)		
Test system	Species	Carp (<i>Cyprinus carpio</i>), length 28.8±1.5 mm.	
	No. of fish	7/treatment.	
	Concentrations	Nominal: 10, 18, 32, 56 and 100 mg/L (no vehicle), untreated control.	
	Test conditions	96-h flow-through test with ~10 renewals/24 h; 21-22°C in 38 L glass vessels containing 30 L of water (hardness 250 mg/L CaCO ₃); 16 h light; unfed; not aerated; loading 0.011 g/L/d.	
	Analysis	At 0 h and 24 h or 96 h for all treatments by extraction/dilution/GC-FID.	
	Phys. meas.	Daily in all replicates: overall ranges for pH 8.0-8.1; O ₂ 100-105%; temperature (only in control vessel) 21-22°C.	
	Observations	Mortality/symptoms at 2, 20, 44, 72 and 96 h.	
Results	Analysis	Mean measured concentrations for 10, 18, 32, 56 and 100 mg/L were respectively 79, 68, 63, 49 and 27% of nominal (note 1). At test concentrations 32, 56 and 100 mg/L a test substance film was observed.	
	Reference test	Method recovery of QCs fortified at 1, 10 and 100 mg/L: 88-108% (n = 3-6). A reference test was performed with pentachlorophenol about 11 months earlier; 24-h LC ₅₀ 0.18 mg/L, which is in accordance with historical control values.	

Biological results.

Parameter	Time [h]	Mean measured concentration [mg/L]					
		0	7.9	12.3	20.1	27.3	27.2
Mortality [%]	96	0	0	71	100	100	100
Symptoms*	0-96		+	+	+	+	+

* Symptoms included hypoactive swimming, loss of equilibrium and/or immobility.

Conclusion 96-h LC₅₀ 11 mg/L (95% CI, 10-14 mg/L).

- Rev. note**
- The analytical results at 24 h were not reported. For treatment levels in which all fish had died at 24 h (32, 56 and 100 mg/L) only the 0-h result was available. Since it is a flow-through test and the duplicate analytical results in the lowest two concentrations were comparable, the single values are acceptable in this test.
 - The measured concentrations were below the nominal, because of the maximum solubility of the test substance and because of hydrolysis of the test substance. In a static pretest a large amount of a hydrolysis product was found. In the flow-through test the same hydrolysis product was observed, but in significant lower amounts.
 - Minor remarks.* Food was withheld for 48 h before the start of the test (OECD 203, 24 h).

Reliability 1

Critical study for SIDS endpoint

4.2. Acute Toxicity to Aquatic Invertebrates (Daphnia)

Title	Report on the acute toxicity test of linalylacetat on <i>Daphnia</i> (<i>Daphnia magna</i> Straus 1820)		
Date of report	December 16, 1994.		
GLP	Yes.		
Reference	5.		
Test substance	Linalyl acetate, purity 97.1%.		
Test method	OECD 202, part I.		
Stat. method	Berkson, Jasa 48 (1953), 569-599.		
Test system	Species	<i>Daphnia magna</i> , ≤24 h old.	
	No. of daphnids	5/replicate, 4 replicates/treatment.	
	Concentrations	Nominal: 10, 18, 32, 58 and 100 mg/L (no vehicle), untreated controls.	
	Test conditions	Static without aeration in the dark; at 20±1°C in beakers, covered with glass	

Results	Analyses	watches and containing 100 mL of reconstituted water of hardness 240 mg/L (CaCO ₃), unfed.					
	Phys. meas.	At 0 and 48 h from all concentrations (composite sample of replicates) by extraction/GC-MS.					
	Observations	At 0 and 48 h in all treatments; overall ranges for pH 7.7-8.0; O ₂ 94-100%; temperature 21-22°C.					
	Analyses	Immobility at 24 and 48 h.					
	Biol. results	Measured concentrations were 82-88% of nominal at 0 h and ≤3.5% of nominal at 48 h. These values were corrected for a recovery rate of 96% (n=10, s _{rel} =2.1%); LOD 0.2 mg/L See table below.					

Parameter	Time [h]	Mean measured concentration [mg/L]					
		0	4.2	7.8	13.3	24.4	45.7
Immobility [%]	48	10	5	85	100	100	100

Conclusions 48-h EC₅₀ based on nominal concentrations calculated by the reviewer using untrimmed Spearman Karber was 15 mg/L (95% CI: 13-17 mg/L) ⇔ 6.2 mg/L (95% CI: 5.5-7.0 mg/L) corrected for mean measured concentrations (41-46% of nominal).

Rev. note Limited information was reported about the validation of the analytical method. Further test concentrations at 48 h were below the LOD for all concentrations except the highest one. The mean measured concentrations are probably still an overestimation of the mean exposure concentrations. Probably the concentrations of test substance disappeared in less than 48 h, which lowers the study reliability.

Reliability 2 Overestimation concentration.

Critical study for SIDS endpoint

4.3. Toxicity to Aquatic Plants

Title	Report on the growth inhibition test of linalylacetat to green algae (<i>Scenedesmus subspicatus</i>)						
Date of report	November 28, 1994.						
GLP	Yes.						
Reference	6.						
Test substance	Linalylacetat, purity 97.1%.						
Guideline	OECD 201.						
Stat. method	Logit model (Mc Cullagh & Nelder, 1983).						
Test system	Species	Green algae (<i>Scenedesmus subspicatus</i>).					
	Initial cell conc.	0.93 x 10 ⁴ cells/mL.					
	No. of replicates	3 per treatment, 6 for controls.					
	Concentrations	Nominal: 4.4, 9.6, 21, 46 and 100 mg/L (no vehicle); water treated controls.					
	Test conditions	100 mL flasks containing 50 mL of algal medium; temperature: 23±1°C; continuous illumination (8000 lux).					
	Analysis	At 0 and 72 h from all concentrations (composite sample of replicates) by extraction/GC-MS.					
	Phys. meas.	At 0 and 72 h in all treatments; overall ranges for pH 7.7-9.0; temperature 23±1°C.					
	Observations	Cell density at 24, 48 and 72 h by cell counting.					
Results	Analytical	Measured concentrations at 0 h were 49-55% of nominal for 4.4-21 mg/L, 35% and 76% of nominal for respectively 46 and 100 mg/L (mean recovery 26%). These values were corrected for a recovery rate of 96% (n=10, s _{rel} =2.1%, tested concentrations 4-102 mg/L); LOD 0.2 mg/L (note 1).					
	Biological	See table below.					

Parameter	Time [h]	Mean measured concentration [mg/L]					
		0	1.2	2.4	5.9	8.0*	37.8*
Mean cell density [10 ⁴ cells/mL]	24	5	5	4	4	2	1
	48	27	16	22	6	5	6
	72	144	107	120	28	10	11
<i>Inhibition [%]</i> - area under curve	0-72	0	29	17	78	91	91
- growth rate	0-72	0	6	4	32	54	51

* Precipitate at 72 h.

Conclusions E_bC₅₀ (0-72h): 16 mg/L based on nominal concentrations ⇔ 4.2 mg/L corrected for mean measured concentrations (26% of nominal);
E_rC₅₀ (0-72h): 62 mg/L based on nominal concentrations ⇔ 16 mg/L corrected for mean measured concentrations (26% of nominal);
72-hour NOEC <4.4 mg/L. based on nominal concentrations ⇔ <1.2 mg/L corrected for mean measured concentrations (26% of nominal).

Rev. note 1. The LOD was reported to be 0.7 mg/L in a separate analytical report, but 0.2 mg/L in the study report itself. Further test concentrations at 72 h were below the LOD for all concentrations. The mean measured concentrations are probably still an overestimation of the mean exposure concentrations. Probably the concentrations of test substance disappeared in less than 72 h from the test solutions. The study reliability is lowered because of this.
2. *Minor remarks.* The test flasks were not reported to been shaken during the study; this is necessary for the transfer of CO₂ formed during the test. Since the rises in pH of >1 unit were only seen in the two lowest test concentrations (1.1 unit), the algae in test flasks were probably kept in suspension. The pH rises of 1.1 unit were probably associated with strong cell growth, due to CO₂ depletion from test media.

Reliability 2 Overestimation concentration.
Critical study for SIDS endpoint

4.4. Toxicity to Bacteria

Title Linalyl acetate Activated sludge, respiration inhibition test

Date of report March 2, 1989 (completion study).

GLP No.

Reference 4.

Test substance Linalyl acetate, purity 96.7%.

Test method OECD 209, ISO 8192.

Procedure The test was performed in duplicate 500 mL flasks (Reference product single flask). To each flask were added synthetic sewage feed, a solution of linalyl acetate or reference product 2,5-dichlorophenol in ethanol and sludge. The test concentrations were 96, 304 and 912 mg/L (for the reference product 272 mg/L). Each flask was aerated at 20±2°C for 3 hours, after which period the O₂ consumption was measured (at low concentrations for 10 minutes).

Results See table below.

Test solution	Concentration [mg/L]	Respiration rate	Inhibition [%]
Control	0	1.212	-
Control	0	1.250	-
Linalylacetate-100	96	1.212	3
Linalylacetate-100	96	1.176	6
Linalylacetate-300	304	0.667	47
Linalylacetate-300	304	0.657	48
Linalylacetate-900	912	0.471	62
Linalylacetate-900	912	0.494	60
Reference product	272	0.375	70

Conclusions 3-h EC₅₀ 415 mg/L (calculated by the reviewer using 39% trimmed SPK).

Rev. note Only limited information was available. The information provided was essentially confined to the above included. There was no information about the composition of the synthetic sewage feed and also the source of the sludge was not reported. The study reliability is lowered.

Reliability 2 Limited information.

5.1. Pharmacokinetics

5.2. Acute Toxicity

Title Food flavourings and compounds of related structure I. acute oral toxicity
Date of report 1964
GLP No.
Reference 31.
Test substance Linalyl acetate, purity not indicated.
Guideline Not indicated.
Stat. method Lichfield & Wilcoxon
Test system **Species** Rat (Osborne-Mendel).
No. of animals 5/sex/treatment.
Dosage Oral by gavage not specified
Observations Mortality, clinical signs (frequency not indicated).
Results Deaths occurred between 4 hours and 4 days after treatment.
Clinical signs: Depression soon after treatment, coma, wet posterior.
Conclusions LD₅₀ 14,550 mg/kg bw (95% CI 12,300-17,1070 mg/kg bw)
Rev. note The report is limited to the above mentioned.
Reliability 4
Critical study for SIDS endpoint

Title Food flavourings and compounds of related structure I. acute oral toxicity
Date of report 1964
GLP No.
Reference 31.
Test substance Linalyl acetate, purity not indicated.
Guideline Not indicated.
Stat. method Lichfield & Wilcoxon
Test system **Species** Mouse.
No. of animals 5/sex/treatment.
Dosage Oral by gavage not specified, not fated before treatment.
Observations Mortality, clinical signs (frequency not indicated).
Results Deaths occurred between 1 and 3 days after treatment.
Clinical signs: Depression within 10-15 min. after treatment.
Conclusions LD₅₀ 13,360 mg/kg bw (95% CI 11,920-15,000 mg/kg bw)
Rev. note The report is limited to the above mentioned.
Reliability 4
Critical study for SIDS endpoint

Title Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation
Date of report August 8, 1991
GLP No.
Reference 16.
Test substance Linalyl acetate, purity not indicated.
Guideline Not indicated.
Stat. method Student's *t*-test, F-test.
Test system **Species** Mouse (Swiss), 6-8 weeks and 6 months, mean weight 28.5 g.
No. of animals 4/dose level.
Dosage Inhalation exposure to 2.74 mg/L during 90 minutes; untreated controls.
Analysis By GC and GC-MS after capture in activated charcoal and elution with carbon disulfide.
Procedure After 60 min adaptation, animals were exposed (young and old animals separately) (whole body, 8.4L). Motor activity was determined pretest (during adaptation) and at 30, 60 and 90 min. during treatment by the number of interruptions of a light beam. Blood was withdrawn at 0, 30, 60 and 90 min. and analysed by GC.

Results The results of analyses (air and blood) were not reported.
Motor activity of control animals was arbitrarily fixed as 100%.
An additional test with old animals (6 months) was performed at a higher dose (not specified), leading to a significant reduction of motility.

Exposure level (mg/l)		0		2.74	
Effect	Time	Young	Old	Young	Old
% of activity	30	100	100	42	76
	60	100	100	11	65
	90	100	100	0	19

Conclusions Effect at 2.74 mg/l.

Rev. note 1. Both male and female mice were included in the test without specification. It was indicated that no sex difference exists between the motor activity of male and female mice.
2. Exposure was limited to 90 minutes. It is not clear what the result of longer exposure times will be.
3. The differences between young and old animals may be explained by the higher amount of fat in older animals and a concomitant higher storage in fat and thus lower plasma levels.

Reliability 4 Limited report, secondary literature.

Critical study for SIDS endpoint

5.3. Corrosiveness/irritation

A Skin irritation/corrosion

Title Comparative studies on the irritancy of oils and synthetic perfumes to the skin of rabbit, guinea pig, rat, miniature swine and man

Date of report August 1979.

GLP No.

Reference 11.

Test substance Linalyl acetate, purity >95%.

Guideline Not applicable.

Stat. method Not applicable.

Test system *Test 1*
Species Angora rabbit (weight 2.3-3.0 kg).
No. of animals 6 (sex not indicated).
Procedure Application of 0.1 g test substance on the clipped dorsal skin (3 x 3 cm) without dressing (solvent not indicated) at 0, 24 and 72 h; after 72 h Evans blue was injected i.v., animals were killed after 1 h and dorsal skin was removed.
Observations Skin reaction on living skin at 24 and 72 h; on removed skin dilation rate, oedema, capillary permeability and bleeding rate; skin histopathology.

Test system *Test 2*
Species Guinea pig (Hartley, weight 350-500 g), rat (Wistar, weight 250-350 g).
No. of animals 6 males/test.
Procedure Application of 0.1 g test substance on the clipped dorsal skin without dressing (solvent not indicated) at 0, 24 and 72 h; after 72 h Evans blue was injected i.v., animals were killed after 1 h and dorsal skin was removed.
Observations Skin reaction on living skin at 24 and 72 h; on removed skin dilation rate, oedema, capillary permeability and bleeding rate; skin histopathology.

Test system *Test 3*
Species Miniature swine (Pitman-Moore, age 1 month).
No. of animals 6 (sex not indicated).
Procedure Application of 0.05 g test substance on the clipped and cleaned dorsal skin under a Ø 15 mm patch (solvent not indicated) for 48 h; after 48 h Evans blue was injected i.v., animals were killed after 1 h and dorsal skin was removed.
Observations Skin reaction on living skin at 48 h; on removed skin dilation rate, oedema, capillary permeability and bleeding rate; skin histopathology.

Test system *Test 4*
Species Human (male volunteers without known allergies).
No. 50

	Procedure	Application of 0.05 mL test substance (32% solution in acetone) on the back under a Ø 15 mm patch for 48 h.
	Observations	Skin reaction at 48, 72, 96 and 120 h.
Results		Animal scores based on erythema, oedema, dilation rate and capillary permeability: <4 mildly irritating, 4-8 moderate irritating, >8 severely irritating. Human scores: positive reactions <10% non-irritating 10-40% mildly irritating, 40-70% moderate irritating, >70% severely irritating.

Species	Test results				
	Rabbit	Guinea pig	Rat	Miniature swine	Human
	Severely irritating	Moderately irritating	Not reported	Not irritating	Not irritating

Conclusion	-
Rev. note	1. <i>Minor remark.</i> In the rabbit test each animal was treated with 3 different test substances and a positive control (n-hexadecane). Two areas were left free and served as untreated controls. 2. The method used to calculate scoring was not fully described.
Reliability	2 Scoring method not fully elucidated (note 2), secondary literature.

B Eye irritation/corrosion

5.4. Skin Sensitisation

5.5. Repeated Dose Toxicity

Title	28-Day oral toxicity study in rats, compound B10.	
Date of report	January 26, 1990.	
GLP	Yes.	
Reference	23.	
Test substance	B10: essential oil of coriander containing 72.9% of natural linalool. Additional constituents were identified as 3.9% alpha-pinene, 0.6% camphene, 0.9% myrcene, 4.0% p-cymene, 2.7% limonene, 3.6% gamma-terpinene, 4.6% camphor, 0.8% alpha-terpineol and 1.2% geranyl acetate. The total of ingredients identified by gas chromatography is 95.5% (area-%), the reminder being minor peaks in the chromatogram.	
Guideline	Not indicated.	
Stat. method	ANOVA.	
Test system	Species	Rat (CrI:CD/BR), 4-week-old males and females.
	No. of animals	10/sex/treatment.
	Dosage	Daily oral administration by gavage of 160, 400 and 1000 mg/kg bw (vehicle is 1% methyl cellulose in dist. water; dosing volume 10 ml/kg). Test mixtures were prepared fresh weekly.
	Exposure period	28 days.
	Post exposure period	1 day.
Investigations	General	Clinical signs (daily); mortality (twice daily); food consumption (weekly); body weight (weekly); physical examination and clinical observation (weekly).
	Clinical pathology	10 Animals per sex were taken at random from the pool of healthy animals not selected for the study as baseline group for clinical chemistry and haematology. Haematology: leukocyte count, erythrocyte count, haemoglobin, haematocrit, platelet count, leukocyte differential count, cell morphology and, for the control and high-dosage groups at week 5 (after test) only, the myeloid/erythroid ratio. Clinical chemistry: Na, K, Ca, Cl, total CO ₂ , total protein, albumin, total bilirubin, blood urea nitrogen, creatinine, glucose, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase and alkaline phosphatase.
	Necropsy	Macroscopy: external body surface, orifices, cranial cavity, carcass, nasal cavity and paranasal sinuses, cervical tissues and organs, external surface of brain and

spinal cord, thoracic, abdominal and pelvic cavities and viscera.
Organ weights: brain, spleen, liver, heart, kidneys, testes with epididymides, thyroid with parathyroids, adrenal glands, ovaries and pituitary.
Microscopy: all organs investigated macroscopically and femoral bone marrow, lung, any lesion, oesophagus, stomach, duodenum, jejunum, ileum, colon, caecum, rectum, pancreas, urinary bladder and mesenteric lymph nodes.

Analysis Results Concentration of the test substance in each concentration for administration was verified by analysis.

Dose	0 mg/kg		160 mg/kg		400 mg/kg		1000 mg/kg		Dose related	
Sex	M	F	M	F	M	F	M	F	M	F
Mortality (A)	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10		
Clinical Signs										
Body weight										
Food consumption										
Haematology										
Clinical chemistry										
Total protein					i		i	i	x	x
Albumin					i		i	i	x	x
Calcium							i		x	
Glucose					d		d		x	
Necropsy										
Macroscopy (B)					+	+	+	+	x	x
Liver weight			i	i	ic	ic	ic	ic	x	x
Kidney weight					ic ^r		ic ^a	ic ^a	x	x
Microscopy (C)				+		+	+	+	+	

Where i=increase; d=decrease.

- (A) One female was found dead on day 2 and was replaced by another female that was dosed for the full time of the test. One male was found dead on day 9, probably by a handling accident.
(B) Thickened liver lobes, pale areas in the kidneys and thickened stomach mucosa.
(C) All treated female groups showed hepatocellular cytoplasmic vacuolisation while the high-dose males had an increase in degenerative lesions in the renal cortex. Middle- and high-dose females also had lesions in the nonglandular part of the stomach, with some erosion, subacute inflammation and acanthosis.

Conclusions NOAEL = 160 mg/kg bw based on histological effects in liver and kidney (equivalent to ~ 148 mg/kg bw linalyl acetate)
LOAEL = 400 mg/kg bw based on histological effects in liver and kidney (equivalent to ~371 mg/kg linalyl acetate)

- Rev. note**
- No remarkable effects on the primary reproductive organs in both females (ovaries and uteri) and males (testes and epididymides) were noted in any animal from any dosage group up to 1000 mg/kg bw, both macroscopically at dissection and also microscopically during histopathology of every single (10 male, 10 female) high-dose animal.
 - No treatment-related effects on survival, clinical observations, body weight or food consumption were observed in any of the treatment groups. There were some treatment-related increases in total serum protein and albumin, with a concomitant increase in calcium; the pathogenesis of these increases is unknown. Liver and kidneys were the organs affected both macroscopically and histopathologically, with dose-related increase in expression of those findings. Based on these findings, treatment-related effects were found in all groups except the low-dose males. However, the severity of the incidences was low. Due to the study layout, any potential reversibility of the effects could not be tested.
 - The dose was converted to equivalents linalyl acetate, making a correction for the purity (72.9%) and the molecular weight.

Reliability 1.
Critical study for SIDS endpoint

Title Effect of linalool on hepatic drug-metabolizing enzymes in the rat.
Date of report 1974.
GLP No.
Reference 21.
Test substance Linalool, purity ≥96%.
Guideline Not indicated.

Stat. method	Not indicated.
Test system	Species Rat (Wistar), 4-week-old males. No. of animals At least 24. Dosage Daily intragastric intubation of 500 mg/kg bw as a 25% (w/v) solution in propylene glycol. Controls: similar volume of propylene glycol. Exposure period 0-64 days.
Method	At intervals of 0, 3, 7, 14, 30 and 64 days after first dose, 4 animals from each of the test and control groups were killed by cervical dislocation, the livers rapidly excised, freed from adhering connective tissue and weighed. Liver homogenates and microsomal fraction were then prepared according to published literature.
Analysis	No data.
Results	There were no deaths over the 64-day period, nor was there any significant effect on body weight gain. No clinical signs were observed. Both the absolute and relative liver weights remained unaffected up to the 30th day of exposure, but by the 64th day there was a slight but significant ($P < 0.05$) increase in these parameters. From liver homogenates and microsomal fractions the following biochemical changes were derived: The microsomal protein concentration was unaffected up to day 14, but was increased by 20% ($P < 0.02$) and remained at this elevated level to the 64th day. Cytochrome P-450 and cytochrome b5 showed a biphasic response, both being depressed on day 7 ($P < 0.02$ in each case), but subsequently increased by 50% ($P < 0.01$) by day 30; CYP450 remained at this elevated level while CYb5 had further increased to 70% ($P < 0.002$) by day 64. 4-Methylumbelliferone glucuronyl transferase increased on chronic exposure to linalool to 17% ($P < 0.02$) on day 3, with a further dramatic rise to 150% ($P < 0.001$) by day 64. Alcohol (ethanol) dehydrogenase showed a biphasic response, being initially depressed by 33% ($P < 0.002$) on day 3, then increased by 36% ($P < 0.001$) on day 7; normal values were regained by day 14 and thereafter there was no significant difference between test animals and controls.
Conclusions	NOAEL = 500 mg/kg bw
Rev. note	- The information available is limited to the above. - The results show that, with the exception of alcohol dehydrogenase, prolonged exposure to linalool was required before significant effects were observed. The biphasic effect on alcohol dehydrogenase, in contrast to the steady increase in 4-methylumbelliferone glucuronyl transferase and the delayed induction of CYP450 and CYb5, may indicate that initially linalool is not readily metabolised and inhibits alcohol dehydrogenase. Subsequently, when the activities of drug-metabolising enzymes (especially 4-methylumbelliferone glucuronyl transferase) were increased, hepatic concentrations of free linalool may have fallen sufficiently to enable the adaptive increase in alcohol dehydrogenase to be observed. Still later in the study, 4-methylumbelliferone glucuronyl transferase was able to meet the whole of the increased metabolic demand and no effects on alcohol dehydrogenase were observed any longer. In corroboration of the importance of glucuronidation, it had been observed in an earlier study that linalool is excreted largely in urine and bile in the form of conjugates with glucuronic acid. Based on this reasoning, the observed effects of linalool are interpreted to represent a physiological adaptation to exposure and not toxicity in a strict sense.
Reliability	2.

5.6. Genetic Toxicity

A Chromosomal Aberration

Title	Evaluation of the ability of linalylacetate to induce chromosome aberrations in cultured peripheral human lymphocytes
Date of report	June 22, 2000.
GLP	Yes.
Reference	19.
Test substance	Linalylacetate, purity 96.7%.
Guideline	OECD 473, 67/548/EEC B10, Draft EEC 23.07.1999.
Stat. method	Chi-square test.
Test system	Cell line Human lymphocytes. Metabolic activation Rat S9 mix (Aroclor 1254-induced). Test concentrations 10-180 µg/ml (based on precipitation).

Controls Negative: vehicle controls (DMSO).
Positive: mitomycin-C (-S9), cyclophosphamide (+S9).
Procedure -S9: 3 h exposure + 24 h fixation.
24 and 48 h exposure + 24 and 48 fixation.
+S9: 3 h exposure + 24 h fixation.
3 h exposure + 48 h fixation.
Colchicine was added for the last 3 hours.

Results

Exposure/fixation (h)	Metabolic activation	Doses evaluated [$\mu\text{g/ml}$]	Aberrations [%]	Test result ^(A)
3/24	Without	33, 100, 130	0.5, 0.5, 1	-
3/24	With	100, 130, 180	2.5, 0.5, 3	-
3/48	With	100, 130, 180	1, 1.5, 0.5	-
24/24	Without	33, 100, 130	1.5, 1.5, 2.5	-
48/48	Without	56, 100, 130	3.5, 3, 1	-

(A) +/- : positive/negative result; positive controls gave expected responses.

Conclusion Not clastogenic.**Rev. note** The 3.5% aberrations seen at the lowest examined dose (48 h treatment, 48 h fixation) was statistically significant. Since no increase was seen at the higher concentrations, it was considered to be without biological significance.**Reliability** 1**Critical study for SIDS endpoint****B Gene Mutation****Title** Mutagenicity evaluation of Ro 02-8282/000 in the Ames test**Date of report** June 15, 1998.**GLP** Yes.**Reference** 12.**Test substance** Linalyl acetate, purity 97.6% (solvent DMSO).**Guideline** OECD 471, 79/831/EEC. B.14, ICH.**Stat. method** Not performed.**Test system** **Bacterial strains** TA97, TA98, TA100, TA1535 and TA102.**Metabolic activation** Male rat liver S9 mix (phenobarbital/ β -naphthoflavone induced).**Test concentration** 5, 15, 50, 150 and 500 $\mu\text{g/plate}$ or tube (based on toxicity).**Controls** Negative: vehicle (DMSO).Positive:

Without activation: sodium azide (TA1535, TA100), ICR191 (TA97), 2 nitrofluorene (TA98), mitomycin C (TA102) and 2-aminoanthracene (all strains).

With activation: 2-aminoanthracene (all strains).

Procedure Plate incorporation and preincubation tests according to OECD 471.**Results** Toxicity in the plate incorporation assay at $\geq 500 \mu\text{g/plate}$, in the preincubation test $\geq 50 \mu\text{g/plate}$ (without S9) and $\geq 100 \mu\text{g/plate}$ (with S9).

Tester strain	Test result ^(A)			
	Plate incorporation assay		Preincubation test	
	Without activation	With activation	Without activation	With activation
TA97	-	-	-	-
TA98	-	-	-	-
TA100	-	-	-	-
TA1535	-	-	-	-
TA102	-	-	-	-

(A) +/- : positive/negative result; positive controls gave expected responses.

Conclusion Not mutagenic.**Rev. note** *Minor remark.* The initial number of cells (0.37-1.65E08) was only reported in an EEC-Sheet which was included in the report.**Reliability** 1**Critical study for SIDS endpoint**

Title	An evaluation of food flavoring ingredients in a genetic toxicity screening battery
Date of report	1989.
Reference	36.
Test substance	Linalyl acetate, purity indicated.
GLP	No data.
Result	63 substances were tested in the Ames test, mouse lymphoma test and in the UDS test. Linalylacetate was not among substances with a positive result in any of these tests
Rev. note	Abstract
Reliability	It is not clear whether linalyl acetate was tested in the assay 4.

5.7. Carcinogenicity

Title	Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice
Date of report	December 1973.
GLP	No.
Reference	8.
Test substance	Linalyl acetate, purity ≥85%.
Guideline	Not indicated.
Stat. method	Chi-quadrat test, likelihood principle (Zweifel, 1966).
Test system	Species Mouse, (A/He), age 6-8 weeks, mean weight 18-20 g. No. of animals 30 females/treatment. Dosage 3-weekly injection (i.p.) for 8 weeks of 200 and 1000 mg/kg bw (total 24 doses → 4.8 and 24 g/kg bw); untreated and vehicle (tricaprylin) controls. Observations Body weights every 2 weeks. At termination (24 weeks after study initiation): Fixation of the lungs to count white nodules on the surface; macroscopic and microscopic examination of the lungs; macroscopy of liver, kidney, spleen, thymus, intestine and salivary and endocrine glands.

Results

Dose [mg/kg bw] \ effect	0 (untr.)	0 (veh.)	200	1000	DR
Mortality	2/50	3/80	6/30	4/30	
Body weight	Not reported				
Tumour bearing animals	8/50	16/80	4/30	4/30	

Conclusions	No evidence of carcinogenic activity
Rev. note	1. The report was limited to the above mentioned. 2. The test duration was too short for full development of possible carcinogenic effects. However, urethan used as a positive control showed a 100% response. 3. The number of animals in the treated groups was too small to allow proper statistical evaluation of the results. No histopathological evaluation of the tumours found was reported. 4. Dose selection was based on the maximum tolerated dose in 5 mice receiving 6 i.p. injections of the test substance over a 2-week period (recovery 1-2 months). 5. The route of administration is not representative for the possible routes of exposure (mainly dermal and/or inhalatory).
Reliability	4 Route of exposure less suitable (note 5).

Title	Cocarcinogenesis studies on mouse skin and inhibition of tumor induction.
Date of report	1971.
Reference	27.
GLP	No data.
Test substance	Linalyl acetate, purified by distillation.
Guideline	Not indicated.
Test system	Species ICR/Ha Swiss mice, age: 6-8 weeks; female. Source Millerton Research Farms, Millerton, N.Y. No. of animals 20/treatment. Dosage 3 mg linalyl acetate with and without 5 µg benzo[a]pyrene; application by

	micropipet in 0.1 ml acetone 3 times weekly for the duration of the experiment; controls: benzo[a]pyrene or acetone only and no treatment
Exposure period	67 weeks.
Investigations	Tumors greater than 1 mm in diameter were counted; only tumors persisting for 30 days or more were counted in the cumulative totals. Animals bearing carcinomas were killed ca. 2 months after the tumors were classified as malignant. All animals were necropsied. Tumor specimens were analysed histologically.
Results	The available table states that coapplication of linalyl acetate and benzo[a]pyrene yields 10 papillomas and 4 carcinomas on the skin (it takes 276 days to the first papilloma). Application of benzo[a]pyrene alone takes 266 days to show the first papilloma and 8 papillomas and 1 carcinoma in total have been formed.
Conclusions	Linalyl acetate has weak cocarcinogenic activity.
Rev. note	1. The information in the report is limited to the above. 2. No necropsy data were presented. Histological evaluation is not reported.
Reliability	4.

5.8. Reproductive toxicity

A Fertility

B Developmental Toxicity

Title	Reproductive and developmental toxicity screening test of B10 administered orally via gavage to Crl:CD(SD)BR female rats.				
Date of report	April 12, 1989.				
GLP	Yes.				
Reference	22.				
Test substance	B10: essential oil of coriander containing 72.9% of natural linalool. Additional constituents were identified as 3.9% alpha-pinene, 0.6% camphene, 0.9% myrcene, 4.0% p-cymene, 2.7% limonene, 3.6% gamma-terpinene, 4.6% camphor, 0.8% alpha-terpineol and 1.2% geranyl acetate. The total of ingredients identified by gas chromatography is 95.5% (area-%), the reminder being minor peaks in the chromatogram.				
Guideline	US Food and Drug Administration (1966): Guidelines for reproduction studies for safety evaluation of drugs for human use.				
Stat. method	Not indicated.				
Test system	Species	Rat (Crl:CD(SD)BR), 71-day-old at gestation day 0, weight 228-383 g.			
	Source	Charles River Laboratories.			
	No. of animals	10 mated females/dose group.			
	Dosage	Oral gavage at 0 (vehicle only), 250, 500 and 1000 mg/kg bw; the vehicle is corn oil.			
	Procedures	Female rats were mated with untreated stock males (1/1) from the same strain and source. The day of observation of a vaginal plug or vaginal smear was defined as day 0 of gestation. Females were treated daily from 7 days prior to and subsequently through cohabitation, gestation, delivery and a 4-day lactation period. All dams were observed for clinical signs, death and/or delivery of a litter. Body weights were recorded during the whole dosage period. Feed consumption was determined on days 0, 6, 10, 14, 16, 20 and 25 of presumed gestation as well as on days 1 and 4 of lactation. On day 4, all females were subjected to macroscopic examination. All litters were examined for number, viability, body weight, sex ratio and external anatomy of the pups. Delivered pups were also examined for viability, clinical observations and body weight during the 4-day postpartum period.			

Results

Dose (mg/kg bw)	0	250	500	1000	DR
<i>Maternal data</i>					
Mortality	0/10	0/10	1/10	0/10	
Clinical signs			+(A)	+(A), (B)	x
Body weight gain		ic	ic	dc/ic ^(C)	x

Feed consumption	ic	ic	dc/ic ^(C)	x
Necropsy	No treatment related effects			
No. of pregnant females	No treatment related effects			
Duration of cohabitation	No treatment related effects			
No. of implantation sites /dam	No treatment related effects			
Duration of gestation	No treatment related effects			
No. live pups/ dam	dc			x
Foetal data				
No. of pups	dc			x
Pup sex ratio/ pup weight/gross morphology	No treatment related effects			

(A) Excessive salivation (p<0.05).

(B) Urine-stained abdominal fur (p<0.01) during the premating period, one or two showed ataxia and/or decreased motor activity during premating and gestation.

(C) Significant decrease during premating for high dosage group, and significant increase during gestation and small increase during lactation for all treated groups.

Conclusions NOAEL for maternal toxicity is 500 mg/kg/d based on body weight gain, feed consumption and clinical signs (equivalent to ~464 mg/kg bw linalyl acetate).
NOAEL for developmental/reproductive toxicity is 500 mg/kg based on increased fetal mortality in utero and after parturition (equivalent to ~464 mg/kg bw linalyl acetate).

Rev. note 1. Effects on offspring are only present at maternally toxic doses.
2. The dose was converted to equivalent linalyl acetate, making a correction for the purity (72.9%) and the molecular weight.
3. Males were not treated before mating.

Reliability 2**Critical study for SIDS endpoint**

5.9. Other Relevant Information

Title Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents
Date of report 1967.
GLP No.
Reference 10.
Test substance Linalyl acetate, purity ≥ 90% or ≥ 96%.
Guideline Not indicated.
Results *Metabolism:*
Probably hydrolysed to linalool and acetic acid. Linalool is converted to geraniol and its metabolites, 1,5-dimethyl-hexadien-1,6-dicarboxylic acid and 7-carboxy-5-methylocto-6-enoic acid.
Acute toxicity (oral):
Mouse: LD50 13360 mg/kg bw
Rat: LD50 14550 mg/kg bw
Repeated dose:
Rat: 12 week feeding study: LOAEL 24.3 mg/kg (growth retardation)

ADI: 0.25 mg/kg bw.
Rev. note The repeated dose study was performed with mixed esters, without further explanation.
Reliability 4 Secondary literature

Title Occupational and environmental health hazard review: linalylacetate (Ro 02-8282)
Date of report 1981.
GLP No.
Reference 18.
Test substance Linalyl acetate.
Guideline Not applicable.

Results	<i>Acute oral (oral LD₅₀):</i> Rat 14500 mg/kg bw. Mouse 13360 mg/kg bw, >8000 mg/kg bw, 15000 mL/kg bw. <i>Skin irritation:</i> see ref. 11. Rabbit (5% solution): not irritating. <i>Subacute toxicity:</i> Rabbits were exposed by inhalation of steam containing 9 and 27 mg/kg H₂O: effects at 27 mg/kg H₂O consisted of weak bronchomucotropic action (some augmentation of volume output of respiratory tract fluid). <i>Chronic toxicity:</i> LOAEL female rats 24 mg/kg bw (growth inhibition). Linalyl cinnamate and linalylisobutyrate were fed in diet to rats during 17-18 weeks. No effect on growth, haematology and macro- and microscopic findings. <i>Carcinogenicity:</i> Not carcinogenic in strain A mice (ref. 8). Weak carcinogenic when applied simultaneously and repeatedly with benzo(a)pyrene.
Rev. note	ADI 0.25 mg/kg bw. 1. The report is limited to the above mentioned. 2. From the subacute toxicity study no actual dose levels can be established. 3. It is not clear whether the reported results from the chronic study came from a study with linalyl acetate.
Reliability	4. Secondary literature
Title	Local anaesthetic activity of the essential oil of <i>Lavandula angustifolia</i> .
Date of report	1999.
Reference	25.
Test substance	Linalyl acetate, purity 43.6% as constituent of essential oil of <i>Lavandula angustifolia</i> and pure linalyl acetate, purity 97%.
GLP	No data.
Result	The local anaesthetic activity of the essential oil of <i>Lavandula angustifolia</i> is evaluated. 1. This essential oil and pure linalyl acetate (0.01-10 µg/ml) were able to drastically reduce, in a dose-dependent manner, the electrically evoked contractions of rat phrenic-hemidiaphragm. This finding excludes antimuscarinic activity of linalyl acetate. 2. A rabbit conjunctival reflex test treatment with a solution of the same test substances (30-2500 µg/ml administered in the conjunctival sac) results in a dose-dependent increase in the number of stimuli necessary to provoke the reflex (i.e. suppression of the reflex).
Conclusion	The local anaesthetic activity is not caused by antimuscarinic activity of these compounds, but is suggested to be related to effects on the Na ⁺ and/or Ca ⁺⁺ channels.
Rev. note	Secondary literature (journal article)
Reliability	4.
Title	<i>In vitro</i> permeation through porcine buccal mucosa of <i>Salvia desoleana</i> Atzei & Picci essential oil from topical formulations.
Date of report	2000.
Reference	26.
Test substance	Linalyl acetate, 26.8% as constituent of essential oil of <i>S. desoleana</i> .
GLP	No data.
Result	In this article it was determined that permeation of the test substance through the porcine buccal mucosa is possible <i>in vitro</i> .
Rev. note	Secondary literature (journal article)
Reliability	4.
Title	Report on the hydrolysis of linalylacetate and ethylformate by artificial gastrointestinal juices
Date of report	April 1979.
GLP	No.
Reference	34.
Test substance	Linalyl acetate, purity not indicated.
Test method	Not specified.

Procedure	Test system: Artificial gastric and pancreatic juices (made according to method of Longland, 1977)			
	Method: Linalyl acetate was added to 110 mL gastric or pancreatic juices (37°C). At intervals 10 mL aliquots were removed and analysed by GC with FID.			
	Half-lives were calculated from the exponential concentration vs. time curve (A) and using the least square method based on log concentration vs. time (B)			
Results	Gastric		Pancreatic	
	Time (min)	Concentration (mg/mL)	Time (min)	Concentration (mg/mL)
	0	30	0	27
	2.5	16.6	2.1	7
	10.4	9.7	2.8	25
	17.6	5.8	17.6	16.6
	25.7	2.4	42.2	15.0
			76.6	10.4
			106.3	6.8
			136.2	4.1
Conclusion	Half-life in gastric fluid 3.6 (A) and 7.3 (B) minutes			
	Half-life in pancreatic fluid 52.2 (A) and 52.8 (B) minutes			
Rev. note	For gastric juices first order kinetics and for pancreatic juices zero order kinetics followed by first order kinetics. The first phase of the test in pancreatic fluids was assumed to be influenced by reduced solubility due to the less acidic environment compared to the situation in gastric fluids.			
Reliability	2.			

5.10. Experience with Human Exposure

Title	Clinical toxicology of commercial products Acute poisoning
Date of report	1981.
GLP	No.
Reference	9.
Test substance	Oil of Bergamot, linalyl acetate 45%.
Guideline	Not applicable.
Results	Repeated skin exposure leads to nervous and digestive symptoms. Persons sensitive develop photodermatitis, eczema and pigment changes.
Rev. note	Statement, limited to the above mentioned.
Reliability	4
Title	Occupational allergy to lavender oil
Date of report	1986
Reference	28.
Test substance	Lavender oil (one of the constituents was linalyl acetate)
GLP	No data.
Result	Case study: a hairdresser showed a strong positive response in a patch test, both 48 and 96 hours after application of lavender shampoo. Lavender oil was the positive substance in the shampoo. The allergen of lavender oil has not been identified, but linalyl acetate is suspected (but was not tested).
Rev. note	Secondary literature (journal article)
Reliability	4.
Title	Health Hazard Evaluation Report
Date of report	1983
Reference	29.
Test substance	Lavender oil (main constituent linalyl acetate)
GLP	Not applicable
Result	For the exposure due to the use in oil paints, area sampling was requested by representatives of artists (painters and potters). 12 artists were interviewed and some of them complained about the odour of the paint and slight dermatitis (no area samples were taken).
Rev. note	The complaint was related to odour only and was withdrawn before the end of the project.
Reliability	4.

	Author	Title	Performing laboratory / source	Year
1.	-	Linalyl acetate MSDS	Roche	1996
2.	-	Internal report	Roche	1979
3.	Schmiedel U.	Determination of the partition coefficient of linalylacetate in n octanol/water	RCC UmweltChemie AG	
4.	Calame R, Ronchi W.	Linalyl acetate activated sludge, respiration inhibition test	Givaudan	1989
5.	Grade R.	Report on the acute toxicity of linalylacetat on daphnia (<i>Daphnia magna</i> Strauss 1820)	Ciba-Geigy Ltd.	1993
6.	Grade R.	Report on the growth inhibition test of linalylacetat to green algae (<i>Scenedesmus subspicatus</i>)	Ciba-Geigy Ltd.	1994
7.	Rudio J.	Linalyl acetate synthetic determination of the ready biodegradability	Givaudan	1991
8.	Stoner G., Shimkin M., Kniazeff A., et al	Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A Mice	Cancer Research 33, 3069-3085	1973
9.	Gosselin R., Hodge H., Smith R., et al.	Clinical toxicology of commercial products Acute poisoning	Oil of Bergamot, 154	1981
10.	FAO	Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents	FAO Nutrition Meetings Report Series No. 44A	1967
11.	Motoyoshi K., Toyoshima Y, Sato M., et al.	Comparative studies on the irritancy of oils and synthetic perfumes to the skin of rabbit, guinea pig, rat, miniature swine and man	Cosm. Toil. 94: 41-48	1979
12.	Kirchner S.	Mutagenicity evaluation of Ro-8282/000 in the Ames test	Roche	1998
13.	Bogers M.	96-hour acute toxicity study with linalylacetate (flow-through)	NOTOX	1998
14.	Jüttner F.	Flavour compounds in weakly polluted rivers as a means to differentiate pollution sources	Wat. Sci. Tech. 25 (2): 155-164	1992
15.	Weidenhamer J., Macias F., Fischer N., et al.	Just how insoluble are monoterpenes?	J. Chem. Ecology 19 (8):1799-1807	1993
16.	Buchbauer G., Jirovetz L., Jäger W.	Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation	Z. Naturforsch. 46c: 1067-1072	1991

17. Clark B., Chamblee T., Iacobucci G.	Micellar-induced selectivity and rate enhancement in the acid-catalyzed cyclization and rearrangement of monoterpenes. The solvolysis of linalyl and geranyl acetates	J. Org. Chem. 54 (5):1032-1036	1989
18. Bauer D.	Occupational and environmental health hazard review: linalylacetate (Ro 02-8282)	Roche	1981
19. Bertens A.	Evaluation of the ability of linalylacetate to induce chromosome aberrations in cultured peripheral human lymphocytes	NOTOX	2000
20. Lewis RJ Sr.		Sax's Dangerous properties of industrial materials, Ninth edition: 2051.	1996
21. Parke DV	Effect of linalool on hepatic drug-metabolizing enzymes in the rat.	Biochem Soc Trans 2: 615-618.	1974
22. Hoberman A.,	Reproductive and developmental toxicity screening test of B10 administered orally via gavage to Crl:CD(SD)BR female rats	Lorillard Inc.	1989
23. Serota D.	28-day oral toxicity study in rats, compound B10.	Lorillard Inc.	1990
24. Brekelmans M.	Determination of the hydrolysis of linalyl acetate as a function of pH	F. Hoffmann-La Roche Ltd.	2001
25. Ghelardini C., et al,	Local anaesthetic activity of the essential oil of <i>Lavandula angustifolia</i>		
26. Ceschel G., et al.	<i>In vitro</i> permeation through porcine buccal mucosa of <i>Salvia desoleana</i> Atzei & Picci essential oil from topical formulations	Intern. J. Pharmaceutics 195: 171-177	2000
27. Van Duuren B., et al.	Cocarcinogenesis studies on mouse skin and inhibition of tumor induction	J. Nat. Cancer Inst. 46(5): 1039-1044	1971
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30.	The Merck Index 12 th Ed vs 12.3		2000
31. Jenner P., et al.	Food flavourings and compounds of related structure I. acute oral toxicity	Fd Cosmet. Toxicol. 2:327-343	1964
32. Ishidate M., et al.	Primary mutagenicity screening of food additives currently used in Japan*	Fd Cosmet. Toxicol. 22:623-636	1984
33. Florin I., et al.	Screening of tobacco smoke constituents for mutagenicity using the Ames' test*	Toxicology 15:219-232	1980

34. Hall	Unpublished report from McCormick & Co. Inc. to the flavor and extracts manufacturers' association of the United States	McCormick & Co. Inc. to the flavor and extracts manufacturers' association of the United States, Washington DC, US	1979
35. Oda et al.	Mutagenicity of food flavors in bacteria **	Shokuhin Eisi Hen, 9, 177-181	1978
36. Heck et al.	An evaluation of food flavoring ingredients in a genetic toxicity screening battery	Toxicologist 9, 257-?	1989
37. Oser	Unpublished report**		1967

* Reference was not included because it did not provide information on linalyl acetate.

** Reference was not retrievable.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.8

Revision Date 07.03.2024

Print Date 19.11.2024

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 : Linalyl acetate

Product Number : 49599

Brand : Sigma-Aldrich

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

CAS-No. : 115-95-7

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science Sp.z.o.o.
Szelągowska 30
PL-61-626 POZNAN

Telephone : +48 61 8290-100

Fax : +48 61 8290-120

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(48)-223988029 (CHEMTREC) 112
(numer alarmowy)

SECTION 2: Hazards identification**2.1 Classification of the substance or mixture**

Skin irritation, (Category 2) H315: Causes skin irritation.

Eye irritation, (Category 2) H319: Causes serious eye irritation.

Skin sensitization, (Sub-category 1B) H317: May cause an allergic skin reaction.



2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

Pictogram



Signal Word

Warning

Hazard Statements

H315

Causes skin irritation.

H317

May cause an allergic skin reaction.

H319

Causes serious eye irritation.

Precautionary Statements

P261

Avoid breathing mist or vapors.

P264

Wash skin thoroughly after handling.

P272

Contaminated work clothing should not be allowed out of the workplace.

P280

Wear protective gloves/ eye protection/ face protection.

P302 + P352

IF ON SKIN: Wash with plenty of water.

P305 + P351 + P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Supplemental Hazard Statements

none

Reduced Labeling (≤ 125 ml)

Pictogram



Signal Word

Warning

Hazard Statements

H317

May cause an allergic skin reaction.

Precautionary Statements

P261

Avoid breathing mist or vapors.

P272

Contaminated work clothing should not be allowed out of the workplace.

P302 + P352

IF ON SKIN: Wash with plenty of water.

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.

Ecological information:

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.

Toxicological information:



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.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : 3,7-Dimethyl-1,6-octadien-3-yl acetate

Formula : $C_{12}H_{20}O_2$

Molecular weight : 196,29 g/mol

CAS-No. : 115-95-7

EC-No. : 204-116-4

Component		Classification	Concentration
3-acetoxy-3,7-dimethyl-1,6-octadiene			
CAS-No.	115-95-7	Skin Irrit. 2; Eye Irrit. 2; Skin Sens. 1B; H315, H319, H317	<= 100 %
EC-No.	204-116-4		

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. Consult a physician.

In case of eye contact

After eye contact: rinse out with plenty of water. Call in ophthalmologist. Remove contact lenses.

If swallowed

After swallowing: caution if victim vomits. Risk of aspiration! Keep airways free. Pulmonary failure possible after aspiration of vomit. Call a physician immediately.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available



SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Carbon dioxide (CO₂) Foam Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

Combustible.

Vapors are heavier than air and may spread along floors.

Forms explosive mixtures with air on intense heating.

Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters

Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information

Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapors, aerosols. 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 with liquid-absorbent material (e.g. Chemisorb®). Dispose of properly. Clean up affected area.

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.

Storage stability Recommended storage temperature

2 - 8 °C



Storage class

Storage class (TRGS 510): 10: Combustible liquids

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 EN 16523-1 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: 0,4 mm

Break through time: 480 min

Material tested: Camatril® (KCL 730 / Aldrich Z677442, Size M)

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 EN 16523-1 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Splash contact

Material: Latex gloves

Minimum layer thickness: 0,6 mm

Break through time: 30 min

Material tested: Lapren® (KCL 706 / Aldrich Z677558, Size M)

Body Protection

protective clothing

Respiratory protection

required when vapours/aerosols 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 ABEK



The entrepreneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure

Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	liquid
b) Color	colorless
c) Odor	No data available
d) Melting point/freezing point	Melting point/range: < -100 °C at 1.013 hPa - OECD Test Guideline 102
e) Initial boiling point and boiling range	220 °C - lit.
f) Flammability (solid, gas)	No data available
g) Upper/lower flammability or explosive limits	No data available
h) Flash point	94 °C - closed cup
i) Autoignition temperature	No data available
j) Decomposition temperature	No data available
k) pH	No data available
l) Viscosity	Viscosity, kinematic: 1,81 mm ² /s at 40 °C - OECD Test Guideline 114 Viscosity, dynamic: 2,5 mPa.s at 20 °C - OECD Test Guideline 114 1141,6 mPa.s at 40 °C - OECD Test Guideline 114
m) Water solubility	0,3 g/l at 20 °C
n) Partition coefficient: n-octanol/water	log Pow: 3,9 at 25 °C - Bioaccumulation is not expected.
o) Vapor pressure	1,33 hPa at 20 °C
p) Density	0,901 g/cm ³ at 25 °C - lit.
Relative density	No data available
q) Relative vapor density	No data available
r) Particle	No data available



characteristics

- s) Explosive properties No data available
- t) Oxidizing properties none

9.2 Other safety information

Relative vapor density 6,78 - (Air = 1.0)

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.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions

Violent reactions possible with:
Strong oxidizing agents

10.4 Conditions to avoid

Strong heating.

10.5 Incompatible materials

Strong oxidizing agents

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

LD50 Oral - Rat - male and female - > 9.000 mg/kg

Remarks: (ECHA)

Inhalation: No data available

LD50 Dermal - Rabbit - > 5.000 mg/kg

Remarks: (ECHA)

Skin corrosion/irritation

Skin - Rabbit

Result: Irritating to skin. - 4 h
(OECD Test Guideline 404)

Serious eye damage/eye irritation

Eyes - Rabbit

Result: Causes serious eye irritation.
Remarks: Causes serious eye irritation.



(ECHA)

Respiratory or skin sensitization

Local lymph node assay (LLNA) - Mouse

Result: positive

(OECD Test Guideline 429)

Germ cell mutagenicity

Test Type: Ames test

Test system: Salmonella typhimurium

Metabolic activation: with and without metabolic activation

Method: OECD Test Guideline 471

Result: negative

Test Type: In vitro mammalian cell gene mutation test

Test system: Mouse lymphoma test

Metabolic activation: with and without metabolic activation

Method: OECD Test Guideline 476

Result: negative

Test Type: Chromosome aberration test in vitro

Test system: Human lymphocytes

Metabolic activation: with and without metabolic activation

Method: OECD Test Guideline 473

Result: negative

Test Type: Micronucleus test

Species: Mouse

Cell type: Bone marrow

Application Route: Oral

Method: OECD Test Guideline 474

Result: negative

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.

Repeated dose toxicity - Rat - male and female - Oral - 28 Days - NOAEL (No observed adverse effect level) - 160 mg/kg

Repeated dose toxicity - Rat - male and female - 91 Days - NOAEL (No observed adverse effect level) - 250 mg/kg

RTECS: RG5910000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Other dangerous properties can not be excluded.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish flow-through test LC50 - Cyprinus carpio (Carp) - 11 mg/l - 96 h
(OECD Test Guideline 203)

Toxicity to daphnia static test EC50 - Daphnia magna (Water flea) - 59 mg/l - 48 h
and other aquatic (OECD Test Guideline 202)
invertebrates

12.2 Persistence and degradability

Biodegradability aerobic - Exposure time 28 d
Result: 70 - 80 % - Readily biodegradable.
(OECD Test Guideline 301F)

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

Discharge into the environment must be avoided.



SECTION 13: Disposal considerations

13.1 Waste treatment methods

No data available

SECTION 14: Transport information

14.1 UN number

ADR/RID: -

IMDG: -

IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: -

IMDG: -

IATA: -

14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: -

14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

No data available

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

Other regulations

Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements

Sigma-Aldrich- 49599

Page 10 of 12

The life science business of Merck operates as MilliporeSigma in the US and Canada



H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The information is believed to be correct but is not exhaustive and will be used solely as a guideline, which is based on current knowledge of the chemical substance or mixture and is applicable to appropriate safety precautions for the product. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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