



Toxicological profile for 4-Terpinenol

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

4-Methyl-1-propan-2-ylcyclohex-3-en-1-ol (PubChem)

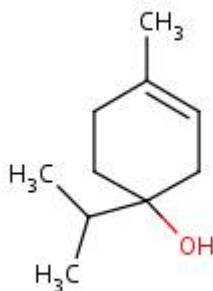
1.2. Synonyms

4-Carvomenthenol; Terpinen-4-ol; 4-Terpineol; p-Menth-1-en-4-ol; Terpinenol-4; 1-Terpinen-4-ol; 1-p-Menthen-4-ol; Terpene-4-ol; 1-Menthene-4-ol; TERPINENE-4-OL; 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-; 1-para-Menthen-4-ol; (+-)-p-Menth-1-en-4-ol; rac Terpinen-4-ol; (+/-)-Terpinen-4-ol; 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol; 4-methyl-1-propan-2-ylcyclohex-3-en-1-ol; FEMA No. 2248; 4-Methyl-1-(propan-2-yl)cyclohex-3-en-1-ol; (+/-)-4-Terpineol; para-Menth-1-en-4-ol; 1-Methyl-4-isopropyl-1-cyclohexen-4-ol; 4-Methyl-1-isopropyl-3-cyclohexen-1-ol; Terpineol-4; Terpin-4-en-1-ol; (+/-)-1-Isopropyl-4-methyl-3-cyclohexen-1-ol; 4-Terpinenol; 1-isopropyl-4-methylcyclohex-3-enol; 1-isopropyl-4-methylcyclohex-3-en-1-ol; L-4-terpineneol; Terpinenolu-4; dl-4-Terpineol; 4-Carvomenthenol (natural); EINECS 209-235-5; EINECS 248-910-9; Origanol; Terpinen 4-ol; alpha-terpinen-4-ol; 1-isopropyl-4-methyl-cyclohex-3-en-1-ol; (1)-1-(Isopropyl)-4-methylcyclohex-3-en-1-ol; TERPINEN-4-OL [FCC]; (-)-p-Menth-1-en-4-ol; (+/-)-p-Menth-1-en-4-ol; 4-TERPINEOL, (+/-)-; 4-Carvomenthenol, >=95%, FCC, FG; 1-Isopropyl-4-methyl-3-cyclohexen-1-ol; 4-Carvomenthenol, natural, >=95%, FG; (-)-1-Isopropyl-4-methyl-3-cyclohexen-1-ol; (-)-4-Hydroxy-4-isopropyl-1-methyl-1-cyclohexene; 1-(ISOPROPYL)-4-METHYLCYCLOHEX-3-EN-1-OL; (+/-)-4-Hydroxy-4-isopropyl-1-methyl-1-cyclohexene; Terpinen 4-ol, primary pharmaceutical reference standard; 3-CYCLOHEXEN-1-OL; 4-METHYL-1-(1-METHYLETHYL)- (PubChem)

1.3. Molecular formula

C₁₀H₁₈O (EPISuite, 2017)

1.4. Structural Formula



1.5. Molecular weight (g/mol)

154.25

1.6. CAS registration number

562-74-3

1.7. Properties

1.7.1. Melting point

(°C): 14.7 (BPDB2022; HSDB, 2015); 14.86 (estimated) (EPISuite, 2017)

1.7.2. Boiling point

(°C): 209 at 760 mmHg (ChemSpider; EPISuite, 2017; HSDB, 2015); 212 or 88–90 (ChemSpider); 211 (BPDB, 2022)

1.7.3. Solubility

1767 mg/L at 20°C (BPDB,2022); 386.6 mg/L at 25°C (estimated) (EPISuite, 2017)

1.7.4. pKa

No dissociation (BPDB, 2022)

1.7.5. Flashpoint

(°C): 79 (closed cup) (BPDB, 2022; ChemSpider)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

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

1.7.8. Decomposition temperature

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

1.7.9. Stability

Stable, combustible, incompatible with strong oxidizing agents (ChemSpider)

1.7.10. Vapor pressure

0.0±0.9 mmHg at 25°C (estimated) (ChemSpider); 0.0427 mmHg at 25°C (estimated) (EPISuite, 2017); 53200 mPa (0.399 mmHg) at 20°C (BPDB, 2022)

1.7.11. log Kow

3.26 (EPISuite, 2017; HSDB, 2015); 3.33 (BPDB, 2022); 1.947 (ChemSpider)

2. General information

2.1. Exposure

4-Terpineol is reported used as a fragrance agent in cosmetics in the EU (CosIng).

Reported uses (ppm): (FEMA, 1994)

Food Category	Usual	Max.
Baked goods	6.85	40.66
Chewing gum	0.25	0.25
Frozen dairy	3.47	11.02
Gelatins, puddings	3.79	10.11
Gravies	3.00	6.00
Meat products	10.00	20.00
Nonalcoholic beverages	1.49	2.67
Soft candy	3.68	5.63

Estimated intake from flavouring use: 0.0008333 mg/kg bw/day.

As taken from Burdock GA, 2010.

Maximised Survey-derived Daily Intakes (MSDI-EU): 150 µg/capita/day.

As taken from EFSA, 2015.

It is used ... in artificial geranium and pepper oils and in perfumery for creating herbaceous and lavender notes. [Fahlbusch K-G et al; Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry 7th ed. (1999-2015). NY, NY: John Wiley & Sons. Online Posting Date: January 15, 2003].

In public use since the 1890's. In the United States, use in fragrances amounts for about 1 million lbs/ year. [Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 695].

As taken from HSDB, 2015.

4-Terpineol is listed as an ingredient in inside the home and auto products by the CPID.

Terpinen-4-ol (CAS RN 562-74-3) is used as a masking agent in non-medicinal natural health products (Health Canada, 2021).

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2004).

Ingredient Name & CAS Number	Chemical class	Mol. Wt. (M), bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
4-Carvomenthenol 562-74-3	Unsaturated cyclic alcohol	M=154 bp 88 at 6 mm Hg	5	92	4-Carvomenthenol 96.0 Methylisopropylbenzenes 1.7 Terpinenes 0.5 Cuminaldehyde? 0.3 Carvacrol 0.2 Benzyl isovalerate 0.2 6 minor compound (3unidentified) 1.1	0.5 0.009 0.003 0.002 0.001 0.001

						0.006

2.3. Ingredient(s) from which it originates

One of several terpinenol isomers, depending on the position of the double bond and that of the hydroxyl group, this terpene, whose structure has been identified by Wallach, can be isolated by fractional distillation. It exists in nature as the dextro, levo and racemic isomer; the synthetic product is always optically inactive.

Reported present in the oil of *Cupressus macrocarpa* lavender, Spanish *origanum*, *Ledum palustre*, *Eucalyptus australiana* var. A., *Thuja occidentalis*, etc. The l-form is present in the oil of *Eucalyptus dives* and in some other essences such as *Xanthoxylum rhetsa*, together with the racemic form. The racemic form is found in camphor oil. Reported found in fresh apple, apricots, orange juice, peel oils of orange, lemon, grapefruit, tangerines, anise, cinnamon, ginger and nutmeg.

As taken from Burdock GA, 2010.

".....volatile organic compounds in 14 honey samples (rosemary, eucalyptus, orange, thyme, sage, and lavender) were identified..... Ten chiral compounds (linalool, trans-linalool oxide, cis-linalool oxide, 4-terpineol, α -terpineol, hotrienol, and four stereoisomers of lilac aldehydes) were selected for further chiral separation." As taken from Pazitná A et al. 2014. Chirality 26(10), 670-674. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25099214>

"The essential oil obtained from *Pulicaria gnaphalodes* by hydrodistillation and analyzed by GC/Mass. Fifty-eight compounds representing 90.7% of total was identified. Main ingredient in the oil were involved α -Pinene (30.2%), 1,8-Cineole (12.1%), Beta-Citronellol (9.6%), Mertenol (6.6%), α -Terpineol (6.1%), 4-Terpineol (5.9%) and Chrysanthenone (2.9%)." As taken from Shariatifar Net al. 2014.Pak. J. Pharm. Sci.27(4), 807-812. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25015444>

"The enantiomer ratios of chiral volatile organic compounds in rapeseed, chestnut, orange, acacia, sunflower and linden honeys were determined by multi-dimensional gas chromatography using solid phase microextraction (SPME) as a sample pre-treatment procedure. Linalool oxides, linalool and hotrienol were present at the highest concentration levels, while significantly lower amounts of α -terpineol, 4-terpineol and all isomers of lilac aldehydes were found in all studied samples." As taken from Spánik I et al. 2014.Food Chem.158, 497-503. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/24731375>

Present in oil of *Melaleuca cajuputi* and its subspecies *cajuputi* (up to 5.6%), *Melaleuca alternifolia* leaf oil and its quantifications (37%), carrot seed oil, cedar leaf oil, the bark and leaf oils of *Cinnamomum burmanii* (8.5 and 4.1%, respectively), the leaf oil of *Cinnamomum cassia*, lemon oil, pepper oil, tamarind, the volatile oils of cascarilla bark, juniper berries, labdanum, magnolia flower, sweet marjoram, spearmint, olibanum, *Ligusticum* (2.7-8.0%).

As taken from Khan IA and Abourashed EA, 2010.

"In this article, contact allergy to, and the chemical composition of, tea tree oil (TTO) are reviewed. This essential oil is a popular remedy for many skin diseases, and may be used as neat oil or be present in cosmetics, topical pharmaceuticals and household products. Of all essential oils, TTO has caused most (published) allergic reactions since the first cases were reported in 1991. In routine testing, prevalences of positive patch test reactions have ranged from 0.1% to 3.5%. Nearly 100 allergic patients have been described in case reports and case series. The major constituents of commercial TTO are terpinen-4-ol, γ -terpinene, 1,8-cineole, α -terpinene, α -terpineol, p-cymene, and α -pinene. Fresh TTO is a weak to moderate sensitizer, but oxidation increases its allergenic

potency. The major sensitizers appear to be ascaridole, terpinolene, α -terpinene, 1,2,4-trihydroxymenthane, α -phellandrene, and limonene. The clinical picture of allergic contact dermatitis caused by TTO depends on the products used. Most reactions are caused by the application of pure oil; cosmetics are the culprits in a minority of cases. Patch testing may be performed with 5% oxidized TTO. Co-reactivity to turpentine oil is frequent, and there is an overrepresentation of reactions to fragrance mix I, *Myroxylon pereirae*, colophonium, and other essential oils." As taken from de Groot AC and Schmidt E. 2016. *Contact Dermatitis* 75(3), 129-43. PubMed, 2016 available at <https://www.ncbi.nlm.nih.gov/pubmed/27173437>

"Laurel, *Laurus nobilis* L. is an evergreen plant belonging to the Lauraceae family, native to Southern Europe and the Mediterranean area. This is the first report on the composition and bioactivity of laurel essential oil (EO) from Bulgaria. The oil yield was 0.78%, 0.80%, and 3.25% in the fruits, twigs, and leaves, respectively. The main constituents in the fruit EO were 1,8-cineole (33.3%), α -terpinyl acetate (10.3%), α -pinene (11.0%), β -elemene (7.5%), sabinene (6.3%), β -phellandrene (5.2%), bornyl acetate (4.4%), and camphene (4.3%); those in the twig EO were 1,8-cineole (48.5%), α -terpinyl acetate (13.1%), methyl eugenol (6.6%), β -linalool (3.8%), β -pinene (3.4%), sabinene (3.3%) and terpinene-4-ol (3.3%); and the ones in the leaf EO were 1,8-cineole (41.0%), α -terpinyl acetate (14.4%), sabinene (8.8%), methyl eugenole (6.0%), β -linalool (4.9%), and α -terpineol (3.1%). The antibacterial and antifungal properties of laurel EOs were examined according to the agar well diffusion method. The leaf EO showed antibacterial and antifungal activities against almost all strains of the microorganisms tested, whereas the twig EO was only able to inhibit *Staphylococcus aureus*. *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 8739 were the bacterial strains that showed the highest resistance to the laurel EO. The results can benefit the EO industry and biopesticide development." As taken from Fidan H et al. 2019. *Molecules* 24(4), E804. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30813368>

"The chemical composition of 18 oil samples of *Santolina africana* isolated from aerial parts at full flowering, collected in three locations in eastern Algeria was determined by GC(RI), GC/MS and ^{13}C -NMR analysis. The major components were: germacrene D, myrcene, spathulenol, α -bisabolol, β -pinene, 1,8-cineole, cis-chrysanthenol, capillene, santolina alcohol, camphor, terpinen-4-ol and lyratol. The chemical composition appeared homogeneous and characterized by the occurrence of four derivatives which exhibited a conjugated alkene dialkyne moiety. They were identified for the first time in an essential oil from *S. africana*. The collective oil sample exhibited moderate antimicrobial and antioxidant activities whereas the anti-inflammatory activity presented a real potential. IC₅₀ value of *Santolina africana* essential oil (0.065 ± 0.004 mg/mL) is 5-fold higher than IC₅₀ value of NDGA used as positive control." As taken from Malti CEW et al. 2019. *Molecules* 24(1), E204. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30626015>

"Even though essential oils from *Piper aduncum* (Piperaceae) have different biological activities, little is known about their application to agricultural areas. White mold is a plant disease caused by the phytopathogen *Sclerotinia sclerotiorum*, which needs to be controlled by alternative measures. This study aimed at evaluating the effect of essential oils from *P. aduncum* leaves (PL-EO) and inflorescences (PI-EO) on the mycelial growth of *S. sclerotiorum*. Essential oils from *P. aduncum* were obtained by hydrodistillation by a Clevenger-type apparatus while their chemical composition was analyzed by GC-MS and GC-FID. Piperitone (23.4 %), myristicin (12.4 %), terpinen-4-ol (12.3 %), β -caryophyllene (7.2 %), α -humulene (6.9 %), germacrene-D (6.9 %) and dillapiol (6.3 %) were the main constituents found in oils from *P. aduncum*. The in vitro antifungal activity showed that PI-EO dose above 30 μL inhibited mycelial growth in 100 %, whereas PL-EO at 50 μL inhibited it in 98.74 %. This is the first report of the chemical composition of PI-EO and results suggest that the essential oils under evaluation have high potential to control the phytopathogenic fungus *S. sclerotiorum*." As taken from Valadares ACF et al. 2018. *An. Acad. Bras. Cienc.* 90(3), 2691-2699. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30304214>

"The chemical composition of essential oils isolated from the aerial parts of *Heracleum lehmannianum*, *Prangos pabularia*, *Pseudohandelia umbellifera* and *Pulicaria salviifolia*, all of them growing in Uzbekistan, were determined by GC-MS analysis. The main components of the oil from *H. lehmannianum* were α -phellandrene (10.5%), 1-butanol (9.0%), δ -cadinene (6.2%), α -cadinol (5.7%), τ -muurolol (3.1%), 4-terpineol (2.4%) and α -muurolene (2.6%), while *cis*-allo-ocimene (17.6%), δ -3-carene (14.2%), limonene (7.6%), 2,4,6-trimethylbenzaldehyde (6.8%), α -terpinolene (6.1%), β -ocimene (4.3%), α -ocimene (4.2%), α -phellandrene (4.2%) were the major oil components in *P. pabularia*, and borneol (4.4%), τ -cadinol (4.1%), α -humulene oxide (4.0%), caryophyllene oxide (3.6%), bornyl chloride (3.1%), β -pinene (2.9%) in *P. umbellifera*. The essential oil of *P. salviifolia* had a much more complex composition which was dominated by 4-terpineol (13.4%), α -cadinol (5.7%), 6-epi-shyobunol (5.2%), γ -terpinene (5.0%), δ -cadinene (4.4%), α -terpinene (3.5%)." As taken from Mamadalieva NZ et al. 2018. Nat. Prod. Res. 32(9), 1118-1122. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/28906143>

"*Hypericum* L. (Hypericaceae) extracts have been used for their therapeutic effects; however, not much is known about the immunomodulatory activity of essential oils extracted from this plant. We isolated essential oils from the flowers and leaves of *H. perforatum* and analyzed their chemical composition and innate immunomodulatory activity. Analysis of flower (HEOFI) versus leaf (HEOLv) essential oils using gas chromatography-mass spectrometry revealed that HEOFI was comprised mainly of monoterpenes (52.8%), with an abundance of oxygenated monoterpenes, including *cis*-p-menth-3-en-1,2-diol (9.1%), α -terpineol (6.1%), terpinen-4-ol (7.4%), and limonen-4-ol (3.2%), whereas the sesquiterpenes were found in trace amounts. In contrast, HEOLv was primarily composed of sesquiterpenes (63.2%), including germacrene D (25.7%) and β -caryophyllene (9.5%). HEOLv also contained oxygenated monoterpenes, including terpinen-4-ol (2.6%), while monoterpene hydrocarbons were found in trace amounts. Both HEOFI and HEOLv inhibited neutrophil Ca^{2+} mobilization, chemotaxis, and reactive oxygen species (ROS) production, with HEOLv being much more active than HEOFI. Furthermore, the pure sesquiterpenes germacrene D, β -caryophyllene, and α -humulene also inhibited these neutrophil responses, suggesting that these compounds represented the active components of HEOLv. Although reverse pharmacophore mapping suggested that potential protein targets of germacrene D, β -caryophyllene, bicyclogermacrene, and α -humulene could be PIM1 and mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MAPKAK2), a kinase binding affinity assay did not support this finding, implying that other biological targets are involved. Our results provide a cellular and molecular basis to explain at least part of the beneficial immunotherapeutic properties of the *H. perforatum* essential oils." As taken from Schepetkin IA et al. 2020. Biomolecules 10(6), 916. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32560389/>

"The chemical composition and biological activities of the essential oil (EO) from the rhizomes of *Zingiber striolatum* Diels were reported for the first time. Forty-five compounds were identified, and represented 95.7% of the total composition of the EO. The predominant components of the EO were β -phellandrene (24.0%), sabinene (17.3%), β -pinene (11.4%), geranyl linalool (8.6%), terpinen-4-ol (8.3%), α -pinene (5.6%) and crypton (4.5%). The EO revealed a weak DPPH and ABTS radical-scavenging activity. The EO exhibited significant antimicrobial activity with the inhibition zones (12.86-24.62 mm) and MIC (0.78-3.12 mg/mL) against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*. The EO showed significant cytotoxicity against human leukemic (K562), lung cancer (A549) and prostatic carcinoma (PC-3) cell lines with the IC_{50} values of 29.67, 48.87 and 86.05 $\mu\text{g/mL}$, respectively. Thus, the EO could be regarded as a bioactive natural product with potential for utilization in the cosmetic and pharmaceutical industry." As taken from Tian M et al. 2020. Nat. Prod. Res. 34(18), 2621-2625. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/30908095/>

" $\pm$ -Terpinene-4-ol is a natural product that can be found in a number of plants, such as allspice, anise, apple, basil, cardamom, cinnamon and *Melaleuca alternifolia* (also called tea tree) and is the

main bioactive component of tea tree oil (PMID 22083482). $\pm$ -Terpinene-4-ol is also one of the monoterpenes found in cannabis plants (PMID:6991645).”

HMDB (2022).

3. Status in legislation and other official guidance

4-Carvomenthenol is included on the FDA's inventory of “Substances Added to Food (formerly EAFUS)” as a flavor enhancer and a flavoring agent or adjuvant and is included under 21 CFR section 172.515 (Synthetic flavoring substances and adjuvants) (FDA, 2024).

4-Carvomenthenol has been reported as used in fragrance compounds (IFRA).

CoE: Used provisionally. Food: 50 ppm (Burdock GA, 2010).

There is a REACH dossier on p-menth-1-en-4-ol (CAS RN 562-74-3) (ECHA).

p-Menth-1-en-4-ol (CAS RN 562-74-3) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2025).

4-Carvomenthenol (CAS RN 562-74-3) is listed in the US EPA InertFinder Database as approved for food and non-food use as well as for fragrance use pesticide products (US EPA InertFinder Database).

4-Terpinenol is authorised for use as a flavouring substance in all categories of flavoured foods in the EU under EU No 872/2012.

CAS No.	0000562-74-3
Chemical name	4-Terpinenol
Purity of the named substance at least 95% unless otherwise specified	
FI No.	02.072
CoE No.	2229
JECFA No.	439
Flavouring type	substances
Mixture	No
Reference body	

As taken from European Commission.

3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (CAS RN 562-74-3) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2024 CDR list (Chemical Data Reporting Rule).

US EPA Substance Registry Services (SRS).4-Carvomenthenol (FEMA no. 2248) has been given GRAS (generally recognized as safe) status by FEMA (Hall and Oser, 1965).

Terpinen-4-ol (CAS RN 562-74-3) is “classified as an NHP under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations” (Health Canada, 2021).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

The in vitro metabolism of (-)-terpinen-4-ol was examined in human liver microsomes and recombinant enzymes. The biotransformation of (-)-terpinen-4-ol was investigated by gas chromatography-mass spectrometry. (-)-Terpinen-4-ol was found to be oxidized to (-)-(1S,2R,4R)-1,2-epoxy-p-menthan-4-ol, major metabolic product by human liver microsomal P450 enzymes. The formation of metabolites of (-)-terpinen-4-ol was determined by relative abundance of mass fragments and retention times on GC. CYP2A6 in human liver microsomes was a major enzyme involved in the oxidation of (-)-terpinen-4-ol by human liver microsomes, based on the following lines of evidence. First, of 11 recombinant human P450 enzymes tested, CYP2A6 had the highest activity for oxidation of (-)-terpinen-4-ol. Second, oxidation of (-)-terpinen-4-ol was inhibited by (+)-menthofuran. Finally, there was a good correlation between CYP2A6 maker activity and (-)-terpinen-4-ol oxidation activities in liver microsomes of 10 human samples. Kinetic analysis showed that the $V(\max)/K(m)$ values for (-)-(1S,2R,4R)-1,2-epoxy-p-menthan-4-ol catalysed by liver microsomes of human sample HH-18 was 2.49 $\mu\text{L}/\text{min}/\text{nmol}$. Human recombinant CYP2A6 catalysed (-)-(1S,2R,4R)-1,2-epoxy-p-menthan-4-ol with $V(\max)$ values of 13.9 $\text{nmol}/\text{min}/\text{nmol}$ P450 and apparent $K(m)$ values of 91 μM (Miyazawa M, Haigou R, 2011).

We examined the in vitro metabolism of (+)-terpinen-4-ol by human liver microsomes and recombinant enzymes. The biotransformation of (+)-terpinen-4-ol was investigated by gas chromatography-mass spectrometry (GC-MS). (+)-Terpinen-4-ol was found to be oxidized to (+)-(1R,2S,4S)-1,2-epoxy-p-menthan-4-ol, (+)-(1S,2R,4S)-1, 2-epoxy-p-menthan-4-ol, and (4S)-p-menth-1-en-4,8-diol by human liver microsomal P450 enzymes. The identities of (+)-terpinen-4-ol metabolites were determined through the relative abundance of mass fragments and retention times on GC-MS. Of 11 recombinant human P450 enzymes tested, CYP1A2, CYP2A6, and CYP3A4 were found to catalyze the oxidation of (+)-terpinen-4-ol. Based on several lines of evidence, CYP2A6 and CYP3A4 were determined to be major enzymes involved in the oxidation of (+)-terpinen-4-ol by human liver microsomes. First, of the 11 recombinant human P450 enzymes tested, CYP1A2, CYP2A6 and CYP3A4 catalyzed oxidation of (+)-terpinen-4-ol. Second, oxidation of (+)-terpinen-4-ol was inhibited by (+)-menthofuran and ketoconazole, inhibitors known to be specific for these enzymes. Finally, there was a good correlation between CYP2A6 and CYP3A4 activities and (+)-terpinen-4-ol oxidation activities in the 10 human liver microsomes (Haigou R, Miyazawa M, 2012).

4.2. Absorption, distribution and excretion

BACKGROUND: The terpenes disturb lipid arrangement in the intercellular region of the stratum corneum (SC) that leads to the increased permeability of the skin. This effect is used in technology of transdermal drug forms and depends on physicochemical properties of terpenes and their amounts penetrated to the stratum corneum; however terpenes do not need penetrate into viable skin tissue and this event is not even desired. **OBJECTIVE:** To correlate skin absorption and elimination kinetics of four cyclic terpenes, namely alpha-pinene, beta-pinene, eucalyptol and terpinen-4-ol, applied as neat substance with their physicochemical properties. **METHODS:** The terpenes were applied onto the human skin in vitro, and after 1-4 h their content in the separated by a tape-stripping method stratum corneum layers and in the epidermis/dermis was determined using GC. Similarly, the amounts of terpenes in the skin were analysed during 4 h following 1 h absorption. **RESULTS:** The fastest and progressive penetration into all skin layers was observed for terpinen-4-ol. All studied terpenes are absorbed in the viable epidermis/dermis, however penetration into this layers is time-dependent process, constantly increasing during 4 h. Like for stratum corneum, the largest cumulation in epidermis/dermis was observed for terpinen-4-ol. The elimination of terpenes from the stratum corneum was fast, especially in deeper layers, and much faster if the initial cumulation was small. **CONCLUSION:** Investigated cyclic terpenes represent different penetration and elimination characteristics and do not permeate across the skin to the acceptor medium due to large cumulation in the skin tissue. The penetration of terpenes into stratum corneum is greater if their log P-value is close to 3 (Cal K, Kupiec K, Sznitowska M, 2006).

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. Penetration of terpinen-4-ol to the skin was better from an oily solution (approximately 90 microg/cm (2)) than from an emulsion (60 microg/cm (2)). 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. In contrast, the cutaneous absorption of linalool was the same from both vehicles (50-60 microg/cm (2)). The skin penetration of alpha-pinene was not traceable when it was applied in an oily solution. Only a small amount (approximately 5 microg/cm (2)) of this terpene was determined in viable skin after application as a hydrogel. Citronellol applied in a hydrogel penetrated into all skin layers in a total amount of 25 microg/cm (2), while no penetration into viable skin layers after application of an oily solution was noted. Only citronellol permeated into the acceptor medium (Cal K, 2006).

The purpose of this study was to investigate dermal pharmacokinetics of terpinen-4-ol in rats following topical administration of plai oil derived from the rhizomes of *Zingiber cassumunar* Roxb. Unbound terpinen-4-ol concentrations in dermal tissue were measured by microdialysis. The dermal pharmacokinetic study of terpinen-4-ol was performed under non-occlusive conditions. The oil was topically applied at a dose of 2, 4, and 8 mg/cm² plai oil corresponding to the amount of 1.0, 1.9, and 3.8 mg/cm² terpinen-4-ol, respectively. Following topical application of the oil, terpinen-4-ol rapidly distributed into the dermis and demonstrated linear pharmacokinetics with no changes in the dose-normalized area under the concentration-time curves across the investigated dosage range. The mean percentages of free terpinen-4-ol distributed in the dermis per amount of administered were 0.39 ± 0.06 %, 0.41 ± 0.08 %, and 0.30 ± 0.03 % for 2, 4, and 8 mg/cm² doses, respectively. The dermal pharmacokinetics of terpinen-4-ol could provide information for its further formulation development and therapy schedules (Chooluck K et al., 2012).

Terpinen-4-ol, a naturally occurring monoterpene, has been shown to possess antibacterial, antioxidant and anti-inflammatory activities. Furthermore, recent reports have demonstrated that terpinen-4-ol could be developed as new therapies against melanoma either in systemic administration or targeted drug delivery. The purpose of this study was to investigate the pharmacokinetics of terpinen-4-ol in rat plasma and dermal tissue following intravenous (i.v.) bolus injection of terpinen-4-ol at a dose of 2 mg/kg. Unbound concentrations of terpinen-4-ol in dermis were continuously determined by dermal microdialysis. Simultaneously, a conventional blood sampling was performed. The concentrations of terpinen-4-ol in plasma and microdialysates were determined by validated gas chromatography-mass spectrometry. Following i.v. bolus administration, terpinen-4-ol rapidly distributed into the dermis and reached relatively low levels with an average maximum concentration (C_{max}) of 0.10 ± 0.06 microg/ml in comparison with a plasma C_{max} of 6.30 ± 1.90 microg/ml. The free terpinen-4-ol concentrations in dermal tissue were lower than the corresponding total and free plasma concentrations for the entire length of study, indicating that plasma levels do not provide information of actual terpinen-4-ol concentrations in the skin. This study demonstrates that dermal microdialysis is an effective and minimally invasive tool to evaluate the dermal pharmacokinetics of terpinen-4-ol following systemic administration (Chooluck K et al., 2013).

"Effective application of the essential oils requires detailed exploration of their mechanism of action and the origin of diverse activity of their components. In this work, the influence of eucalyptol and terpinen-4-ol on artificial membranes was studied to verify whether the differences in the activity of these compounds are related to their effect on membranes. The properties of monolayers formed from structurally different lipids in the presence of terpenes were examined based on the results of the surface pressure-area measurements, penetration studies, and Brewster angle microscopy experiments. Both compounds were able to incorporate into the membrane and alter lipid/lipid

interactions, making the monolayer less stable and more fluid. These effects were determined by monolayer composition (but not by its condensation per se) and the resulting rheological properties and were stronger in the presence of terpinen-4-ol. These findings confirm the hypothesis that differences in the antimicrobial potency of these terpenes are membrane-related, and membrane composition may determine their selectivity.” As taken from H-àc-Wydro K et al. 2017. *Langmuir* 33(27), 6916-6924. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28654274>

4.3. Interactions

This work aimed to evaluate the effect induced by excipients conventionally used for topical dosage forms, namely isopropyl myristate (IPM) or oleic acid (OA) or polyethylene glycol 400 (PEG400) or Transcutol (TR), on the human skin permeability of terpinen-4-ol (T4OL) contained in the pure Tea tree oil. The effect of such excipients was determined by evaluating the absorption of T4OL using human epidermis and the perturbation of the organization of stratum corneum by ATR-FTIR. Among the tested excipients OA enhanced the absorption of T4OL by perturbing the stratum corneum lipid barrier. Other excipients caused a weak enhancement effect and their use should be carefully monitored (Casiraghi A et al., 2010).

“The combined effect of terpinen-4-ol, the main component of tea tree oil, and capric acid against mycelial growth of *Candida albicans* and murine oral candidiasis was evaluated in vitro and in vivo. Mycelial growth of *C. albicans* was estimated by the Cristal violet method. Combination of these compounds revealed a potent synergistic inhibition of growth. Therapeutic efficacy of the combination was evaluated microbiologically in murine oral candidiasis, and its application of the compounds clearly demonstrated therapeutic activity. Based on these results, the combined agent of terpinen-4-ol and capric acid was discussed as a possible candidate for oral candidiasis therapy.” As taken from Ninomiya K et al. 2013a. *Yakugaku Zasshi* 133(1), 133-40. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23292030?dopt=AbstractPlus>

"BACKGROUND: *Glossogyne tenuifolia* (GT) is a perennial herb widely distributed in the areas from south Asia to Australia. Many biological effects of *G. tenuifolia* have been reported; however, the information about antimicrobial activity of the essential oil (EO) of the herb remains unavailable. Therefore, the aims of this study were to investigate the antimicrobial activity of the GT-EO in vitro and food systems, the antimicrobial impact (AI) of its individual compounds, and interactive effects of major active compounds (linalool, 4-terpineol, α -terpineol, p-cymene) on selected Gram-positive (*S. aureus*, *L. monocytogenes*, *S. mutans* and *S. sanguinis*) and Gram-negative (*E. coli* O157:H7, *V. parahaemolyticus* and *S. enterica*) pathogens. RESULTS: The minimal microbicidal concentration (MMC) of the GT-EO ranged from 0.75 to 12 mg mL⁻¹ against the test bacteria in vitro. Except for *L. monocytogenes*, the GT-EO exhibited more inhibitory effect on the selected Gram positive than against the Gram negative bacteria at the GT-EO concentrations $\leq$ 12 mg mL⁻¹. The interactive effects of major active compounds (linalool, 4-terpineol, α -terpineol, p-cymene) are additive instead of synergistic via the checkerboard analysis. The bacteria with a microbial load of ca. 10² CFU mL⁻¹ in the milk tea could be completely inactivated by the GT-EO with the MMC of 1.5 mg mL⁻¹. CONCLUSION: p-Cymene is the largest component in the GT-EO; however, it is not the compound predominantly affecting the entire antimicrobial activity of the EO. Instead, 4-terpineol is the most influential among the test compounds that contribute to the antimicrobial activity of the GT-EO.” As taken from Yang TS et al. 2014. *J. Sci. Food Agric.* 94(14), 2965-2971. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24615540>

“Terpinen-4-ol (4TRP) is a monoterpenoid alcoholic component of essential oils obtained from several aromatic plants. We investigated the psychopharmacological and electrophysiological activities of 4TRP in male Swiss mice and Wistar rats. 4TRP was administered intraperitoneally (i.p.) at doses of 25 to 200 mg/kg and intracerebroventricularly (i.c.v.) at concentrations of 10, 20, and 40 ng/2 μ L. For in vitro experiments, 4TRP concentrations were 0.1mM and 1.0mM. 4TRP (i.p.)

inhibited pentylenetetrazol- (PTZ-) induced seizures, indicating anticonvulsant effects. Electroencephalographic recordings showed that 4TRP (i.c.v.) protected against PTZ-induced seizures, corroborating the behavioural results. To determine whether 4TRP exerts anticonvulsant effects via regulation of GABAergic neurotransmission, we measured convulsions induced by 3-mercapto-propionic acid (3-MP). The obtained results showed involvement of the GABAergic system in the anticonvulsant action exerted by 4TRP, but flumazenil, a selective antagonist of the benzodiazepine site of the GABAA receptor, did not reverse the anticonvulsant effect, demonstrating that 4TRP does not bind to the benzodiazepine-binding site. Furthermore, 4TRP decreased the sodium current through voltage-dependent sodium channels, and thus its anticonvulsant effect may be related to changes in neuronal excitability because of modulation of these channels. [Nobrega FF et al; Oxid Med Cell Longev (2014)]

As taken from HSDB, 2015.

“The purpose of this study was to investigate the protective effects of Terpinen-4-ol (TER) on dextran sulfate sodium (DSS)-induced experimental colitis and clarify the possible mechanisms. In vivo, an acute colitis model was used to confirm the anti-inflammatory activity and the possible mechanisms of TER in C57BL/6 and NLRP3^{-/-} mice. In vitro, we performed further study, using RAW264.7 cells and Caco-2 cells, to confirm the molecular mechanisms of TER on inflammatory response. In C57BL/6 mice, TER alleviated DSS-induced disease activity index (DAI), colon length shortening, colonic pathological damage, and myeloperoxidase (MPO) activities. The production of pro-inflammatory mediators was significantly decreased by TER. Furthermore, TER inhibited NF-κB and NLRP3 inflammasome activation. Surprisingly, TER reduced the plasmatic lipopolysaccharide (LPS) concentration and re-balanced *Escherichia coli* (*E. coli*) and *Lactobacillus* levels. In addition, TER prevented the impairment of colon epithelium barrier by regulating the expression of zonula occludens-1 and occludin. In vitro, the results showed that TER significantly suppressed NLRP3 inflammasome activation in LPS-stimulated RAW264.7 cells, as indicated by decreased expression of NLRP3 and caspase-1, and lowered interleukin-1β secretion. In contrast, mice deficient for NLRP3 were less sensitive to DSS-induced acute colitis, and TER treatment exerted little protective effect on DSS-induced intestinal inflammation in NLRP3^{-/-} mice. The protective effect of TER may be largely attributed to its inhibition of NLRP3 inflammasome activation in colon. Taken together, our findings showed that TER might be a potential agent for the treatment of ulcerative colitis.” As taken from Zhang Z et al. 2017. Front Immunol. 8, 558. PubMed 2018, available at <https://www.ncbi.nlm.nih.gov/pubmed/28553294>

“It has been reported that natural transdermal permeation enhancers (TPEs) are superior in safety compared with synthetic TPEs. The essential oil (EO) of *Ledum palustre* L. var. *angustum* N. Busch had a strong enhancement effect on drug skin permeation based on previous studies. However, their enhancement mechanisms and safety were still unclear. The composition of the EO was determined using GC-MS. By using donepezil (DNP) as a model drug, the enhancement effect of the constituents of the EO and the EO were evaluated by in vitro skin permeation test. Confocal laser scanning microscopy (CLSM), attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) and molecular docking were used to investigate the interaction among drug, enhancers and skin. Skin retention amount, apparent partition coefficient (*K'*) and molecular simulation were used to reflect the effect of the enhancers on drug partition into skin. The skin irritation potential was evaluated using in vivo skin erythema analysis. The results showed that the main constituents of the EO were sabinene (SA), 4-terpineol (TE), p-cymene (CY) and cuminaldehyde (CU). CU was the main active constituent of the EO, which facilitated skin permeation of DNP. CU improved the skin permeation of DNP by increasing the mobility of the stratum corneum (SC) intercellular lipids, decreasing the interaction between DNP and the SC intercellular lipids, and improving the partition of DNP into the SC layer. Besides the superior enhancement effect, CU also showed a lower skin irritation potential compared with the EO. This work gave us some enlightenment that the effectiveness and safety of the natural transdermal permeation enhancers could be improved by understanding their composition and the

enhancement mechanisms.” As taken from Nan L et al. 2018. Eur. J. Pharm. Sci. 124, 105-113. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30153525>

“Terpinen-4-ol, the major constituent of tea tree oil, has been reported to have anti-inflammatory effect. However, whether terpinen-4-ol could attenuate LPS-induced inflammation in lung tissues remains unclear. In the present study, we aimed to investigate the protective effects of terpinen-4-ol on LPS-induced acute lung injury (ALI) in mice. Terpinen-4-ol could inhibit LPS-induced ALI as confirmed by the decreased lung histopathological changes, MPO activity, and lung W/D ratio caused by terpinen-4-ol. The production of TNF- α and IL-1 β in the BALF was suppressed by the treatment of terpinen-4-ol. Western blot analysis showed that terpinen-4-ol significantly attenuated LPS-induced phosphorylation of I κ B α and NF- κ B p65. Furthermore, the expression of PPAR- γ was dose-dependently upregulated by the treatment of terpinen-4-ol. In conclusion, the results of this study indicated that terpinen-4-ol inhibited LPS-induced ALI via activating PPAR- γ , which subsequently attenuated LPS-induced NF- κ B activation and inflammatory response.” As taken from Ning J et al. 2018. Inflammation 41(6), 2012-2017. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30003404>

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose	Effect	Reference
mouse	LD50	intramuscular	780mg/kg	KIDNEY, URETER, AND BLADDER: URINE VOLUME INCREASED	Cesko-Slovenska Farmacie. Vol. 8, Pg. 433, 1959.
mouse	LD50	intraperitoneal	250mg/kg	KIDNEY, URETER, AND BLADDER: URINE VOLUME INCREASED	Cesko-Slovenska Farmacie. Vol. 8, Pg. 433, 1959.
mouse	LD50	oral	1016mg/kg		Zhonghua Jiehe He Huxixi Jibing Zazhi. Chinese Journal of Tuberculosis and Respiratory Diseases. Vol. 4(4), Pg. 203, 1981.
mouse	LD50	subcutaneous	750mg/kg	KIDNEY, URETER, AND BLADDER: URINE VOLUME INCREASED	Cesko-Slovenska Farmacie. Vol. 8, Pg. 433, 1959.
rabbit	LD50	skin	> 2500mg/kg		Food and Chemical Toxicology. Vol. 20, Pg. 833, 1982.
rat	LD50	intramuscular	1500mg/kg	KIDNEY, URETER, AND BLADDER: URINE VOLUME INCREASED	Cesko-Slovenska Farmacie. Vol. 8, Pg. 433, 1959.
rat	LD50	oral	1300mg/kg		Food and Chemical Toxicology. Vol. 20, Pg. 833, 1982.

As taken from PubChem

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Intravenous	Rodent - rat	1 mg/kg	Vascular - BP lowering not characterized in autonomic section	FCLPH* Fundamental & Clinical Pharmacology (Oxford : Blackwell Science) 2001- Volume(issue)/page/year: 17,323,2003
TDLo - Lowest published toxic dose	Unreported	Rodent - mouse	100 mg/kg	Behavioral - analgesia	FCLPH* Fundamental & Clinical Pharmacology (Oxford : Blackwell Science) 2001- Volume(issue)/page/year: 18,51,2004

As taken from RTECS, 2021.

5.2. Repeated dose toxicity

For terpineol a NOAEL of 250 mg/kg bw per day was identified in a 5-week study in rats (doses: 0, 60, 250 and 750 mg/kg bw per day, administration route: gavage; GLP compliant, OECD Guideline 422). Males receiving 750 mg/kg bw per day showed evidence of testicular and epididymal toxicity leading to infertility. The NOAEL was set at 250 mg/kg bw per day where fertility of male was unaffected by the test substance and no other effects were reported. In females, effects on the liver were reported with centrilobular hepatocyte hypertrophy and increased plasma level of bile acids and glucose at 750 mg/kg bw per day indicating an alteration of the metabolic function of the liver following administration of terpineol.

A group NOAEL of 250 mg/kg bw can be used for terpineol, α -terpineol, terpineol acetate and 4-terpinenol (EFSA, 2013).

“The repeated dose toxicity data on 4-carvomenthenol are insufficient for the repeated dose toxicity endpoint. Read-across material terpineol (CAS # 8000-41-7; see Section VI) has sufficient repeated dose toxicity data.

In a GLP/OECD 413 guideline study, Crl:CD(SD) male and female rats (10/sex/group) were exposed to terpineol multiconstituent by snout-only inhalation route at 0.202, 0.572, and 2.23 mg/L (actual levels) for 13 weeks (6 h/day; 5 days/week), corresponding to 0, 52, 148 or 578 mg/kg/day according to standard minute volume and body weight parameters for Sprague Dawley rats. The MMAD was between 0.52 and 1.6 μ M, and the respective GSD was between 2.99 and 1.75. A 4-week, treatment-free recovery group of 10/sex/group of control and high-dose group animals was also included. The nasal cavity was identified as a target organ for local effects. A significant reduction in mean group bodyweight gain among males of the high-dose group was observed. Examination of recovery phase animals showed no changes in the nasal pharynx respiratory epithelium, suggesting complete recovery after 4 weeks which is therefore not considered adverse. The group mean reticulocyte percentage and the absolute reticulocyte count were significantly lower than control values for males of the high-dose group. This alteration was not present among the recovery group animals. In addition, there were no other related hematological alterations reported among treatment or recovery group animals as compared to control. Thus the NOAEL for the repeated dose toxicity endpoint was determined to be 2.23 mg/L, the highest dose tested, equivalent to 578 mg/kg/day according to standard minute volume and body weight parameters for Sprague Dawley rats (ECHA, 2017a; the ECHA dossier on this material uses terpineol data as read-across).

In another study, an OECD 422 gavage combined repeated dose toxicity study with the reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. There were 3 treatment groups. The reproductive subgroup (main phase) consisted of 10 males and 10 females/dose (except for control males and at top dose: 5 males/ dose) administered terpineol at doses of 0, 60, 250, and 750 mg/kg/day. The toxicity subgroup consisted of 5 females/dose group and 10 males/ dose group, administered terpineol at doses of 0, 60, 250, and 750 mg/ kg/day. Main phase males and toxicity phase females were dosed daily for a minimum of 5 consecutive weeks. An additional 5 rats/sex/dose were dosed with the vehicle or 750 mg/kg/day for 5 weeks and then given 2 weeks of recovery before termination. Although there were alterations in liver weight, clinical chemistry, and histopathological alterations, all the effects were reversible hence not considered adaptive and not adverse (Hall et al., 2012). Histopathological changes associated with hyaline droplets were observed in the kidneys of male rats receiving 250 or 750 mg/kg/day, such changes are commonly associated with administration of volatile hydrocarbons and are of no consequence to human risk assessment (Lehman-McKeeman and Caudill, 1992 and Lehman-McKeeman et al., 1990). In addition, the kidney weights and histopathology among recovery group animals were similar to the control. The repeated dose toxicity NOAEL was determined to be 750 mg/kg/day, the highest dose tested (ECHA, 2017a; the ECHA dossier on this material uses terpineol data as read-across).

The most conservative NOAEL of 578 mg/kg/day from the 90-day inhalation toxicity study was selected for the repeated dose toxicity endpoint.

Therefore, the 4-carvomenthenol MOE for the repeated dose toxicity endpoint can be calculated by dividing the terpineol NOAEL in mg/kg/ day by the total systemic exposure to 4-carvomenthenol, 578/0.00043, or 1344186.

In addition, the total systemic exposure to 4-carvomenthenol (0.43 µg/kg/day) is below the TTC (30 µg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.”

“Terpineol (CAS # 8000-41-7) could be used as a structurally similar read-across analog for the target material 4-carvomenthenol (CAS # 562-74-3) for the repeated dose toxicity and reproductive toxicity endpoints.

- o The target material and the read-across analog are structurally similar and belong to a class of unsaturated cyclic tertiary terpene alcohols.
- o The target material and the read-across analog have a cycloalkene (2-(4-methylcyclohex-3-en-1-yl)propan-2-ol) fragment common among them.
- o The key difference between the target material and the read-across analog is that the target material has isopropyl branching near the hydroxy group while the read-across analog has 2 methyl groups flanking the hydroxy group. This makes the target material’s hydroxy group more sterically hindered compared to the hydroxy group in the read-across analog. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
- o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.

o According to the QSAR OECD Toolbox (v4.2), structural alerts for the skin sensitization, repeated dose, and reproductive endpoints are consistent between the target material and the read-across analog. The CAESAR model v.2.1.6 predicts the target and the read-across analog to be sensitizers. Other protein binding alerts for both of the substances are negative. The data described in the skin sensitization section above shows that the read-across analog does not pose a concern for the skin sensitization endpoint. Therefore, this alert will be superseded by the availability of data. In addition, the target material and read-across analog are predicted to be a toxicant for the developmental endpoint with good reliability only by the CAESAR model v.2.1.6. The data described in the developmental and reproductive section supports that the readacross material is safe to use within a given MOE and level of use for developmental toxicity endpoint, so these in silico predictions will be superseded.

o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator. Due to structural differences and more steric hindrance, the target material shows a fewer number of metabolic transformations compared to the read-across analog, which increases in vivo reactivity of the read-across analog.

o The structural differences between the target material and the read-across analog are deemed to be toxicologically insignificant for the skin sensitization, repeated dose, developmental, and reproductive endpoints.” As taken from Api AM. (2022). Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/562-74-3_1.pdf

5.3. Reproduction toxicity

p-Menth-1-en-4-ol is suspected to be toxic for reproduction. The CAESAR developmental toxicity model in VEGA (Q)SAR platform predicts that the chemical is a toxicant (good reliability) (ECHA, 2016).

The reliability and applicability of this QSAR prediction as standalone source of toxicological information is limited and inappropriate for some complex endpoints like reprotoxicity or carcinogenicity. Nevertheless, for the toxicological assessment of this ingredient, this result was still taken into consideration and used within the WoE approach as a supportive tool, in combination with other sources of information when available, like experimental data or appropriate read-across.

ECHA Annex III has been withdrawn as of April 2023: “The list of substances that are unlikely to benefit from the reduced information requirements, the so-called ‘Annex III inventory’, has been withdrawn.”

“There are no reproductive toxicity data on 4- carvomenthenol. Read-across material terpineol (CAS # 8000-41-7) was used to support the reproductive toxicity endpoint.

An OECD 422 gavage combined repeated dose toxicity study with the reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. There were 3 treatment groups. The reproductive subgroup (main phase) consisted of 10 males and 10 females/dose (except for control males and at top dose: 5 males/dose) administered terpineol at doses of 0, 60, 250, and 750 mg/kg/day. The toxicity subgroup consisted of 5 females/dose group and 10 males/dose group, administered terpineol at doses of 0, 60, 250, and 750 mg/kg/day. Main phase males and toxicity phase females were dosed daily for a minimum of 5 consecutive weeks. An additional 10 rats/sex/dose were dosed with the vehicle or 750 mg/kg/day for 5 weeks and then given 2 weeks of recovery before termination. There were no adverse effects on the development of the fetus up to 250 mg/kg/day. At 750 mg/kg/day, no females became pregnant. It is considered that the testicular and epididymal effects observed in males receiving 750 mg/kg/day would have been sufficient to prevent fertilization. Thus the NOAEL for the developmental toxicity endpoint was determined to be

more than 250 mg/kg/day (ECHA, 2017a; the ECHA dossier on this material uses terpineol data as read-across).

In another study, terpineol multiconstituent diluted in corn oil was administered by gavage to groups of mated female Sprague Dawley rats (20 mated females/dose) at the dose levels of 0, 60, 200, 600 mg/kg bw/ day from days 6–19 after mating. The test was conducted according to the OECD 414 protocol. Embryo-fetal growth was slightly reduced by maternal treatment as evidenced by reduced mean male and female fetal weight at 600 mg/kg/day. In addition, mean placental weight in this dose group was slightly low, with differences attaining statistical significance. Mean placental, litter, and fetal weights at 60 or 200 mg/kg/ day were unaffected by maternal treatment with terpineol. The incidence of major and minor abnormalities and skeletal variants showed no relationship to maternal treatment with terpineol. Thus the NOAEL for the developmental toxicity was determined to be 200 mg/kg/day (ECHA, 2017a; the ECHA dossier on this material uses terpineol data as read-across).

The most conservative NOAEL of 200 mg/kg/day was selected for the developmental toxicity endpoint.

Therefore, the terpineol MOE for the developmental toxicity endpoint can be calculated by dividing the terpineol NOAEL by the total systemic exposure to terpineol, $200/0.00043$ or 465116.

An OECD 422 gavage combined repeated dose toxicity study with the reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. There were 3 treatment groups. There were 3 treatment groups. The reproductive subgroup (main phase) consisted of 10 males and 10 females/dose (except for control males and at top dose: 5 males/dose) administered terpineol at doses of 0, 60, 250, and 750 mg/kg/day. The toxicity subgroup consisted of 5 females/dose group and 10 males/dose group, administered terpineol at doses of 0, 60, 250, and 750 mg/kg/day. Main phase males and toxicity phase females were dosed daily for a minimum of 5 consecutive weeks. An additional 10 rats/sex/dose were dosed with the vehicle or 750 mg/kg/day for 5 weeks and then given 2 weeks of recovery before termination. Testis weight was markedly low in males receiving 750 mg/kg/day, and there was also an indication of low epididymal weights at this dose. This effect was also seen in the recovery group males. At 750 mg/kg/day, reduced numbers or complete absence of spermatozoa, accompanied by the presence of degenerate spermatogenic cells in the duct(s), were observed in the epididymides and were still present following the 2- week recovery period. Spermatocoele granuloma(ta) that was seen in 2 males receiving 750 mg/kg/day and one receiving 60 mg/kg/day was not seen at the end of the recovery period. The significance of this change in the single male receiving 60 mg/kg/day is uncertain as spermatocoele granuloma(ta) can occur spontaneously in rats of this age and considering the absence of other degenerative changes in the testes or epididymides of this animal. Moderate to severe seminiferous tubular atrophy/degeneration was seen in the testes of all animals dosed at 750 mg/kg/day, accompanied by minimal to moderate spermatid giant cells and minimal to slight seminiferous tubular vacuolation. Similar findings were still evident following the 2-week recovery period but at a lower incidence and severity, suggesting a degree of recovery. There were no alterations in the female reproductive cycles or the reproductive organs up to the highest dose tested. Thus the NOAEL for the fertility endpoint was determined to be 250 mg/kg/day based on impairment of male fertility at 750 mg/kg/day (ECHA, 2017a; the ECHA dossier on this material uses terpineol data as read-across).

The most conservative NOAEL of 250 mg/kg/day was selected for the fertility endpoint. Therefore, the terpineol MOE for the fertility endpoint can be calculated by dividing the terpineol NOAEL by the total systemic exposure to terpineol, $250/0.00043$, or 581395.

Readacross justification

“Terpineol (CAS # 8000-41-7) could be used as a structurally similar read-across analog for the target material 4-carvomenthenol (CAS # 562-74-3) for the repeated dose toxicity and reproductive toxicity endpoints.

- o The target material and the read-across analog are structurally similar and belong to a class of unsaturated cyclic tertiary terpene alcohols.

- o The target material and the read-across analog have a cycloalkene (2-(4-methylcyclohex-3-en-1-yl)propan-2-ol) fragment common among them.

- o The key difference between the target material and the read-across analog is that the target material has isopropyl branching near the hydroxy group while the read-across analog has 2 methyl groups flanking the hydroxy group. This makes the target material's hydroxy group more sterically hindered compared to the hydroxy group in the read-across analog. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.

- o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.

- o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.

- o According to the QSAR OECD Toolbox (v4.2), structural alerts for the skin sensitization, repeated dose, and reproductive endpoints are consistent between the target material and the read-across analog. The CAESAR model v.2.1.6 predicts the target and the read-across analog to be sensitizers. Other protein binding alerts for both of the substances are negative. The data described in the skin sensitization section above shows that the read-across analog does not pose a concern for the skin sensitization endpoint. Therefore, this alert will be superseded by the availability of data. In addition, the target material and read-across analog are predicted to be a toxicant for the developmental endpoint with good reliability only by the CAESAR model v.2.1.6. The data described in the developmental and reproductive section supports that the readacross material is safe to use within a given MOE and level of use for developmental toxicity endpoint, so these in silico predictions will be superseded.

- o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator. Due to structural differences and more steric hindrance, the target material shows a fewer number of metabolic transformations compared to the read-across analog, which increases in vivo reactivity of the read-across analog.

- o The structural differences between the target material and the read-across analog are deemed to be toxicologically insignificant for the skin sensitization, repeated dose, developmental, and reproductive endpoints.”

As taken from Api AM. (2022). Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/562-74-3_1.pdf

5.4. Mutagenicity

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: NONE

Method: STANDARD PLATE

Dose Regimen: 0-2000 UG/ML (TEST MATERIAL SOLVENT: ACETONE OR ETHANOL)

Results: NEGATIVE

Reference: [FLETCHER,JP, CASSELLA,JP, HUGHES,D AND CASSELLA,S; AN EVALUATION OF THE MUTAGENIC POTENTIAL OF COMMERCIALY AVAILABLE TEA TREE OIL IN THE UNITED KINGDOM; INT. J. AROMATHERAPY]

Test System: AMES SALMONELLA TYPHIMURIUM

Metabolic Activation: RAT, LIVER, S-9

Strain Indicator: TA98

Strain Indicator: TA102

As taken from CCRIS, 2006.

"4-Carvomenthenol was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 4-carvomenthenol has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA97a, and TA102 were treated with 4-carvomenthenol in dimethyl sulfoxide (DMSO) at concentrations up to 5 mg/ plate (5000 µg/plate). No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2000b). Under the conditions of the study, 4-carvomenthenol was not mutagenic in the Ames test.

The clastogenic activity of 4-carvomenthenol was evaluated in an in vitro micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with 4-carvomenthenol in DMSO at concentrations up to 1540 µg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 1540 µg/mL in the presence and absence of metabolic activation. 4-Carvomenthenol did induce statistically significant increases in binucleated cells with micronuclei when tested up to cytotoxic concentrations at 400 µg/mL in the 4-h treatment with S9 (RIFM, 2015). However, the increase was within the historical control range and negative for dose response, so the result was considered not biologically relevant. Under the conditions of the study, 4-carvomenthenol was considered to be non-clastogenic in the in vitro micronucleus test.

Based on the data available, 4-carvomenthenol does not present a concern for genotoxic potential."

As taken from Api AM. (2022). Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/562-74-3_1.pdf

5.5. Cytotoxicity

Terpinen-4-ol, a monoterpene component of the essential oils of several aromatic plants, exhibits antitumor effects. In this study, the antitumor effects of terpinen-4-ol and the cellular and molecular mechanisms responsible for it were evaluated and studied, respectively on human nonsmall cell lung cancer (NSCLC) cells. Our results indicated that terpinen-4-ol elicited a dose-dependent cytotoxic effect, as determined by MTT assay. Increased sub-G1 population and annexin-V binding, activation of caspases 9 and 3, cleavage of poly(ADPribose) polymerase (PARP), and a decrease of mitochondrial membrane potential (MMP) indicated involvement of the mitochondrial apoptotic pathway in terpinen-4-ol-treated A549 and CL1-0 cells. Elevation of the Bax/Bcl-2 ratio and a

decrease in IAP family proteins XIAP and survivin were also observed following terpinen-4-ol treatment. Notably, terpinen-4-ol was able to increase p53 levels in A549 and CL1-0 cells. Diminution of p53 by RNA interference induced necrosis instead of apoptosis in A549 cells following terpinen-4-ol treatment, indicating that terpinen-4-ol-elicited apoptosis is p53-dependent. Moreover, intratumoral administration of terpinen-4-ol significantly suppressed the growth of s.c. A549 xenografts by inducing apoptosis, as confirmed by TUNEL assay. Collectively, these data provide insight into the molecular mechanisms underlying terpinen-4-ol-induced apoptosis in NSCLC cells, rendering this compound a potential anticancer drug for NSCLC (Chieh-Shan Wu, et al., 2012).

“BACKGROUND:.....In this study apoptotic and autophagic types of cell death induced by terpinen-4-ol and associated mechanisms were investigated in human leukemic HL-60 cells. MATERIALS AND METHODS: The cytotoxicity of human leukemic U937 and HL-60 cells was determined by MTT assay. Cytochrome c release, expression of Bax, Bcl-2, Bcl-xl and cleaved Bid were determined by Western blotting. Cell morphology was examined under a transmission electron microscope. LC3-I/II, ATG5 and Beclin-1 levels were detected by immunoblotting. RESULTS: Terpinen-4-ol exhibited cytotoxicity to human leukemic HL-60 but not U937 cells. The apoptotic response to terpinen-4-ol in HL-60 cells was due to induction of cytochrome c release from mitochondria and cleavage of Bid protein after the stimulation of caspase-8. There was a slightly decrease of Bcl-xl protein level. The characteristic cell morphology of autophagic cell death was demonstrated with multiple autophagosomes in the cytoplasm. At the molecular level, the results from Western blot analysis showed that terpinen-4-ol significantly induced accumulation of LC3-I/II, ATG5 and Beclin-1, regulatory proteins required for autophagy in mammalian cells. CONCLUSIONS: Terpinen-4-ol induced-human leukemic HL-60 cell death was via both autophagy and apoptosis.” As taken from Banjerdpongchai R and Khaw-On P. 2013. Asian Pac. J. Cancer Prev. 14(12), 7537-42. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24460330>

AIMS: The aim of this study was to compare both the antimicrobial activity of terpinen-4-ol and tea tree oil (TTO) against clinical skin isolates of meticillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (CoNS) and their toxicity against human fibroblast cells. METHODS AND RESULTS: Antimicrobial activity was compared by using broth microdilution and quantitative in vitro time-kill test methods. Terpinen-4-ol exhibited significantly greater bacteriostatic and bactericidal activity, as measured by minimum inhibitory and bactericidal concentrations, respectively, than TTO against both MRSA and CoNS isolates. Although not statistically significant, time-kill studies also clearly showed that terpinen-4-ol exhibited greater antimicrobial activity than TTO. Comparison of the toxicity of terpinen-4-ol and TTO against human fibroblasts revealed that neither agent, at the concentrations tested, were toxic over the 24-h test period. CONCLUSIONS: Terpinen-4-ol is a more potent antibacterial agent against MRSA and CoNS isolates than TTO with neither agent exhibiting toxicity to fibroblast cells at the concentrations tested. SIGNIFICANCE AND IMPACT OF THE STUDY: Terpinen-4-ol should be considered for inclusion as a single agent in products formulated for topical treatment of MRSA infection. However, further work would initially be required to ensure that resistance would not develop with the use of terpinen-4-ol as a single agent (Loughlin R et al., 2008).

“The combined effect of terpinen-4-ol, the main component of tea tree oil, and capric acid against mycelial growth of *Candida albicans* and murine oral candidiasis was evaluated in vitro and in vivo. Mycelial growth of *C. albicans* was estimated by the Cristal violet method. Combination of these compounds revealed a potent synergistic inhibition of growth. Therapeutic efficacy of the combination was evaluated microbiologically in murine oral candidiasis, and its application of the compounds clearly demonstrated therapeutic activity. Based on these results, the combined agent of terpinen-4-ol and capric acid was discussed as a possible candidate for oral candidiasis therapy.” As taken from Ninomiya K et al. 2013a. Yakugaku Zasshi 133(1), 133-40. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23292030?dopt=AbstractPlus>

“BACKGROUND: *Glossogyne tenuifolia* (GT) is a perennial herb widely distributed in the areas from south Asia to Australia. Many biological effects of *G. tenuifolia* have been reported; however, the information about antimicrobial activity of the essential oil (EO) of the herb remains unavailable. Therefore, the aims of this study were to investigate the antimicrobial activity of the GT-EO in vitro and food systems, the antimicrobial impact (AI) of its individual compounds, and interactive effects of major active compounds (linalool, 4-terpineol, α -terpineol, p-cymene) on selected Gram-positive (*S. aureus*, *L. monocytogenes*, *S. mutans* and *S. sanguinis*) and Gram-negative (*E. coli* O157:H7, *V. parahaemolyticus* and *S. enterica*) pathogens. RESULTS: The minimal microbicidal concentration (MMC) of the GT-EO ranged from 0.75 to 12 mg mL⁻¹ against the test bacteria in vitro. Except for *L. monocytogenes*, the GT-EO exhibited more inhibitory effect on the selected Gram positive than against the Gram negative bacteria at the GT-EO concentrations $\leq$ 12 mg mL⁻¹. The interactive effects of major active compounds (linalool, 4-terpineol, α -terpineol, p-cymene) are additive instead of synergistic via the checkerboard analysis. The bacteria with a microbial load of ca. 102 CFU mL⁻¹ in the milk tea could be completely inactivated by the GT-EO with the MMC of 1.5 mg mL⁻¹. CONCLUSION: p-Cymene is the largest component in the GT-EO; however, it is not the compound predominantly affecting the entire antimicrobial activity of the EO. Instead, 4-terpineol is the most influential among the test compounds that contribute to the antimicrobial activity of the GT-EO.” As taken from Yang TS et al. 2014. J. Sci. Food Agric. 94(14), 2965-2971. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24615540>

“The aim of this study was to examine the antimicrobial potential of three essential oils (EOs: tea tree oil, lemon myrtle oil and *Leptospermum* oil), five terpenoid compounds (α -bisabolol, α -terpinene, cineole, nerolidol and terpinen-4-ol) and polyphenol against two strains of *Campylobacter jejuni* (ACM 3393 and the poultry isolate C338), *Campylobacter coli* and other Gram negative and Gram positive bacteria. Different formulations of neem oil (*Azadirachta indica*) with these compounds were also tested for synergistic interaction against all organisms. Antimicrobial activity was determined by the use of disc diffusion and broth dilution assays....Among the single compounds, terpinen-4-ol showed the highest activity against *Campylobacter* spp. and other reference strains. Based on the antimicrobial activity and potential commerciality of these agents, lemon myrtle oil, α -tops (α -terpineol+cineole+terpinen-4-ol) and terpinen-4-ol were also evaluated using an in vitro fermentation technique to test antimicrobial activity towards *C. jejuni* in the microbiota from the chicken-caecum. EO compounds (terpinen-4-ol and α -tops) were antimicrobial towards *C. jejuni* at high doses (0.05%) without altering the fermentation profile. EOs and terpenoid compounds can have strong anti-*Campylobacter* activity without adversely affecting the fermentation potential of the chicken-caeca microbiota. EOs and their active compounds may have the potential to control *C. jejuni* colonisation and abundance in poultry.” As taken from Kurekci C et al. 2013. Int. J. Food Microbiol. 166(3), 450-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24041998>

“The aim of this paper was to investigate the effect of terpene penetration enhancers on membrane fluidity and membrane potential using HaCaT keratinocytes, and study the potential mechanisms of these terpene compounds using as natural transdermal penetration enhancer. Six terpene compounds, namely menthol, limonene, 1,8-cineole, menthone, terpinen-4-ol and pulegone, were chosen in this study on account of their good penetration-enhancement activities. The cytotoxicity of these terpene compounds was measured using an MTT assay. The fluorescence recovery after photobleaching (FRAP) technique was employed to measure the change of membrane fluidity of HaCaT cells. The flow cytometer was used to study the alteration of membrane fluidity of HaCaT cells, and investigate the effect of terpene compounds on intracellular Ca²⁺. It was found that 6 terpene compounds possessed low cytotoxicity in comparison to the well-established and standard penetration enhancer azone. Those terpene compounds could significantly enhance HaCaT cells membrane fluidity and decrease HaCaT cells membrane potentials. Meanwhile, after treated with various terpene compounds, the Ca²⁺(+)-ATPase activity and intracellular Ca²⁺ of HaCaT cells was decreased significantly. Terpene penetration enhancers perhaps changed the membrane fluidity and potentials of HaCaT cells by altering the Ca²⁺ balance of the cell inside and outside, resulting

in the low skin permeability to increase the drug transdermal absorption.” As taken from Lan Y et al. 2015. *Zhongguo Zhong Yao Za Zhi* 40(4), 643-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26137684>

“PURPOSE: The main purpose of this study was to demonstrate the anticancer effects of 4-terpineol against Hep-G2 hepatocellular carcinoma (HCC) cells by evaluating its effect on apoptosis induction, cell migration, DNA fragmentation and cell cycle phase distribution. METHODS: MTT assay was used to evaluate the cytotoxic effect of 4-terpineol on Hep-G2 cells, while fluorescence microscopy and flow cytometry were used to study apoptosis induction. Wound healing assay was used to study the effects of 4-terpineol on cell migration, while gel electrophoresis was performed to evaluate the effects on DNA fragmentation. Flow cytometry using propidium iodide (PI) as a probe was used to evaluate the effects on cell cycle arrest. Cells treated with dimethylsulfoxide (DMSO) only served as controls. BALB/c nude mice weighing about 35 g each were used for in vivo studies using 10 and 20 mg/kg of 4-terpineol dose. RESULTS: 4-terpineol induced dose-dependent cytotoxicity in Hep-G2 hepatocellular carcinoma cells. Gel electrophoresis indicated that DNA fragmentation was associated with increasing dose of 4-terpineol. It was also observed that a wound scratch in the vehicle-treated control cells was practically entirely closed after 48 hrs of incubation. However, treatment with 0, 25, 50 and 100 μ M dose of 4-terpineol resulted in inhibition of wound healing in a dose-dependent manner. The percentage of apoptotic cells increased from 2.5% in the control cells to 10.3, 64.6 and 78.9% in cells treated with 25, 50 and 100 μ M of 4-terpineol respectively. 4-terpineol-treated cells exhibited increased percentage of cells in sub-G1 phase of the cell cycle. The in vivo mouse results indicated that 10 and 20 mg/kg of 4-terpineol decreased the tumor weight and tumor volume in a dose-dependent manner. CONCLUSION: The results of this study showed that 4-terpineol exhibits anticancer effects in Hep-G2 cells by inducing apoptosis, DNA fragmentation, inhibition of cell migration and sub-G1 cell cycle arrest.” As taken from Liu S et al. 2016. *J. Buon.* 21(5), 1195-1202. PubMed, 2016 available at <https://www.ncbi.nlm.nih.gov/pubmed/27837623>

“Terpinen-4-ol (TP4O) is the main component of the essential oil extracted from *Melaleuca alternifolia*, known as the tea tree, of the botanical family Myrtaceae. The anticancer effects of TP4O have been reported in several cancer cell lines. Previous reports have demonstrated that TP4O exerts anticancer effects by inducing apoptotic cell death in several cell lines; however, the underlying molecular mechanisms of these effects remain unclear. In the present study, the anticancer effects of TP4O against the colorectal cancer (CRC) cell lines HCT116 and RKO were evaluated using WST-8 and bromodeoxyuridine assays. The mechanism of cell death was investigated by the measurement of caspase-3/7, Annexin V and lactate dehydrogenase release. Reactive oxygen species (ROS) levels induced by TP4O were evaluated by electron spin resonance and quantitative measurement of dihydroethidium. Localization of the ROS derived from mitochondria was observed by confocal inverted microscopy. Protein levels of ROS scavengers were assessed by western blotting analysis. To confirm the role of ROS, cell viability was measured in the presence of antioxidant reagents. In an in vivo xenograft model of ICR-SCID mice implanted with HCT116 cells, 200 mg/kg TP4O was injected locally, and tumor growth was compared with that of the control. TP4O induced apoptotic cell death in HCT116 and RKO cells in a dose-dependent manner, and TP4O also increased the levels of ROS generated by mitochondria. TP4O-induced cell death was rescued by administration of antioxidant reagents. In vivo, TP4O inhibited the proliferation of HCT116 xenografts compared with that of the control group. The results of the present study suggest that TP4O induces apoptosis in CRC cells through ROS generation. Furthermore, TP4O is potentially useful for the development of novel therapies against CRC.” As taken from Nakayama K et al. 2017. *Oncol. Lett.* 14(2), 2015-2024. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28781645>

“The measurement of skin electrical resistance (SER) has drawn a great deal of attention for the rapid screening of transdermal penetration enhancers (PEs). However, the mechanisms underlying the SER measurement are still unclear. This study was to investigate the effects and mechanisms

of seven oxygen-containing terpenes on the SER kinetics. Stratum corneum (SC) lipids were proved to play a key role in SER measurement. Then, the factors affecting the SER measurement were optimized. By the determination of SER kinetics, cyclic terpenes (1,8-cineole, terpinen-4-ol, menthol and α -terpineol) were demonstrated to possess higher enhancement ratio (ER) values compared with linear terpenes (linalool, geraniol and citral). For the first time, the linear correlation was found between ER of terpenes and the interaction energy of terpene-ceramide complexes revealed by molecular simulation. The attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) analysis revealed that the effect of cyclic terpenes on SC lipid arrangement was obviously stronger than that of linear terpenes. In addition, by evaluating HaCaT skin cell viability, little difference was found between the toxicities of cyclic and linear terpenes. In conclusion, measurement of SER could be a feasible approach for the efficient evaluation of the PEs that mainly act on SC lipids." As taken from Zhu XM et al. 2019. *Molecules* 24(3), E523. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30709044>

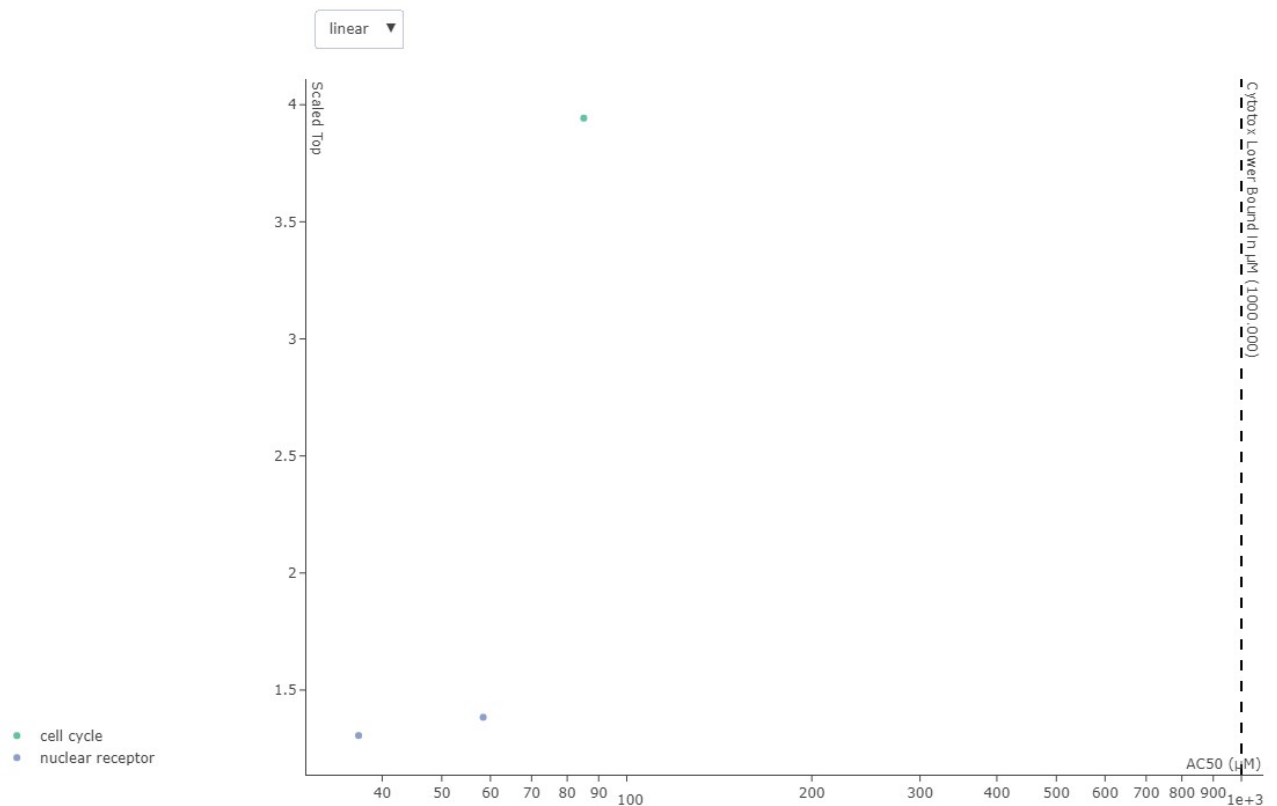
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 4-methyl-1-(propan-2-yl)cyclohex-3-en-1-ol 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 4-methyl-1-(propan-2-yl)cyclohex-3-en-1-ol 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



“Purpose: Infestation with demodex mites has been linked to the development of chalazion, meibomian gland dysfunction, and blepharitis. An effective treatment is the eyelid application of terpinen-4-ol (T4O), a tea tree oil component. However, T4O is also known to be toxic to nonocular epithelial cells. We hypothesize that T4O toxicity also extends to human meibomian gland epithelial cells (HMGECS). Methods: Immortalized (I) HMGECS were cultured with varying concentrations (1.0%-0.001%) of T4O under proliferating or differentiating conditions up to 5 days. Experimental procedures included analyses of cell appearance, survival, P-Akt signaling, lysosome accumulation, and neutral lipid content. Results: Our findings show that T4O causes a dose- and time-dependent decrease in the cell survival of IHMGECS. After 15 minutes of exposure to 1% T4O, IHMGECS exhibited rounding, atrophy, and poor adherence. Within 90 minutes of such treatment, almost all cells died. Reducing the T4O concentration to 0.1% also led to a marked decrease in P-Akt signaling and cell survival of IHMGECS. Decreasing the T4O amount to 0.01% caused a slight, but significant, reduction in the IHMGEC number after 5 days of culture and did not influence the ability of these cells to differentiate. Conclusions: T4O, even at levels 10-fold to 100-fold lower than demodicidal concentrations, is toxic to HMGECS in vitro.” As taken from Chen D et al. 2020. Cornea 39(12), 1541-1546. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32947397/>

5.6. Carcinogenicity

Tumor Inhibition Studies:

Species: RAT

Strain/Sex: SPRAGUE-DAWLEY/FEMALE

Dose (Inhibitor): 10 MG/KG BW DAILY FOR 6 MO AFTER CARCINOGEN ADMINISTRATION

Route (Inhibitor): INTRAGASTRIC (GASTRIC)

Carcinogen: 7,12-DIMETHYLBENZ[A]ANTHRACENE (57-97-6)

Route (Carcinogen): INTRAGASTRIC

Dose (Carcinogen): 75 MG/KG BW AT 55 D OF AGE (STUDY DURATION: 1 YR)

Target Tissue: Type of Lesion: MAMMARY: ADENOCARCINOMA

Endpoint (Incidence): 75%, 58%

Endpoint (Multiplicity): 4.0, 1.58, 60.5% $P < 0.05$

Reference: [LENOIR,V, DE JONAGE-CANONICO,MBY, PERRIN,MH, MARTIN,A, SCHOLLER,R AND KERDELHUE,B; PREVENTIVE AND CURATIVE EFFECT OF MELATONIN ON MAMMARY CARCINOGENESIS INDUCED BY DIMETHYLBENZ[A]ANTHRACENE IN THE FEMALE SPRAGUE-DAWLEY RAT; BREAST CANCER RES. 7(4):R470-R479, 2005]

Dose (Inhibitor): 10 MG/KG BW DAILY BETWEEN 45 AND 59 D OF AGE (STUDY DURATION: 1 YR)

Route (Inhibitor): INTRAGASTRIC (GASTRIC) IN HYDROXYETHYLCELLULOSE SODIUM

Dose (Carcinogen): 75 MG/KG BW IN SESAME OIL (ONCE) AT 60 D OF AGE

Endpoint (Incidence): 75%, 85%

Endpoint (Multiplicity): 4.0, 1.30, 68% $P < 0.05$

As taken from CCRIS, 2006.

PURPOSE: To examine the in vitro anticancer activity of *Melaleuca alternifolia* (tea tree) oil (TTO), and its major active terpene component, terpinen-4-ol, against two aggressive murine tumour cell lines, AE17 mesothelioma and B16 melanoma. **METHODS:** Effects of TTO and terpinen-4-ol on the cellular viability of two tumour cell lines and fibroblast cells were assessed by MTT assay. Induction of apoptotic and necrotic cell death was visualised by fluorescent microscopy and quantified by flow cytometry. Tumour cell ultrastructural changes were examined by transmission electron microscopy and changes in cell cycle distribution were assessed by flow cytometry, with changes in cellular morphology monitored by video time lapse microscopy. **RESULTS:** TTO and terpinen-4-ol significantly inhibited the growth of two murine tumour cell lines in a dose- and time-dependent manner. Interestingly, cytotoxic doses of TTO and terpinen-4-ol were significantly less efficacious against non-tumour fibroblast cells. TTO and terpinen-4-ol induced necrotic cell death coupled with low level apoptotic cell death in both tumour cell lines. This primary necrosis was clarified by video time lapse microscopy and also by transmission electron microscopy which revealed ultrastructural features including cell and organelle swelling following treatment with TTO. In addition, both TTO and terpinen-4-ol induced their inhibitory effect by eliciting G1 cell cycle arrest. **CONCLUSION:** TTO and terpinen-4-ol had significant anti-proliferative activity against two tumour cell lines. Moreover, the identification of primary necrotic cell death and cell cycle arrest of the aggressive tumour cells highlights the potential anticancer activity of TTO and terpinen-4-ol (Greay SJ et al., 2010).

The search for innovative therapeutic approaches based on the use of new substances is gaining more interest in clinical oncology. In this in vitro study the potential anti-tumoral activity of tea tree oil, distilled from *Melaleuca alternifolia*, was analyzed against human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamycin-resistant cells. Both sensitive and resistant cells were grown in the presence of tea tree oil at concentrations ranging from 0.005 to 0.03%. Both the complex oil (tea tree oil) and its main active component terpinen-4-ol were able to induce caspase-dependent apoptosis of melanoma cells and this effect was more evident in the resistant variant cell population. Freeze-fracturing and scanning electron microscopy analyses suggested that the effect of the crude oil and of the terpinen-4-ol was mediated by their interaction with plasma membrane and subsequent reorganization of membrane lipids. In conclusion, tea tree oil and terpinen-4-ol are able to impair the growth of human M14 melanoma cells and appear to be more

effective on their resistant variants, which express high levels of P-glycoprotein in the plasma membrane, overcoming resistance to caspase-dependent apoptosis exerted by P-glycoprotein-positive tumor cells (Calcabrini A et al., 2004).

“PURPOSE: The main purpose of this study was to demonstrate the anticancer effects of 4-terpineol against Hep-G2 hepatocellular carcinoma (HCC) cells by evaluating its effect on apoptosis induction, cell migration, DNA fragmentation and cell cycle phase distribution. METHODS: MTT assay was used to evaluate the cytotoxic effect of 4-terpineol on Hep-G2 cells, while fluorescence microscopy and flow cytometry were used to study apoptosis induction. Wound healing assay was used to study the effects of 4-terpineol on cell migration, while gel electrophoresis was performed to evaluate the effects on DNA fragmentation. Flow cytometry using propidium iodide (PI) as a probe was used to evaluate the effects on cell cycle arrest. Cells treated with dimethylsulfoxide (DMSO) only served as controls. BALB/c nude mice weighing about 35 g each were used for in vivo studies using 10 and 20 mg/kg of 4-terpineol dose. RESULTS: 4-terpineol induced dose-dependent cytotoxicity in Hep-G2 hepatocellular carcinoma cells. Gel electrophoresis indicated that DNA fragmentation was associated with increasing dose of 4-terpineol. It was also observed that a wound scratch in the vehicle-treated control cells was practically entirely closed after 48 hrs of incubation. However, treatment with 0, 25, 50 and 100 μ M dose of 4-terpineol resulted in inhibition of wound healing in a dose-dependent manner. The percentage of apoptotic cells increased from 2.5% in the control cells to 10.3, 64.6 and 78.9% in cells treated with 25, 50 and 100 μ M of 4-terpineol respectively. 4-terpineol-treated cells exhibited increased percentage of cells in sub-G1 phase of the cell cycle. The in vivo mouse results indicated that 10 and 20 mg/kg of 4-terpineol decreased the tumor weight and tumor volume in a dose-dependent manner. CONCLUSION: The results of this study showed that 4-terpineol exhibits anticancer effects in Hep-G2 cells by inducing apoptosis, DNA fragmentation, inhibition of cell migration and sub-G1 cell cycle arrest.” As taken from Liu S et al. 2016. J. Buon. 21(5), 1195-1202. PubMed, 2016 available at <https://www.ncbi.nlm.nih.gov/pubmed/27837623>

“Terpinen-4-ol (TP4O) is the main component of the essential oil extracted from *Melaleuca alternifolia*, known as the tea tree, of the botanical family Myrtaceae. The anticancer effects of TP4O have been reported in several cancer cell lines. Previous reports have demonstrated that TP4O exerts anticancer effects by inducing apoptotic cell death in several cell lines; however, the underlying molecular mechanisms of these effects remain unclear. In the present study, the anticancer effects of TP4O against the colorectal cancer (CRC) cell lines HCT116 and RKO were evaluated using WST-8 and bromodeoxyuridine assays. The mechanism of cell death was investigated by the measurement of caspase-3/7, Annexin V and lactate dehydrogenase release. Reactive oxygen species (ROS) levels induced by TP4O were evaluated by electron spin resonance and quantitative measurement of dihydroethidium. Localization of the ROS derived from mitochondria was observed by confocal inverted microscopy. Protein levels of ROS scavengers were assessed by western blotting analysis. To confirm the role of ROS, cell viability was measured in the presence of antioxidant reagents. In an in vivo xenograft model of ICR-SCID mice implanted with HCT116 cells, 200 mg/kg TP4O was injected locally, and tumor growth was compared with that of the control. TP4O induced apoptotic cell death in HCT116 and RKO cells in a dose-dependent manner, and TP4O also increased the levels of ROS generated by mitochondria. TP4O-induced cell death was rescued by administration of antioxidant reagents. In vivo, TP4O inhibited the proliferation of HCT116 xenografts compared with that of the control group. The results of the present study suggest that TP4O induces apoptosis in CRC cells through ROS generation. Furthermore, TP4O is potentially useful for the development of novel therapies against CRC.” As taken from Nakayama K et al. 2017. Oncol. Lett. 14(2), 2015-2024. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28781645>

5.7. Irritation/immunotoxicity

This paper highlights the role of plant volatile organic compounds, found in essential oils, for the treatment of bacteria related inflammation. This report is focused on tea tree oil, particularly its main compound terpinen-4-ol. Analysis of the published literature shows that many essential oils have significant antibacterial, antifungal and anti-inflammatory effects. Some of their major components, such as terpinen-4-ol, act by inhibiting pro-inflammatory cytokine expression while stimulating production of anti-inflammatory cytokines. Such observations may be exploited to encourage biotherapy against mastitis. The use of synthetic antibiotics is being increasingly discouraged because their presence in dairy milk may have potential downstream effects on population health and the agri-food chain. In the context of inflammation and related mammalian responses, understanding the interplay between volatile organic compounds, especially terpinen-4-ol, and cytokines during bacteria related inflammation should clarify their mode of action to control mastitis (Taga I et al., 2012).

“The onset of oral candidiasis is accompanied by inflammatory symptoms such as pain in the tongue, edema or tissue damage and lowers the quality of life (QOL) of the patient. In a murine oral candidiasis model, the effects were studied of terpinen-4-ol (T-4-ol), one of the main constituents of tea tree oil, *Melaleuca alternifolia*, on inflammatory reactions. When immunosuppressed mice were orally infected with *Candida albicans*, their tongues showed inflammatory symptoms within 24 h after the infection, which was monitored by an increase of myeloperoxidase activity and macrophage inflammatory protein-2 in their tongue homogenates. Oral treatment with 50 µL of 40 mg/mL terpinen-4-ol 3h after the *Candida* infection clearly suppressed the increase of these inflammatory parameters. In vitro analysis of the effects of terpinen-4-ol on cytokine secretion of macrophages indicated that 800 µg/mL of this substance significantly inhibited the cytokine production of the macrophages cultured in the presence of heat-killed *C. albicans* cells. Based on these findings, the role of the anti-inflammatory action of T-4-ol in its therapeutic activity against oral candidiasis was discussed” As taken from Ninomiya K et al. 2013b. *Biol. Pharm. Bull.* 36(5), 838-44. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23649340>

Type of Test	Route of Exposure	Species Observed	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration onto the skin	Rodent - rabbit	500 mg/24H	Moderate	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 20,833,1982
Standard Draize test	Administration onto the skin	Rodent - rabbit	100%	Moderate	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 46,S1,2008

As taken from RTECS, 2021.

Terpinen-4-ol is a known skin, eye and respiratory tract irritant, and a skin sensitiser.

As taken from BPDB, 2022.

“Contact hypersensitivity response and skin swelling in mice were inhibited by topical application of terpinen-4-ol; however, ultraviolet B- or irritant-induced swelling was not suppressed.”

As taken from Khan IA and Abourashed EA, 2010.

“In this article, contact allergy to, and the chemical composition of, tea tree oil (TTO) are reviewed. This essential oil is a popular remedy for many skin diseases, and may be used as neat oil or be

present in cosmetics, topical pharmaceuticals and household products. Of all essential oils, TTO has caused most (published) allergic reactions since the first cases were reported in 1991. In routine testing, prevalences of positive patch test reactions have ranged from 0.1% to 3.5%. Nearly 100 allergic patients have been described in case reports and case series. The major constituents of commercial TTO are terpinen-4-ol, γ -terpinene, 1,8-cineole, α -terpinene, α -terpineol, p-cymene, and α -pinene. Fresh TTO is a weak to moderate sensitizer, but oxidation increases its allergenic potency. The major sensitizers appear to be ascaridole, terpinolene, α -terpinene, 1,2,4-trihydroxymenthane, α -phellandrene, and limonene. The clinical picture of allergic contact dermatitis caused by TTO depends on the products used. Most reactions are caused by the application of pure oil; cosmetics are the culprits in a minority of cases. Patch testing may be performed with 5% oxidized TTO. Co-reactivity to turpentine oil is frequent, and there is an overrepresentation of reactions to fragrance mix I, Myroxylon pereirae, colophonium, and other essential oils." As taken from de Groot AC and Schmidt E. 2016. Contact Dermatitis 75(3), 129-43. PubMed, 2016

p-Menth-1-en-4-ol is a suspected skin sensitizer. The CAESAR skin sensitisation model in VEGA (Q)SAR platform predicts that the chemical is a sensitizer (good reliability) (ECHA, 2016).

The reliability and applicability of this QSAR prediction as standalone source of toxicological information is limited and inappropriate for some complex endpoints like reprotoxicity or carcinogenicity. Nevertheless, for the toxicological assessment of this ingredient, this result was still taken into consideration and used within the WoE approach as a supportive tool, in combination with other sources of information when available, like experimental data or appropriate read-across.

ECHA Annex III has been withdrawn as of April 2023: "The list of substances that are unlikely to benefit from the reduced information requirements, the so-called 'Annex III inventory', has been withdrawn."

"Objective: Microbial infection has been reported to cause blepharitis, conjunctivitis and keratitis. We evaluated the safety and efficacy of a foam formulation of 2% 4-terpineol (T4O) against common ocular microorganisms. Material and methods: The antimicrobial effect of a 2% T4O formulation was evaluated by the United States Pharmacopeia 51 (USP <51>) antimicrobial effectiveness test for 14 and 28 days, as well as by a Time Kill Study (ASTM E2315) with a 60 s exposure time. Its potential of causing skin and ocular irritation was evaluated by the Repeated Insult Patch Test and the Hen's Egg Chorioallantoic Membrane Test, respectively. Results and discussion: It was seen that 2% T4O formulation did not cause ocular irritation, skin irritation, sensitisation or allergic contact dermatitis in human subjects. Most importantly, it killed microorganisms listed in USP <51> at both 14 and 28 days and exerted a rapid killing effect within 60 s against 13 bacteria, 1 fungus and Acanthamoeba castellanii. Conclusion: The above finding suggests that 2% T4O formulation is safe and effective in killing microorganisms related to common ocular and skin infective diseases. Translational relevance: Although the clinical efficacy in treating ocular disease was not directly studied; this foam formulation containing 2% T4O, based on the in vitro results of this work, demonstrated that it can potentially be used as a preservative-free cleansing agent for ocular hygiene maintenance due to its ability to exert a broad-spectrum antimicrobial effect without causing ocular or skin irritation." As taken from Su CW et al. 2018. BMJ Open Ophthalmol. 3(1), e000094. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30123842>

"Terpinen 4-ol, a phytochemical is a monoterpene which has been reported for its anti-inflammatory effect. Present research was planned to check its effect against arthritis through in vitro and in vivo models. Terpinen 4-ol was evaluated through in-vitro procedures including blocking of protein (BSA and egg albumin) denaturation and human RBC membrane stabilization. In in vivo study, terpinen 4-ol (15, 30 and 60 mg/kg) was evaluated using formaldehyde and CFA arthritic models. Terpinen 4-ol significantly inhibited increase in paw and joint swelling as compared to diseased group. Terpinen 4-ol showed remarkable antioxidant effect (SOD, reducing power) and also improved body weight, haematological, histopathological and radiological parameters in CFA model. Also,

moreover, the excess production of IL-1 β , TNF- α , IRAK, and NF-kB were noticeably attenuated in all terpinen 4-ol treated rats, however, IL-17 and IL-10 were distinctly increased compared to arthritic control rats in RT-PCR. Also, terpinen 4-ol showed promising antioxidant effect in DPPH assay. Henceforth, it might be concluded that terpinen 4-ol has anti-arthritic effect which can be attributed to the downregulation of pro-inflammatory cytokines.” As taken from Aslam S et al. (2022). Available at <https://pubmed.ncbi.nlm.nih.gov/35320496/>

“Based on the existing data, 4-carvomenthenol is not considered a skin sensitizer. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). 4-Carvomenthenol was found to be negative in an in vitro direct peptide reactivity assay (DPRA) and KeratinoSens, but positive in the human cell line activation test (h-CLAT) (RIFM, 2017a; RIFM, 2017c; RIFM, 2016c). In an open epicutaneous test in guinea pigs, no reactions indicative of skin sensitization were observed with 4-carvomenthenol (Klecak, 1985). Additionally, in 2 human maximization tests, no reactions indicative of sensitization were observed with 5% (3450 $\mu\text{g}/\text{cm}^2$) 4-carvomenthenol in petrolatum (RIFM, 1977).

Based on the weight of evidence (WoE) from structural analysis and in vitro, animal, and human studies, 4-carvomenthenol does not present a concern for skin sensitization under the current, declared levels of use.”

As taken from Api AM. (2022). Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/562-74-3_1.pdf

5.8. All other relevant types of toxicity

AIMS: The aim of this study was to compare both the antimicrobial activity of terpinen-4-ol and tea tree oil (TTO) against clinical skin isolates of meticillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (CoNS) and their toxicity against human fibroblast cells. METHODS AND RESULTS: Antimicrobial activity was compared by using broth microdilution and quantitative in vitro time-kill test methods. Terpinen-4-ol exhibited significantly greater bacteriostatic and bactericidal activity, as measured by minimum inhibitory and bactericidal concentrations, respectively, than TTO against both MRSA and CoNS isolates. Although not statistically significant, time-kill studies also clearly showed that terpinen-4-ol exhibited greater antimicrobial activity than TTO. Comparison of the toxicity of terpinen-4-ol and TTO against human fibroblasts revealed that neither agent, at the concentrations tested, were toxic over the 24-h test period. CONCLUSIONS: Terpinen-4-ol is a more potent antibacterial agent against MRSA and CoNS isolates than TTO with neither agent exhibiting toxicity to fibroblast cells at the concentrations tested. SIGNIFICANCE AND IMPACT OF THE STUDY: Terpinen-4-ol should be considered for inclusion as a single agent in products formulated for topical treatment of MRSA infection. However, further work would initially be required to ensure that resistance would not develop with the use of terpinen-4-ol as a single agent (Loughlin R et al., 2008).

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. Penetration of terpinen-4-ol to the skin was better from an oily solution (approximately 90 $\mu\text{g}/\text{cm}^2$) than from an emulsion (60 $\mu\text{g}/\text{cm}^2$). 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. In contrast, the cutaneous absorption of linalool was the same from both vehicles (50-60 $\mu\text{g}/\text{cm}^2$). The skin penetration of alpha-pinene was not traceable when it was applied in an oily solution. Only a small amount (approximately 5 $\mu\text{g}/\text{cm}^2$) of this terpene was

determined in viable skin after application as a hydrogel. Citronellol applied in a hydrogel penetrated into all skin layers in a total amount of 25 microg/cm (2), while no penetration into viable skin layers after application of an oily solution was noted. Only citronellol permeated into the acceptor medium (Cal K, 2006).

“PURPOSE: To determine the active ingredient in tea tree oil (TTO) responsible for its reported killing effect on Demodex mites, the most common ectoparasite found in the human skin extending to the eye. METHODS: Using a reported in vitro killing assay to measure the survival time of adult Demodex folliculorum up to 150 minutes, we have screened serial concentrations of 13 of the 15 known ingredients of TTO (ISO4730:2004) that were soluble in mineral oil and examined their synergistic relationships in killing mites. The most potent ingredient was then tested for its efficacy in killing Demodex in vivo. RESULTS: All ingredients exhibited a dose-dependent killing effect. Besides Terpinen-4-ol, the order of relative potency did not correlate with the order of relative abundance in TTO for the remaining 12 ingredients. Terpinen-4-ol was the most potent ingredient followed by α -Terpineol, 1,8-Cineole and Sabinene. Terpinen-4-ol, the most abundant ingredient in TTO, was more potent than TTO at equivalent concentrations and its killing effect was even observable at a mere concentration of 1%. Terpinen-4-ol exhibited a significant synergistic effect with Terpinolene, but an antagonistic effect with α -Terpineol in killing mites (both $P < 0.05$). In vivo, Terpinen-4-ol was shown to eradicate mites. CONCLUSIONS: The above finding suggests that deployment of Terpinen-4-ol alone should enhance its potency in killing Demodex mites by reducing the adverse and antagonistic effects from other ingredients in TTO. TRANSLATIONAL RELEVANCE: Terpinen-4-ol can be adopted in future formulations of acaricides to treat a number of ocular and cutaneous diseases caused by demodicosis.” As taken from Tighe S et al. 2013. Transl. Vis. Sci. Technol. 2(7), 2. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24349880>

“This study aimed to evaluate the susceptibility in vitro and in vivo of Trypanosoma evansi to terpinen-4-ol, γ -terpinene and α -terpinene, the three main compounds of tea tree oil (Melaleuca alternifolia) with known efficacy in the treatment of trypanosomiasis. In vitro, a trypanocidal effect of terpinen-4-ol, γ -terpinene, and α -terpinene was observed when used alone or associated at 0.5, 1 and 2% concentrations i.e., the α -terpinene showed a faster trypanocidal effect when compared to chemotherapy (diminazene aceturate - D.A.). In vivo studies were performed in two experiments: I and II where experiment I used T. evansi infected mice treated with terpinen-4-ol, γ -terpinene and α -terpinene alone (at a dose of 1.0 mL kg⁻¹) or associated (two compounds, dose of 0.5 mL kg⁻¹ of each compound; three compounds, dose of 0.335 mL kg⁻¹ of each compound); Treatment with α -terpinene was able to extend animal longevity, but showed no curative efficacy. In experiment II, T. evansi infected mice were treated with D.A. associated with α -terpinene, where a curative efficacy of 57.14% was found, a much better result when D.A. was used alone (14.28%). In summary, α -terpinene associated with D.A. can be used as an alternative treatment for T. evansi infection. The compound α -terpinene from M. alternifolia essential oil is the one responsible for the trypanocidal effect, a fact confirmed by in vitro results and the increased longevity observed on treated mice.” As taken from Baldissera MD et al. 2016. Exp. Parasitol. 162, 43-8. PubMed, 2016 available at <https://www.ncbi.nlm.nih.gov/pubmed/26773165>

“Effective application of the essential oils requires detailed exploration of their mechanism of action and the origin of diverse activity of their components. In this work, the influence of eucalyptol and terpinen-4-ol on artificial membranes was studied to verify whether the differences in the activity of these compounds are related to their effect on membranes. The properties of monolayers formed from structurally different lipids in the presence of terpenes were examined based on the results of the surface pressure-area measurements, penetration studies, and Brewster angle microscopy experiments. Both compounds were able to incorporate into the membrane and alter lipid/lipid interactions, making the monolayer less stable and more fluid. These effects were determined by monolayer composition (but not by its condensation per se) and the resulting rheological properties and were stronger in the presence of terpinen-4-ol. These findings confirm the hypothesis that

differences in the antimicrobial potency of these terpenes are membrane-related, and membrane composition may determine their selectivity.” As taken from H-àc-Wydro K et al. 2017. Langmuir 33(27), 6916-6924. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28654274>

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - skin	221.48 mg/L/48H	In Vitro Toxicity Studies - cell protein synthesis	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 186,351,2016

As taken from RTECS, 2021

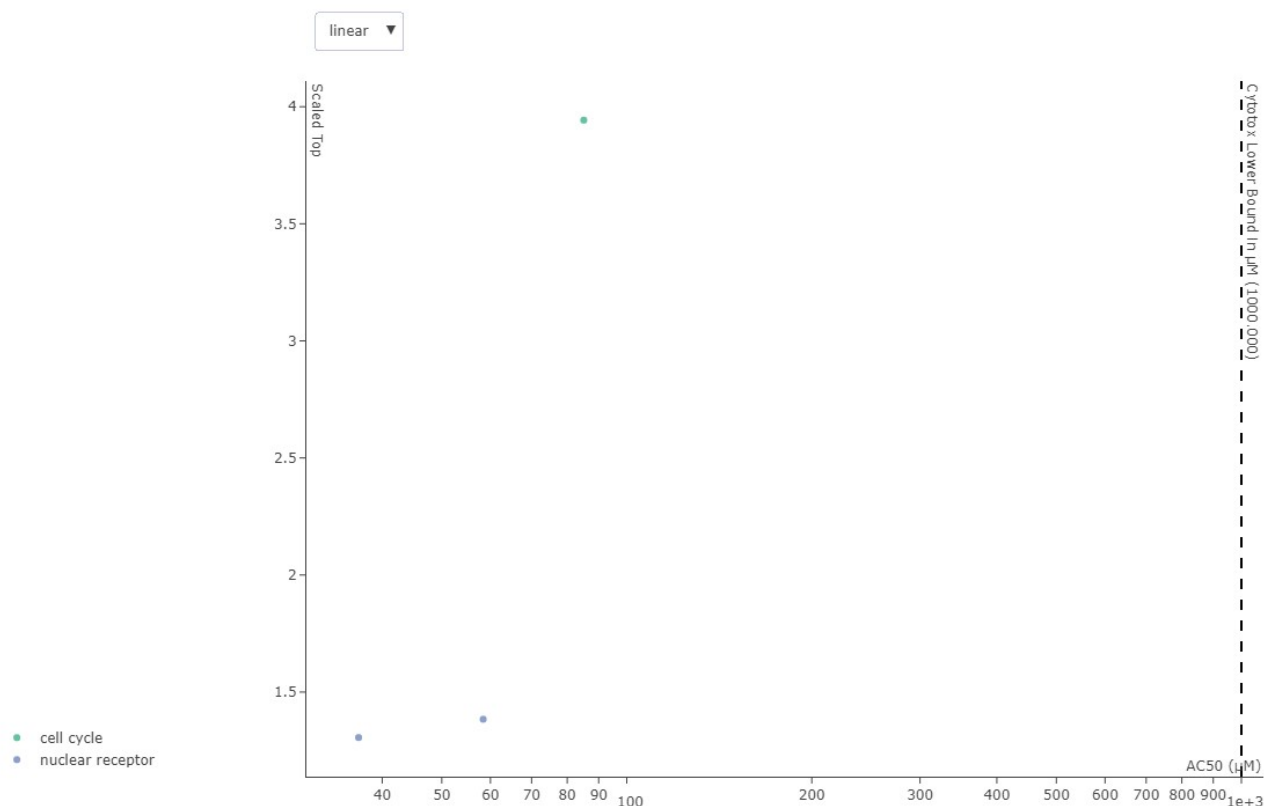
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated 4-methyl-1-(propan-2-yl)cyclohex-3-en-1-ol 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 4-methyl-1-(propan-2-yl)cyclohex-3-en-1-ol is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA’s CompTox Dashboard.

Figure 1



6. Functional effects on

6.1. Broncho/pulmonary system

Summary: The use of this material under current conditions is supported by existing information. This material was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, as well as environmental safety. Data show that this material is not genotoxic. Data on the target material and the suitable read across material terpineol (CAS # 8000-41-8) show that this material does not have skin sensitization potential. The local respiratory toxicity endpoint was completed using the TTC (Threshold of Toxicological Concern) for a Cramer Class I material (1.4 mg/day). The repeated dose, developmental and reproductive toxicity endpoints were completed using terpineol (CAS # 8000-41-7) as a suitable read across analogue, which provided a MOE > 100. The phototoxicity/photoallergenicity endpoint was completed based on suitable UV spectra. The environmental endpoint was completed as described in the RIFM Framework. As taken from Api AM et al. (2017). RIFM fragrance ingredient safety assessment, 4-Carvomenthenol, CAS Registry Number 562-74-3. Food Chem. Toxicol. 110(Suppl. 1), S403-S411. available at <https://www.ncbi.nlm.nih.gov/pubmed/28743524>

“Tobacco products containing flavorings, such as electronic nicotine delivery devices (ENDS) or e-cigarettes, cigars/cigarillos, waterpipes, and heat-not-burn devices (iQOS) are continuously evolving. In addition to increasing the exposure of teenagers and adults to nicotine containing flavoring products and flavoring enhancers, chances of nicotine addiction through chronic use and abuse also increase. These flavorings are believed to be safe for ingestion, but little information is available about their effects on the lungs. In this review, we have discussed the in vitro and in vivo data on toxicity of flavoring chemicals in lung cells. We have further discussed the common flavoring agents, such as diacetyl and menthol, currently available detection methods, and the

toxicological mechanisms associated with oxidative stress, inflammation, mucociliary clearance, and DNA damage in cells, mice, and humans. Finally, we present potential biomarkers that could be utilized for future risk assessment. This review provides crucial parameters important for evaluation of risk associated with flavoring agents and flavoring enhancers used in tobacco products and ENDS. Future studies can be designed to address the potential toxicity of inhaled flavorings and their biomarkers in users as well as in chronic exposure studies.” As taken from Kaur G et al. 2018. *Toxicology Lett.* 288, 143-155. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29481849>

6.2. Cardiovascular system

The present study investigated the hypotensive responses to intravenous (i.v.) treatment with the essential oil of *Alpinia zerumbet* (EOAZ) and its main constituent, terpinen-4-ol (Trp-4-ol), in the experimental model of deoxycorticosterone-acetate (DOCA)-salt hypertensive rat. In both DOCA-salt hypertensive and uninephrectomized, normotensive rats, i.v. bolus injections of EOAZ (1-20 mg/kg) or Trp-4-ol (1-10 mg/kg) decreased mean aortic pressure (MAP) in a dose-related manner. However, hypotensive responses to Trp-4-ol were significantly greater than those evoked by the same doses of EOAZ (1-10 mg/kg). Treatment with DOCA-salt significantly enhanced the maximal percentage decreases in MAP evoked by EOAZ or Trp-4-ol. Likewise, both maximal percentage and absolute decreases in MAP elicited by i.v. injection of the ganglion blocker, hexamethonium (30 mg/kg), were significantly greater in DOCA-salt hypertensive than in control rats. In DOCA-salt hypertensive rats, neither hexamethonium (30 mg/kg, i.v.) nor methylatropine (1 mg/kg, i.v.) pretreatment affected the enhancement of EOAZ-induced hypotension. These results show that i.v. treatment with either EOAZ or Trp-4-ol dose-dependently decreases blood pressure in conscious DOCA-salt hypertensive rats, and this action is enhanced when compared with uninephrectomized controls. This enhancement could be related mainly to an increase in EOAZ-induced vascular smooth muscle relaxation rather than to enhanced sympathetic nervous system activity in this hypertensive model. The data further support our previous hypothesis that hypotensive effects of EOAZ are partially attributed to the actions of Trp-4-ol (Lahlou S et al., 2003).

“AIMS: This study investigated the mechanisms underlying the vascular effects of terpinen-4-ol in isolated rat aortic ring preparations. MAIN METHODS: The thoracic aortae of healthy rats were submitted to isometric tension recording. Membrane resting potential and input membrane resistance were measured by conventional microelectrode technique. KEY FINDINGS: Terpinen-4-ol reversibly relaxed endothelium-containing preparations pre-contracted with high K(+) and phenylephrine with IC₅₀ values of 421.43 μ M and 802.50 μ M, respectively. These effects were significantly reduced by vascular endothelium removal. In Ca(2+)-free and high K(+) (80 mM) medium, the contractions produced by Ba(2+) were reduced by terpinen-4-ol (100-1000 μ M) in a concentration-dependent manner. In aortic rings maintained under Ca(2+)-free conditions, terpinen-4-ol significantly reduced the contractions induced by either phenylephrine (1 μ M) or phorbol 12,13-dibutyrate (1 μ M). Terpinen-4-ol (10-1000 μ M) also relaxed the contractions evoked by BAYK-8644 (3 μ M) with an IC₅₀ of 454.23 μ M. Neither membrane resting potential nor input resistance of smooth muscle cells was altered by terpinen-4-ol exposure. SIGNIFICANCE: The present results suggest that terpinen-4-ol induced vascular smooth muscle relaxation that was preferentially due to the inhibition of electromechanical pathways related to calcium influx through voltage-operated calcium channels.” As taken from Maia-Joca RP et al. 2014. *Life Sci.* 115(1-2), 52-58. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25219882>

“Both *M. alternifolia* leaf oil and terpinen-4-ol induced differentiation of white blood cells in vitro in human myelocytic (HL-60) cells.”

As taken from Khan IA and Abourashed EA, 2010.

“(–)-Terpinen-4-ol is a naturally occurring plant monoterpene and has been shown to have a plethora of biological activities. The objective of this study was to investigate the effects of (–)-

terpinen-4-ol on the rat heart, a key player in the control and maintenance of arterial blood pressure. The effects of (-)-terpinen-4-ol on the rat heart were investigated using isolated left atrium isometric force measurements, in vivo electrocardiogram (ECG) recordings, patch clamp technique, and confocal microscopy. It was observed that (-)-terpinen-4-ol reduced contraction force in an isolated left atrium at millimolar concentrations. Conversely, it induced a positive inotropic effect and extrasystoles at micromolar concentrations, suggesting that (-)-terpinen-4-ol may have arrhythmogenic activity on cardiac tissue. In anaesthetized animals, (-)-terpinen-4-ol also elicited rhythm disturbance, such as supraventricular tachycardia and atrioventricular block. To investigate the cellular mechanism underlying the dual effect of (-)-terpinen-4-ol on heart muscle, experiments were performed on isolated ventricular cardiomyocytes to determine the effect of (-)-terpinen-4-ol on L-type Ca^{2+} currents, Ca^{2+} sparks, and Ca^{2+} transients. The arrhythmogenic activity of (-)-terpinen-4-ol in vitro and in vivo may be explained by its effect on intracellular Ca^{2+} handling. Taken together, our data suggest that (-)-terpinen-4-ol has cardiac arrhythmogenic activity.” As taken from Gondim ANS et al. 2017. Eur. J. Pharmacol. 807, 56-63. PubMed 2018, available at <https://www.ncbi.nlm.nih.gov/pubmed/28435092>

6.3. Nervous system

“The biological and pharmacological activities of the terpenoid terpinen-4-ol (1), which include depressant effects in the central nervous system, are of potential therapeutic interest. In the present study, the effects of 1 on neuronal excitability and voltage-dependent $\text{K}(+)$ currents in the somatic sensory system were investigated. Intact and dissociated neurons of rat dorsal root ganglia (DRG) were used for intracellular and patch-clamp recordings, respectively. In neurons of intact DRG, 1 caused concentration-dependent depolarization of the resting membrane potential and increased input resistance. 1 also inhibited action potentials (AP) and decreased AP parameters, with the exception of AP duration, which was increased. In dissociated DRG neurons, 1 partially blocked the total $\text{K}(+)$ current in a concentration-dependent manner. 1 inhibited $\text{I}(\text{A})$, $\text{I}(\text{D})$, and $\text{I}(\text{K})$ with IC_{50} values of 3.2 ± 0.3 , 0.7 ± 0.1 , and 1.6 ± 0.7 mM, respectively. 1 did not shift either the steady-state activation or inactivation curves of $\text{I}(\text{A})$, $\text{I}(\text{D})$, and $\text{I}(\text{K})$ but reduced the decay time course of $\text{I}(\text{A})$. The alterations in DRG reported here are consistent with the inhibition of $\text{K}(+)$ currents and might partially explain the effect of 1 on excitable tissues.” As taken from dos Santos-Nascimento T et al. 2015. J. Nat. Prod. 78(2), 173-80. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25607610>

“Terpinen-4-ol (4TRP) is a monoterpene alcoholic component of essential oils obtained from several aromatic plants. We investigated the psychopharmacological and electrophysiological activities of 4TRP in male Swiss mice and Wistar rats. 4TRP was administered intraperitoneally (i.p.) at doses of 25 to 200 mg/kg and intracerebroventricularly (i.c.v.) at concentrations of 10, 20, and 40 ng/2 μL . For in vitro experiments, 4TRP concentrations were 0.1mM and 1.0mM. 4TRP (i.p.) inhibited pentylenetetrazol- (PTZ-) induced seizures, indicating anticonvulsant effects. Electroencephalographic recordings showed that 4TRP (i.c.v.) protected against PTZ-induced seizures, corroborating the behavioural results. To determine whether 4TRP exerts anticonvulsant effects via regulation of GABAergic neurotransmission, we measured convulsions induced by 3-mercaptopropionic acid (3-MP). The obtained results showed involvement of the GABAergic system in the anticonvulsant action exerted by 4TRP, but flumazenil, a selective antagonist of the benzodiazepine site of the GABAA receptor, did not reverse the anticonvulsant effect, demonstrating that 4TRP does not bind to the benzodiazepine-binding site. Furthermore, 4TRP decreased the sodium current through voltage-dependent sodium channels, and thus its anticonvulsant effect may be related to changes in neuronal excitability because of modulation of these channels. [Nobrega FF et al; Oxid Med Cell Longev (2014)]

As taken from HSDB, 2015.

Terpinen-4-ol is a known neurotoxicant

As taken from BPDB, 2022

"The monoterpenoid terpinen-4-ol (4TERP) is known to inhibit cell excitability, has low toxicity and important pharmacological activities, which are likely related to neural excitability, such as anti-inflammatory, antiepileptic and antinociceptive effects. However, the pharmacological characteristics and mechanisms underlying the effects of 4TERP on blockade of neural action potential are not completely elucidated. Since Na^+ current (I_{Na}) through voltage-dependent Na^+ channels (Na_V) is a major mechanism for excitability, the present study investigated the pharmacological characteristics and mechanisms of the action of 4TERP on I_{Na} through Na_V . For this aim, dissociated small neurons of dorsal root ganglia of adult rats were used for whole cell patch-clamp recordings. 4TERP concentration-dependently inhibits I_{Na} (IC_{50} 0.8 ± 0.3 mM; pharmacological efficacy $42.89 \pm 5.54\%$). 4TERP interfered with I_{Na} through a mechanism with various components, which includes predominantly channel pore block and sensitivity to frequency of use. In presence of 4TERP (3 mM), decreasing stimulation from 5 Hz to very low frequency (75 s of quiescence previously to stimulation) induced I_{Na} decrease to $65.17 \pm 5.86\%$ of control. 4TERP also altered (left shift) voltage sensitivity of the steady state activation of Na_V . Data are discussed aiming to interpret the importance of blockade of I_{Na} through Na_V as participant of 4TERP-induced inhibition of membrane excitability." As taken from dos Santos-Nascimento T et al. 2020. Chem. Biol. Interact. 315, 108890. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31678597/>

"The monoterpenoid terpinen-4-ol (4TERP) is known to inhibit cell excitability, has low toxicity and important pharmacological activities, which are likely related to neural excitability, such as anti-inflammatory, antiepileptic and antinociceptive effects. However, the pharmacological characteristics and mechanisms underlying the effects of 4TERP on blockade of neural action potential are not completely elucidated. Since Na^+ current (I_{Na}) through voltage-dependent Na^+ channels (Na_V) is a major mechanism for excitability, the present study investigated the pharmacological characteristics and mechanisms of the action of 4TERP on I_{Na} through Na_V . For this aim, dissociated small neurons of dorsal root ganglia of adult rats were used for whole cell patch-clamp recordings. 4TERP concentration-dependently inhibits I_{Na} (IC_{50} 0.8 ± 0.3 mM; pharmacological efficacy $42.89 \pm 5.54\%$). 4TERP interfered with I_{Na} through a mechanism with various components, which includes predominantly channel pore block and sensitivity to frequency of use. In presence of 4TERP (3 mM), decreasing stimulation from 5 Hz to very low frequency (75 s of quiescence previously to stimulation) induced I_{Na} decrease to $65.17 \pm 5.86\%$ of control. 4TERP also altered (left shift) voltage sensitivity of the steady state activation of Na_V . Data are discussed aiming to interpret the importance of blockade of I_{Na} through Na_V as participant of 4TERP-induced inhibition of membrane excitability." As taken from Santos-Nascimento TD et al. 2020. Chem. Biol. Interact. 315, 108890. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/31678597/>

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

"The aim of this paper was to investigate the effect of terpene penetration enhancers on membrane fluidity and membrane potential using HaCaT keratinocytes, and study the potential mechanisms of these terpene compounds using as natural transdermal penetration enhancer. Six terpene compounds, namely menthol, limonene, 1,8-cineole, menthone, terpinen-4-ol and pulegone, were chosen in this study on account of their good penetration-enhancement activities. The cytotoxicity of these terpene compounds was measured using an MTT assay. The fluorescence recovery after photobleaching (FRAP) technique was employed to measure the change of membrane fluidity of HaCaT cells. The flow cytometer was used to study the alteration of membrane fluidity of HaCaT cells, and investigate the effect of terpene compounds on intracellular Ca^{2+} . It was found that 6 terpene compounds possessed low cytotoxicity in comparison to the well-established and standard penetration enhancer azone. Those terpene compounds could significantly enhance HaCaT cells membrane fluidity and decrease HaCaT cells membrane potentials. Meanwhile, after treated with various terpene compounds, the Ca^{2+} -ATPase activity and intracellular Ca^{2+} of HaCaT cells was decreased significantly. Terpene penetration enhancers perhaps changed the membrane fluidity

and potentials of HaCaT cells by altering the Ca²⁺ balance of the cell inside and outside, resulting in the low skin permeability to increase the drug transdermal absorption.” As taken from Lan Y et al. 2015. Zhongguo Zhong Yao Za Zhi 40(4), 643-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26137684>

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 4-Terpinenol 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 4-Terpinenol. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	12	Baker R et al., 2004a
	<1	Carmines, 2002 & Rustemeier et al., 2002
	6	Roemer et al., 2014
In vitro genotoxicity	12	Baker R et al., 2004c
	<1	Carmines, 2002 & Roemer et al., 2002
	6	Roemer et al., 2014
In vitro cytotoxicity	12	Baker R et al., 2004c
	<1	Carmines, 2002 & Roemer et al., 2002
	6	Roemer et al., 2014
Inhalation study	12	Baker R et al., 2004c
	<1	Carmines, 2002 & Vanscheeuwijck et al., 2002
	6	Schramke et al., 2014
In vivo genotoxicity	6	Schramke et al., 2014

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing Terpinen-4-ol 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	0.045	Labstat International Inc. (2021a) JTI Heated Tobacco Stick Study Report(s)
In vitro genotoxicity	0.045	Labstat International Inc. (2021b)

		JTI Heated Tobacco Stick Study Report(s)
In vitro cytotoxicity	0.045	Labstat International Inc. (2021b) JTI Heated Tobacco Stick Study Report(s)

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following data:

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

Bond Method :	1.58E-005 atm-m3/mole
Group Method:	3.15E-006 atm-m3/mole
Henry's LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 2.242E-005 atm-m3/mole (2.271E+000 Pa-m3/mole) VP: 0.0427 mm Hg (source: MPBPVP) WS: 387 mg/L (source: WSKOWWIN)

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

Log Kow used:	3.26 (exp database)
Log Kaw used:	-3.190 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	6.450
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):	0.4902 0.2880 2.6462 (weeks-months) 3.4717 (days-weeks) 0.3368 0.3032 - 0.3363
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):	5.15 Pa (0.0386 mm Hg)
Log Koa (Koawin est):	6.450
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	5.83E-007 6.92E-007

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	2.11E-005
Mackay model:	4.66E-005
Octanol/air (Koa) model:	5.53E-005

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

OVERALL OH Rate Constant =	103.9461 E-12 cm3/molecule-sec
Half-Life =	0.103 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	1.235 Hrs

Ozone Reaction:

OVERALL Ozone Rate Constant =	43.000000 E-17 cm3/molecule-sec
Half-Life =	0.027 Days (at 7E11 mol/cm3)
Half-Life =	38.378 Min
Reaction With Nitrate Radicals May Be Important!	
Fraction sorbed to airborne particulates (phi): 3.38E-005 (Junge-Pankow, Mackay avg) 5.53E-005 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	81.56 L/kg (MCI method)
Log Koc:	1.911 (MCI method)
Koc :	207.4 L/kg (Kow method)
Log Koc:	2.317 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]: Rate constants can NOT be estimated for this structure!

Volatilization from Water: Henry LC: 3.15E-006 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	232.1 hours (9.671 days)
Half-Life from Model Lake:	2636 hours (109.8 days)

Removal In Wastewater Treatment:

Total removal:	8.78 percent
Total biodegradation:	0.15 percent
Total sludge adsorption:	8.46 percent
Total to Air:	0.17 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0301	0.508	1000
Water	26	900	1000
Soil	73.8	1.8e+003	1000
Sediment	0.151	8.1e+003	0

Persistence Time: 863 hr Henry's law constant at 25°C (Pa m³ mol⁻¹) = 4.64.

Soil adsorption and mobility: Koc = 61.2.

As taken from BPDB, 2016.

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- is not persistent in the environment:

Media of concern leading to Categorization	Air-Water-Soil
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	37.5
MITI probability of biodegradation	0.3032
EPI Predicted Ozone reaction half-life (days)	0.02665
EPI Predicted Atmospheric Oxidation half-life (days)	0.1029

Data accessed December 2016 on the OECD website.

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	67.5
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	67.5
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	6.427
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	14.5962
Toxicity to fish (LC50 in mg/l) as predicted by Aster	23.412986
Toxicity to fish (LC50 in mg/l) as predicted by PNN	38.57412
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	0.717
Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	0.693
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	6.43E-002

Data accessed December 2016 on the OECD website.

Record for 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (CAS RN 562-74-3):

Spec. Name	Sci. Name	Exp. Type	Media Type	Resp. Site	Endpoint	Trend	Effect	Conc (Standardized)	Stat. Signif.
Spec. Common Name		Chem. Anal.	Loc	Obs. Dur. (Days)	BCF	Eff %	Effect Meas.	Appl. Rate	Sig. Level
Artemia salina		S	SW		LC50	INC	MOR	F 880000 ug/L	
Brine Shrimp		U	LAB	0.25			MORT		
Aedes aegypti		S	FW		NOEC	NEF	MOR	F 100000 ug/L	ANOSIG
Yellow Fever Mosquito		U	LAB	2		0	MORT		<0.001
Aedes aegypti		S	FW		NR-ZERO	NEF	MOR	F 100000 ug/L	
Yellow Fever Mosquito		U	LAB	2		0	MORT		

As taken from the EPA ECOTOX database.

ECOSAR version 1.11 provides the following aquatic toxicity data for CAS RN 562-74-3: Values used to Generate ECOSAR Profile: Log Kow: 3.332 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 386.6 (mg/L, EPISuite WSKowwin v1.43 Estimate) ECOSAR v1.11 Class-specific Estimations: Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Fish	96-hr	LC50	8.068
Neutral Organics :	Daphnid	48-hr	LC50	5.180
Neutral Organics :	Green Algae	96-hr	EC50	6.416
Neutral Organics :	Fish		ChV	0.912
Neutral Organics :	Daphnid		ChV	0.711
Neutral Organics :	Green Algae		ChV	2.210
Neutral Organics :	Fish (SW)	96-hr	LC50	10.238
Neutral Organics :	Mysid	96-hr	LC50	3.087
Neutral Organics :	Fish (SW)		ChV	2.506
Neutral Organics :	Mysid (SW)		ChV	0.180

“This study evaluated the sedative and anesthetic effects of the essential oils (EO) of *Hyptis mutabilis* (Rich.) Briq. and their isolated components on silver catfish (*Rhamdia quelen*). Quantitative chemical differences between the EOs obtained from leaves and inflorescences were verified, and a new chemotype rich in globulol was described....Fractionation of the leaf EO was carried out by column chromatography. The isolated compounds [(+)-1-terpinen-4-ol and (-)-globulol] showed different activity from that detected for the leaf EO in proportional concentrations and similar sedation to a eugenol control at 10 mg/L. However, fish exposed to 1-terpinen-4-ol (3 and 10 mg/L) did not remain sedated for 30 min....” As taken from Silva LL et al. 2013. Braz. J. Med. Biol. Res. 46(9), 771-9. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24068193>

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

“A major component (30-48%) of Tea tree oil which is used to control various fungal pathogens on a wide range of crops.”

Earthworms - Acute 14 day LC50: > 1000 mg/kg. As taken from BPDB, 2016. Record for 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (CAS RN 562-74-3):

Spec. Sci. Name Spec. Common Name	Resp. Site Exp. Dur. (Days)	Media Type Test Loc.	Exp. Type Chem. Anal.	Dose# Res. Sample Unit	Endpoint BAF/BCF	Effect Effect Meas.	Signif. Sig. Level	Dose Dose Stat. Meth.
Lactuca sativa Lettuce	3	AGR			EC50	REP GERM		NC 0.14 mM
Ostrinia nubilalis European Corn Borer	6	LAB	FD U	7	LC50	MOR MORT		F > 20.0 mg/eu
Dermatophagoides farinae American House Dust Mite	1	FAB LAB	EN U	5-7	LD50	MOR MORT		F 304.8 (261.8-377.1) mg/m2
Dermatophagoides pteronyssinus European House Dust Mite	1	FAB LAB	EN U	5-7	LD50	MOR MORT		F 303.2 (259.9-376.2) mg/m2
Ostrinia nubilalis European Corn Borer	(26.4 - 28.4)	LAB	FD U	7	LOEL	DVP PUPA	SIG 0.05	F 20 mg/eu
Ostrinia nubilalis European Corn Borer		LAB	FD U	7	LOEL	DVP EMRG	SIG 0.05	F 20 mg/eu
Ostrinia nubilalis European Corn Borer	26.4	LAB	FD U	7	NOEL	DVP STGE	ANOSIG 0.05	F 20 mg/eu
Ostrinia nubilalis European Corn Borer	(26.4 - 28.4)	LAB	FD U	7	NOEL	DVP PUPA	NOSIG 0.05	F 2 mg/eu
Ostrinia nubilalis			FD	7	NOEL	DVP	NOSIG	F 2 mg/eu

European Corn Borer		LAB	U			EMRG	0.05	
Ostrinia nubilalis			FD	7	NOEL	GRO	ANOSIG	F 20 mg/eu
European Corn Borer		LAB	U			WGHT	0.05	
Dermatophagoides farinae	1	FAB	EN	2		MOR		F 101.8 ug/cm3
American House Dust Mite		LAB	U			MORT		
Varroa jacobsoni	2	NONE	EN	6		MOR		F (0.04-9.9) mg
Mite		LAB	U			MORT		
Dermatophagoides farinae	1	FAB	EN	2	NR-LETH	MOR		F 101.8 ug/cm3
American House Dust Mite		LAB	U			MORT		
Apis mellifera	2	NONE	EN	6		MOR		F (0.04-9.9) mg
Honey Bee		LAB	U			MORT		

As taken from the EPA ECOTOX database.

ECOSAR version 1.11 provides the following terrestrial toxicity data for CAS RN 562-74-3: Values used to Generate ECOSAR Profile: Log Kow: 3.332 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 386.6 (mg/L, EPISuite WSKowwin v1.43 Estimate) ECOSAR v1.11 Class-specific Estimations: Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Earthworm	14-day	LC50	195.133

“BACKGROUND: Dermatophagoides farinae and Tyrophagus putrescentiae are recognised as an important source of allergens. An assessment was made of the toxicity of hiba, Thujopsis dolabrata var. hondai, oil and 13 organic compounds and the control efficacy of four experimental spray formulations containing the oil (5-30 g L⁻¹ spray) against both mite species. RESULTS: In a contact + fumigant mortality bioassay, (-)-thujopsene was the most toxic constituent against D. farinae and T. putrescentiae (24 h LC50 9.82 and 10.92 µg cm⁻²), and the toxicity of the compound was nearly identical to that of benzyl benzoate (9.33 and 10.14 µg cm⁻²). The toxicity was more pronounced in carvacrol, (+)-terpinen-4-ol, β-thujaplicin, (-)-terpinen-4-ol, cedrol and α-terpineol (LC50 12.05-15.20 and 12.74-16.48 µg cm⁻²) than in N,N-diethyl-3-methylbenzamide (LC50 35.53 and 38.42 µg cm⁻²) against both mite species. The hiba oil 30 g L⁻¹ spray and

commercial permethrin (cis:trans 25:75) 2.5 g L(-1) spray treatment resulted in 100 and 11% mortality against the two mite species respectively. In vapour-phase mortality tests, the two compounds were consistently more toxic in closed versus open containers, indicating that toxicity was achieved mainly through the action of vapour. CONCLUSION: Reasonable mite control in indoor environments can be achieved by a spray formulation containing the 30 g L(-1) hiba oil as a potential contact-action fumigant..” As taken from Kim JR et al. 2015. Pest Manag. Sci. 71(5), 737-43. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/24916027>

“The aim of this research was to determine the chemical composition and insecticidal and repellent activity of the essential oil of *Artemisia rupestris* L. aerial parts against the booklice *Liposcelis bostrychophila* Badonnel and isolation of insecticidal and repellent constituents from the essential oil. The essential oil of *A. rupestris* was obtained by hydrodistillation and analyzed by GC-MS. A total of 30 components of the essential oil of *A. rupestris* was identified and the principal compounds in the essential oil were α -terpinyl acetate (37.18%), spathulenol (10.65%), α -terpineol (10.09%), and linalool (7.56%), followed by 4-terpineol(3.92%) and patchoulol (3.05%). Based on bioactivity-guided fractionation, the four active constituents were isolated from the essential oil and identified as α -terpineol, α -terpinyl acetate, 4-terpineol and linalool.... α -Terpinyl acetate (LD₅₀ = 92.59 μ g/cm²) exhibited stronger contact toxicity against booklice than α -terpineol (LD₅₀ = 140.30 μ g/cm²), 4-terpineol (LD₅₀ = 211.35 μ g/cm²), and linalool (LD₅₀ = 393.16 μ g/cm²). The essential oil of *A. rupestris* (LC₅₀ = 6.67 mg/L air) also possessed fumigant toxicity against *L. bostrychophila* while the four constituents, 4-terpineol, α -terpineol, α -terpinyl acetate and linalool had LC₅₀ values of 0.34, 1.12, 1.26 and 1.96 mg/L air, respectively. α -Terpinol and α -terpinyl acetate showed strong repellency against *L. bostrychophila*, while linalool and 4-terpinol exhibited weak repellency. The results indicate that the essential oil of *A. rupestris* aerial parts and its constituent compounds have potential for development into natural insecticides or fumigants as well as repellents for control of insects in stored grains.” As taken from Liu XC et al. 2013. Molecules 18(9), 10733-46. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24005967>

“BACKGROUND: Insect olfactory organs possess many olfactory receptor neurons, which detect many different sets of odorants in nature. In order to feed on blood meals, stable flies locate host animals and humans using chemical cues such as 1-octen-3-ol and butyric acid. In the present study, behavioural and electroantennogram (EAG) response patterns to repellent volatiles from essential oils (EOs) of *Zanthoxylum piperitum* and *Z. armatum* in combination with the attractants were investigated. RESULTS: Components of the EOs such as cuminaldehyde, citronellal, neral, linalool, linalool oxide, terpinen-4-ol, 1,8-cineole, and piperitone induced remarkable repellent behaviours in the stable fly. EAG responses in the fly antenna to these chemicals showed a dose-dependent manner. The patterns of behavioural and EAG responses were significantly altered depending on the ratios of 1-octen-3-ol or butyric acid to the EOs or compounds in the air mixtures. CONCLUSION: The present study demonstrated that the *Zanthoxylum* EOs decreased the levels of response of flight behaviours of the stable fly towards host volatile compounds. The combinations of odorant mixtures of the attractants with the EOs and their components affect the representation of behavioural and EAG responses of the flies....” As taken from Hieu TT et al. 2014. Pest Manag. Sci. 70(1), 163-72. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23564737>

“The aim of this research was to determine the chemical composition of the essential oils of *Dahlia pinnata*, their insecticidal activity against *Sitophilus zeamais* and *Sitophilus oryzae* and to isolate insecticidal constituents. Based on bioactivity-guided fractionation, active constituents were isolated and identified as D-limonene, 4-terpineol and α -terpineol. Essential oils and active compounds tested exhibited contact toxicity, with LD₅₀ values ranging from 132.48 to 828.79 μ g/cm(2) against *S. zeamais* and *S. oryzae*. Essential oils possessed fumigant toxicity against *S. zeamais* and *S. oryzae* with LC₅₀ from 14.10 to 73.46 mg/L. d-Limonene (LC₅₀ = 4.55 and 7.92 mg/L) showed stronger fumigant toxicity against target insects. 4-Terpeneol (88 $\pm$ 8%) and d-limonene (87 $\pm$ 5%) showed the strongest repellency against *S. zeamais* and *S. oryzae*, respectively. The results indicate that essential oils and insecticidal constituents have potential for development into natural

fumigants, insecticides or repellents for control of the stored-product insect pests. .” As taken from Wang DCet al. 2015. Nat. Prod. Res. 29(18), 1748-51. PubMed, 2016 available at <https://www.ncbi.nlm.nih.gov/pubmed/25563135>

“The chemical composition of the essential oil from *Juniperus formosana* leaves and its contact and repellent activities against *Tribolium castaneum* and *Liposcelis bostrychophila* adults were investigated. The essential oil of *J. formosana* leaves was obtained by hydrodistillation and analyzed by GC-MS. A total of 28 components were identified and the main compounds in the essential oil were α -pinene (21.66%), 4-terpineol (11.25%), limonene (11.00%) and β -phellandrene (6.63%). The constituents α -pinene, 4-terpineol and d-limonene were isolated from the essential oil. It was found that the essential oil exhibited contact activity against *T. castaneum* and *L. bostrychophila* adults (LD50 = 29.14 μ g/adult and 81.50 μ g/cm², respectively). The compound 4-terpineol exhibited the strongest contact activity (LD50 = 7.65 μ g/adult). In addition, data showed that at 78.63 nL/cm², the essential oil and the three isolated compounds strongly repelled *T. castaneum* adults. The compounds α -pinene and d-limonene reached the same level (Class V) of repellency as DEET (p = 0.396 and 0.664) against *L. bostrychophila* at 63.17 nL/cm² after 2 h treatment. The results indicate that the essential oil and the isolated compounds have potential to be developed into natural insecticides and repellents to control insects in stored products.” As taken from Guo S et al. 2016. *Molecules* 21(4), 504. PubMed, 2016

“Mosquito-borne diseases represent a deadly threat for millions of people worldwide. However, the use of synthetic insecticides to control *Culicidae* may lead to resistance, high operational costs and adverse non-target effects. Nowadays, plant-borne mosquitocides may serve as suitable alternative in the fight against mosquito vectors. In this study, the mosquito larvicidal activity of *Origanum vulgare* (Lamiaceae) leaf essential oil (EO) and its major chemical constituents was evaluated against the malaria vectors *Anopheles stephensi* and *An. subpictus*, the filariasis vector *Culex quinquefasciatus* and the Japanese encephalitis vector *Cx. tritaeniorhynchus*. The chemical composition of the EO was analyzed by gas chromatography-mass spectroscopy. GC-MS revealed that the essential oil of *O. vulgare* contained 17 compounds. The major chemical components were carvacrol (38.30%) and terpinen-4-ol (28.70%). EO had a significant toxic effect against early third-stage larvae of *An. stephensi*, *An. subpictus*, *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*, with LC50 values of 67.00, 74.14, 80.35 and 84.93 μ g/ml. The two major constituents extracted from the *O. vulgare* EO were tested individually for acute toxicity against larvae of the four mosquito vectors. Carvacrol and terpinen-4-ol appeared to be most effective against *An. stephensi* (LC50=21.15 and 43.27 μ g/ml, respectively) followed by *An. subpictus* (LC50=24.06 and 47.73 μ g/ml), *Cx. quinquefasciatus* (LC50=26.08 and 52.19 μ g/ml) and *Cx. tritaeniorhynchus* (LC50=27.95 and 54.87 μ g/ml). Overall, this research adds knowledge to develop newer and safer natural larvicides against malaria, filariasis and Japanese encephalitis mosquito vectors.” As taken from Govindarajan M et al. 2016. *Res. Vet. Sci.* 104, 77-82. PubMed, 2016 available at <https://www.ncbi.nlm.nih.gov/pubmed/26850541>

10.5. All other relevant types of ecotoxicity

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	1.818 (BCF = 65.76 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-0.2974 days (HL = 0.5042 days)
Log BCF Arnot-Gobas method (upper trophic):	2.001 (BCF = 100.3)
Log BAF Arnot-Gobas method (upper trophic):	2.001 (BAF = 100.3)

log Kow used:	3.26 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- is not bioaccumulative in the environment:

Empirical Log Kow	3.26
Log Kow predicted by KowWin	3.33
Log BAF T2MTL predicted by Gobas	2.07857227120511
Log BCF 5% T2LTL predicted by Gobas	1.97142627829453
Log BCF Max predicted by OASIS	2.68997556630337
Log BCF predicted by BCFWIN	1.81

Data accessed December 2016 on the OECD website.

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Fifty-first report of the
Joint FAO/WHO Expert Committee on
Food Additives



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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

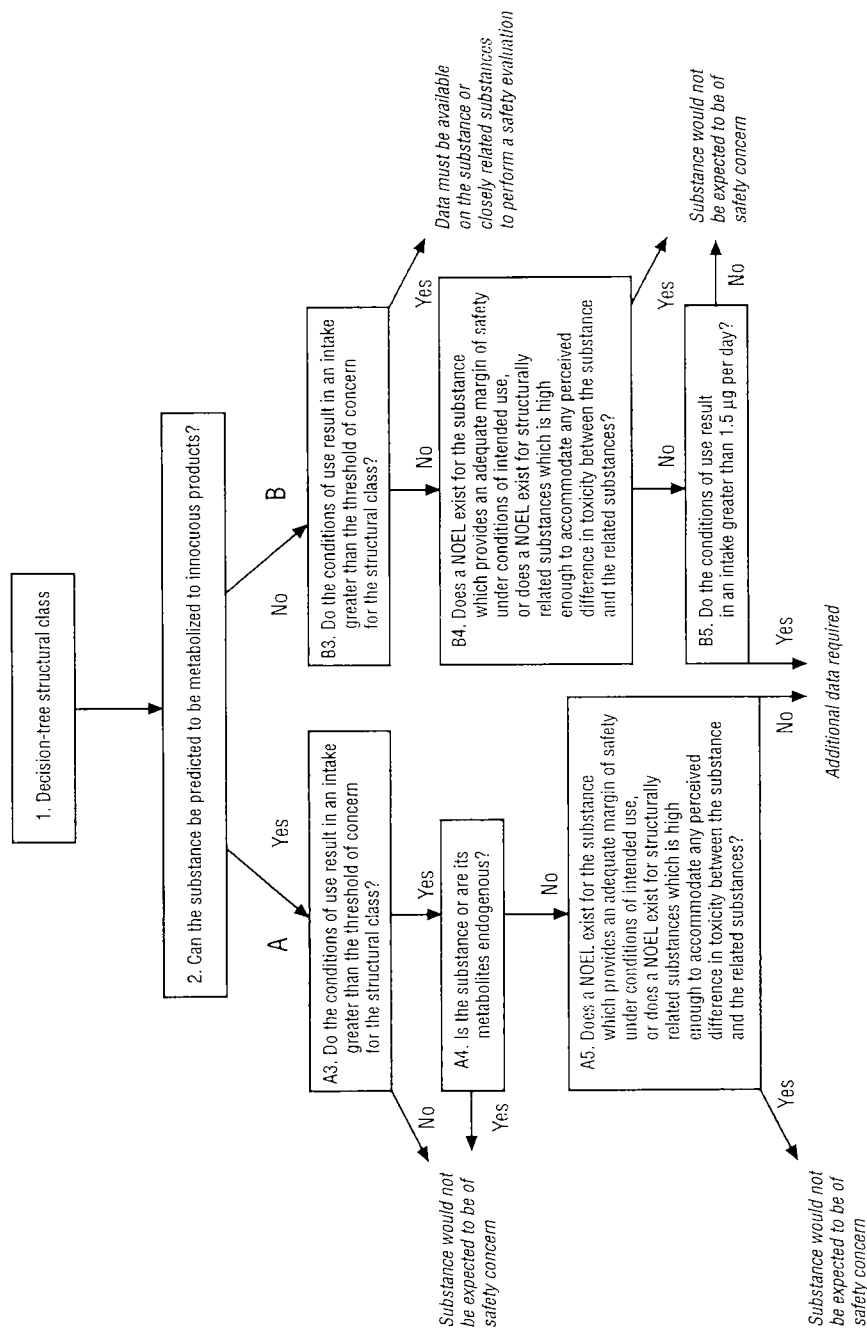


Figure 1
Procedure for the Safety Evaluation of Flavouring Agents

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).	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.
	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.	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 [¹⁴C] *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 I22). 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, Hilderandts 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.

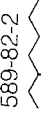
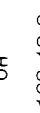
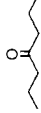
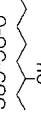
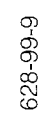
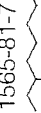
4.2 **Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters**

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: 36000	Yes	NA	No safety concern
Isopropyl alcohol	277	67-63-0 	Yes	Yes Europe: 99000 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

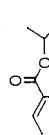
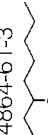
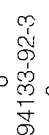
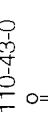
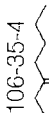
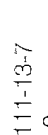
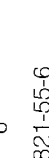
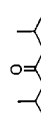
Isopropyl myristate	311	110-27-0		Yes	No Europe: 23 USA: 0.02	NA	NA	No safety concern
Isopropyl tiglate	312	6284-46-4		Yes	No Europe: 0.01 USA: 0.1	NA	NA	
3-Octyl acetate	313	4864-61-3		Yes	No Europe: 0.7 USA: 30	NA	NA	
1-Ethylhexyl liglate	448	94133-92-3		Yes	No Europe: 0.01 USA: 29	NA	NA	
Structural 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.

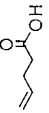
Of the 42 aliphatic unsaturated primary alcohols and unconjugated aldehydes, acids and related esters in this group, 27 are reported to occur naturally in a wide variety of foods, including fruits and vegetables, dairy products, fish and alcoholic beverages. Oleic and linoleic acids (nos 333 and 332) 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		

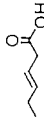
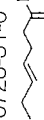
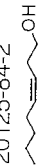
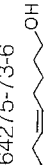
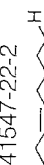
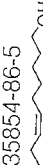
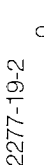
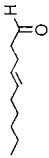
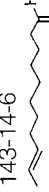
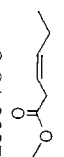
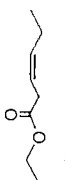
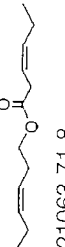
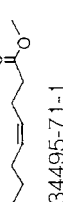
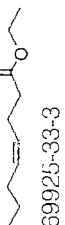
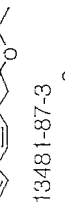
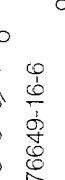
<i>cis</i> -3-Hexenal	316	6789-80-6 	No Europe: 5 USA: 3	NR	NR	See note 2
3-Hexenoic acid	317	4219-24-3 	No Europe: 11 USA: 1	NR	NR	See note 3
4-Hexen-1-ol	318	6126-50-7 	No Europe: 3 USA: 0.04	NR	NR	See note 1
<i>cis</i> -4-Hexenal	319	4634-89-3 	No Europe: 0.03 USA: 0.1	NR	NR	See note 2
4-Heptenal	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	

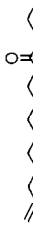
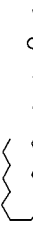
Table 4 (continued)

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	

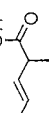
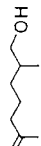
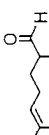
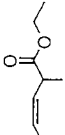
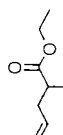
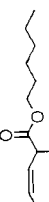
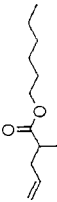
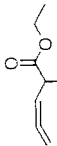
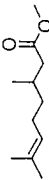
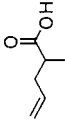
2-Methyl-3-pentenoic acid	347	37674-63-8 	No Europe: 1 USA: 2	NR	NR	See note 7
2,6-Dimethyl-6-hepten-1-ol	348	36806-46-9 	No Europe: ND USA: 0.1	NR	NR	See note 7
2,6-Dimethyl-5-heptenal	349	106-72-9 	No Europe: 31 USA: 250	NR	NR	See note 7
Ethyl 2-methyl-3-pentenoate	350	1617-23-8 	No Europe: 6 USA: 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
						No safety concern
						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.

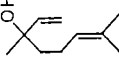
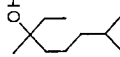
Estimated daily per capita intake

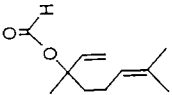
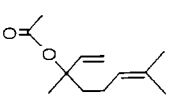
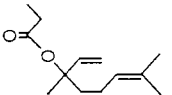
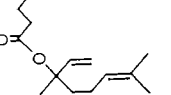
The total annual volume of production of the 23 tertiary alcohols and related esters is approximately 58 tonnes in Europe and 15 tonnes in the USA. Four substances in the group, linalool (no. 356), linalyl acetate (no. 359), α -terpineol (no. 366) and terpinyl acetate (no. 368) account for approximately 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

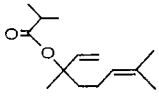
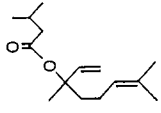
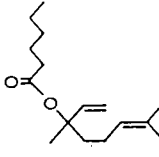
Summary of the results of safety evaluations of 23 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances^a

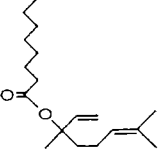
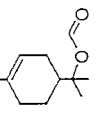
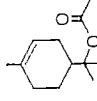
Substance	No.	CAS no. and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step B4/A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
Linalool ^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

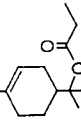
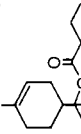
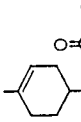
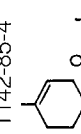
Table 5 (continued)

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.

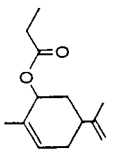
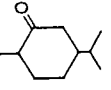
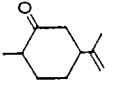
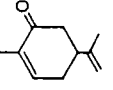
The Committee had previously evaluated carvone on several occasions. A conditional ADI of 0–1.25 mg/kg of body weight for the (+)- and (–)-isomers was established at the eleventh meeting (Annex 1, reference 14). A temporary ADI of 0–1 mg/kg of body weight was established for (+)- and (–)-carvone at the 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

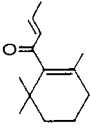
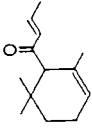
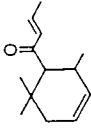
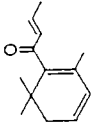
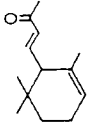
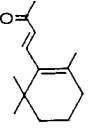
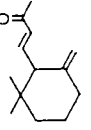
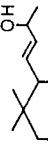
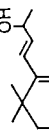
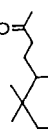
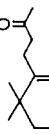
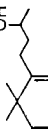
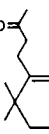
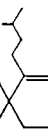
Substance	No.	CAS no. and structure	Step 2 Metabolized to innocuous products?	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step B4 Adequate NOEL for substance or related substance?	Conclusion based on current intake
β -Damascone	384	23726-92-3 	No	No Europe: 43 USA: 10	Yes	No safety 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

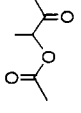
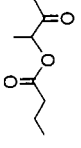
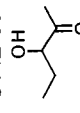
The Committee evaluated a group of 22 flavouring agents that included aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones (Table 8) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1). One member of the group, diacetyl (2,3-butanedione), was previously evaluated 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.

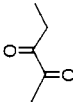
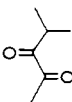
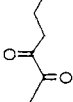
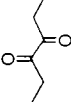
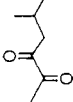
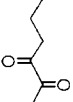
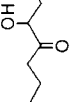
Estimated daily per capita intake

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

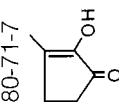
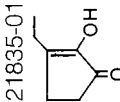
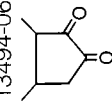
Summary of the results of safety evaluations of 22 aliphatic acyclic and alicyclic α -diketones and related α -hydroxyketones^a

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	

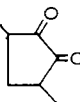
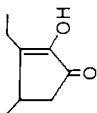
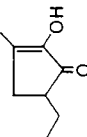
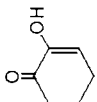
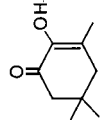
3,5-Dimethyl-1,2-cyclopentanedione	421	13494-07-0		No Europe: 55 USA: 29	NR	NR
3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one	422	42348-12-9		No Europe: ND USA: 0.17	NR	NR
5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one	423	53263-58-4		No Europe: ND USA: 0.38	NR	NR
2-Hydroxy-2-cyclohexen-1-one	424	10316-66-2		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).

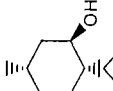
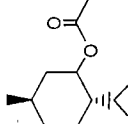
Estimated daily per capita intake

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	

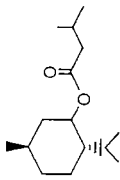
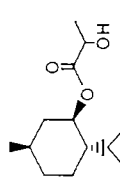
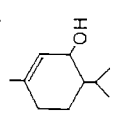
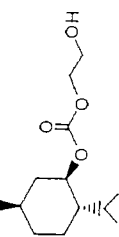
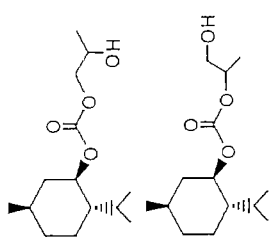
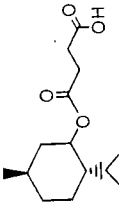
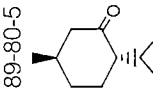
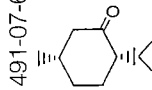
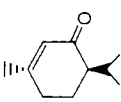
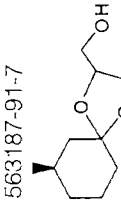
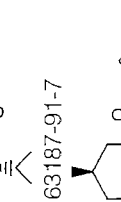
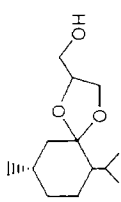
Menthyl isovalerate	432	16409-46-4		No Europe: 9 USA: 27	NR	NR	No safety concern
(-)-Menthyl lactate	433	59259-38-0		No Europe: 26 USA: 0.1	NR	NR	
<i>p</i> -Menth-1-en-3-ol	434	491-04-3		No Europe: 0.02 USA: 0.02	NR	NR	
(-)-Menthol ethylene glycol carbonate	443	156324-78-6		No Europe: ND USA: 760	NR	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-glycocol ketal	445	563187-91-7		No Europe: ND USA: 190	NR	NR	
(±)-Menthone 1,2-glycocol 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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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).
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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).
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¹ The full text is available electronically on the Internet at <http://www.who.int/pes>.

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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	Not evaluated ^f
<i>p</i> -Menth-8-en-1-ol (β -terpineol)	0374	N, T	
4-Thujanol	0441	N, T	
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(5 <i>H</i>)-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(3 <i>H</i>)-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**

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

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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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Chemical Identity

As part of the GRASr2 program, the FEMA Expert Panel (FEXPAN) evaluated a group of 44 flavor ingredients that fit into the group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related flavoring ingredients. This group includes 14 aliphatic terpene tertiary alcohols, 8 alicyclic terpene alcohols, 6 esters of terpene tertiary alcohols, 3 alicyclic tertiary alcohols and 2 esters of alicyclic tertiary alcohols, 2 aliphatic tertiary alcohols, 3 phenyl tertiary alcohols, and 6 esters of phenyl substituted aliphatic tertiary alcohols.

In 1995, the FEXPAN re-evaluated 23 members of this chemical group and all were determined to be GRAS reaffirmed (GRASr) under conditions of use. In the intervening time period, there have been substantial increases in the volume of use of some members of this group as flavor ingredients, as reported in the 2005 FEMA Poundage Update Survey (Gavin and others 2008). Additionally, 20 new substances have been evaluated and determined to be GRAS since the GRASr for this group.

Exposure

From the most recent annual poundage surveys, of the 44 substances in this review 7 have reported volumes of greater than 500 kg/y (see Table 1). More than 76% of the total annual volume of production for this group is accounted for by linalool and linalyl acetate (Gavin and others 2008). The volumes of production reported for linalool and linalyl acetate are 200% and 166%, respectively, greater than the annual volumes reported in the 1995 volume of use survey (Lucas and others 1999; Gavin and others 2008) and 840% and 560%, respectively, greater than the annual volumes evaluated in the original GRASr of the group (National Academy of Science, NAS 1989). Consequently, the estimated daily per capita intakes using maximized survey-derived intake (MSDI) for these 2 materials have increased 5- and 15-fold, respectively, as compared to their original GRASr evaluations. Other substances with high volumes are α -terpineol and the acetate and butyrate esters of α , α -dimethylphenethyl alcohol (see Table 1).

Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances often have a sweet floral rose to a fruity citrus green organoleptic profile. The majority impart a balsamic rose or bergamot citrus flavor and are used as flavor ingredients at average usual use levels of 1 to 10 parts per million (ppm) in a wide range of food categories, but most commonly in baked goods, beverages, candies, chewing gum, frozen dairy and gelatins, and puddings. The few with specialized flavor profiles, such as cool or minty, are used at much higher levels, with average maximum use levels in excess of 1000 ppm in chewing gum.

Twenty-two of the 44 flavor ingredients in this group have been reported to occur naturally, and can be found in chamomile, cocoa, coffee, a variety of fruits and especially citrus fruit varieties and vegetables, lemon juice, black and green teas, calamus, soybean, pepper, strawberry guava, beer and wine (TNO 2013). For some substances within this group, quantitative data are available that indicate that their consumption as naturally occurring constituents of food is far greater than their intake as flavor ingredients (Stofberg and Grundschober 1987).

Annual volumes of production for each ingredient plus the daily per capita intake values calculated using MSDI and reported natural occurrence are summarized in Table 1.

Absorption, Distribution, Metabolism, and Elimination

As described in the previous GRAS affirmation and GRASr group summaries, aliphatic acyclic and alicyclic terpenoids and

related esters undergo efficient metabolism. Based on the results of studies under a wide variety of conditions, including aqueous buffered media, simulated gastric juice, simulated human intestinal fluid, blood plasma, whole hepatocytes and liver microsome preparations, terpene esters formed from tertiary alcohols (for example, linalool), and simple aliphatic carboxylic acids are expected to undergo hydrolysis. Although differences in the rates of hydrolysis occur under *in vitro* conditions in gastric juice and intestinal fluids, ready hydrolysis is observed in tissue preparations that have an abundant concentration of carboxylesterases (CES), especially the liver (Hosokawa 2008; Fukami and Yokoi 2012). The most important class of these enzymes is the B-esterases, which are members of the serine esterase superfamily. Generally, CES enzymes are ubiquitous throughout mammalian tissues and are found at the highest levels in hepatocytes (Hosokawa 2008; Fukami and Yokoi 2012). CES have been classified into 5 major subgroups based on their catalytic capabilities. For the present discussion, the 2 subgroups of interest are CES1, which hydrolyzes esters composed of a large carboxyl group and a small alcohol group, and CES2 with typical substrates composed of a small carboxyl group and a large alcohol (Fukami and Yokoi 2012).

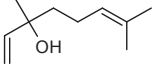
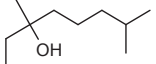
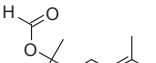
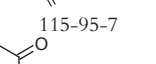
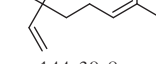
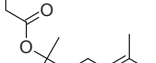
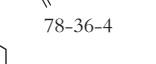
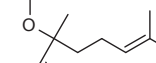
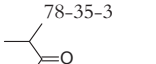
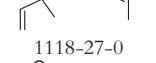
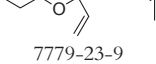
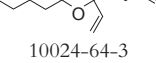
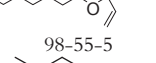
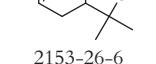
In general, esters are hydrolyzed to their corresponding alcohol and carboxylic acid. It is expected that the tertiary aromatic alcohols will undergo direct conjugation of the hydroxyl group with glucuronic acid (Williams 1959), while the tertiary terpenoid alcohols formed as a result of hydrolysis are rapidly absorbed and converted to the glucuronic acid conjugates which are excreted in the urine, or are further oxidized to CO₂ that is subsequently expired (Phillips and others 1976; Diliberto and others 1988).

Linalyl esters (Nr. 3 to 10) or those derived from α -terpineol (Nr. 12 to 17) are expected to be hydrolyzed in humans to yield the parent terpenoid alcohol (linalool or α -terpineol, respectively) and the corresponding saturated aliphatic carboxylic acid. Methyl 1-acetoxycyclohexyl ketone (Nr. 23) is expected to hydrolyze to acetic acid and methyl 1-hydroxycyclohexyl ketone. Similarly it is expected that esters of α , α -dimethylphenethyl alcohol (Nr. 32 to 35, 38 and 39) will be rapidly hydrolyzed to yield α , α -dimethylphenethyl alcohol and the corresponding acid.

Linalyl acetate was readily 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 constants k = 0.01 to 0.0055/min) compared to the hydrolysis rate in acidic gastric juice (k > 5/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. Both linalool and α -terpineol may then be either conjugated and excreted or oxidized to more polar excretable metabolites.

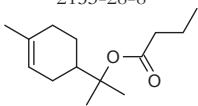
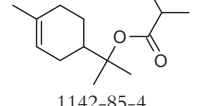
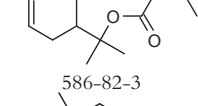
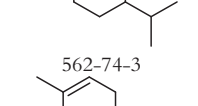
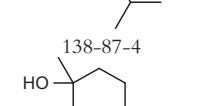
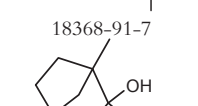
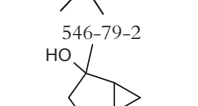
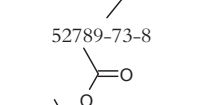
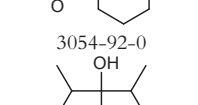
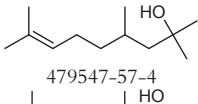
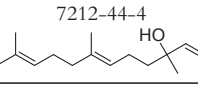
In humans and animals, the principle route of elimination for tertiary alcohols is through formation of glucuronic acid conjugates and excretion in the urine and feces (Williams 1959; Parke

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

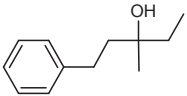
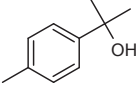
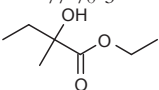
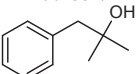
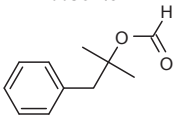
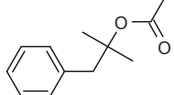
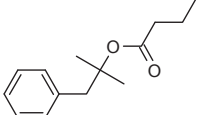
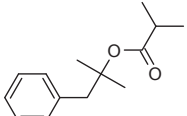
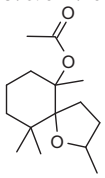
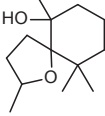
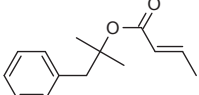
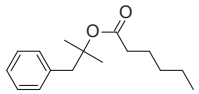
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Table 1–Continued.

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

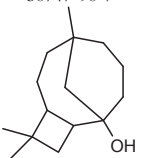
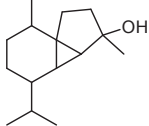
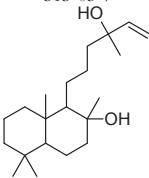
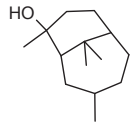
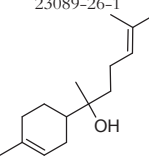
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Table 1–Continued.

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.

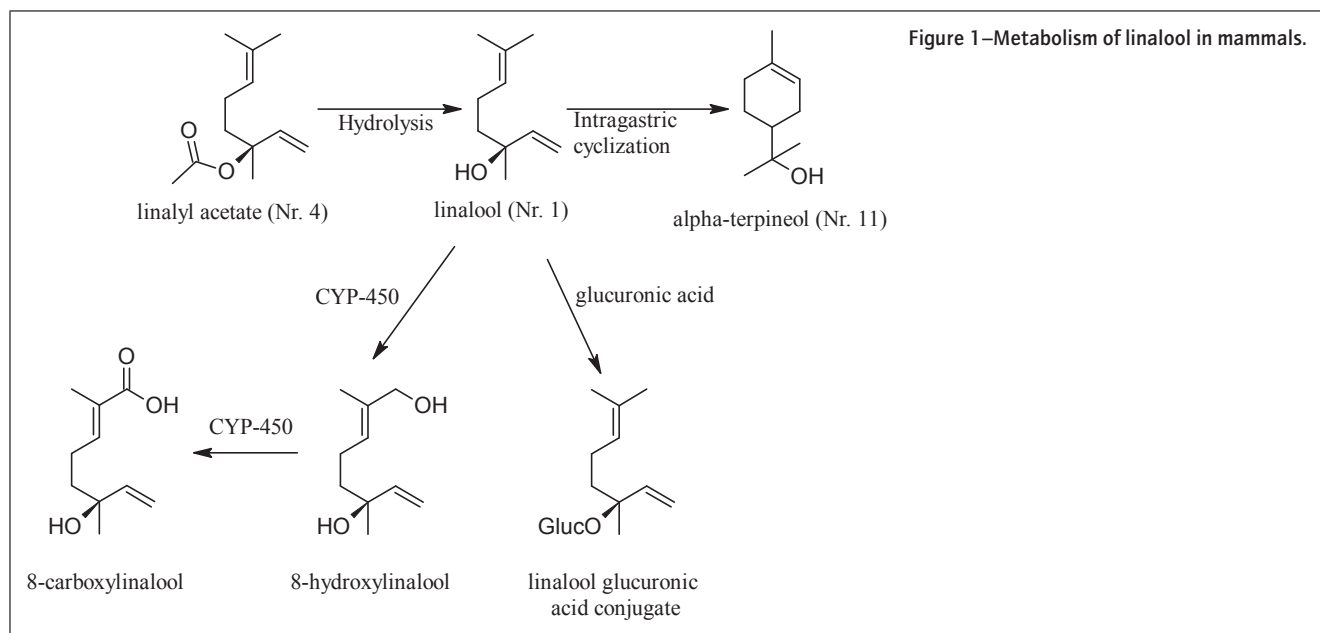
In a minor pathway, epoxidation of the alkene of α -terpineol is followed by hydrolysis to a triol metabolite, 1,2,8-trihydroxy-*p*-menthane, which also has been reported in humans following ingestion of a pine oil disinfectant containing α -terpineol (Horning and others 1976). It is expected that α -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).

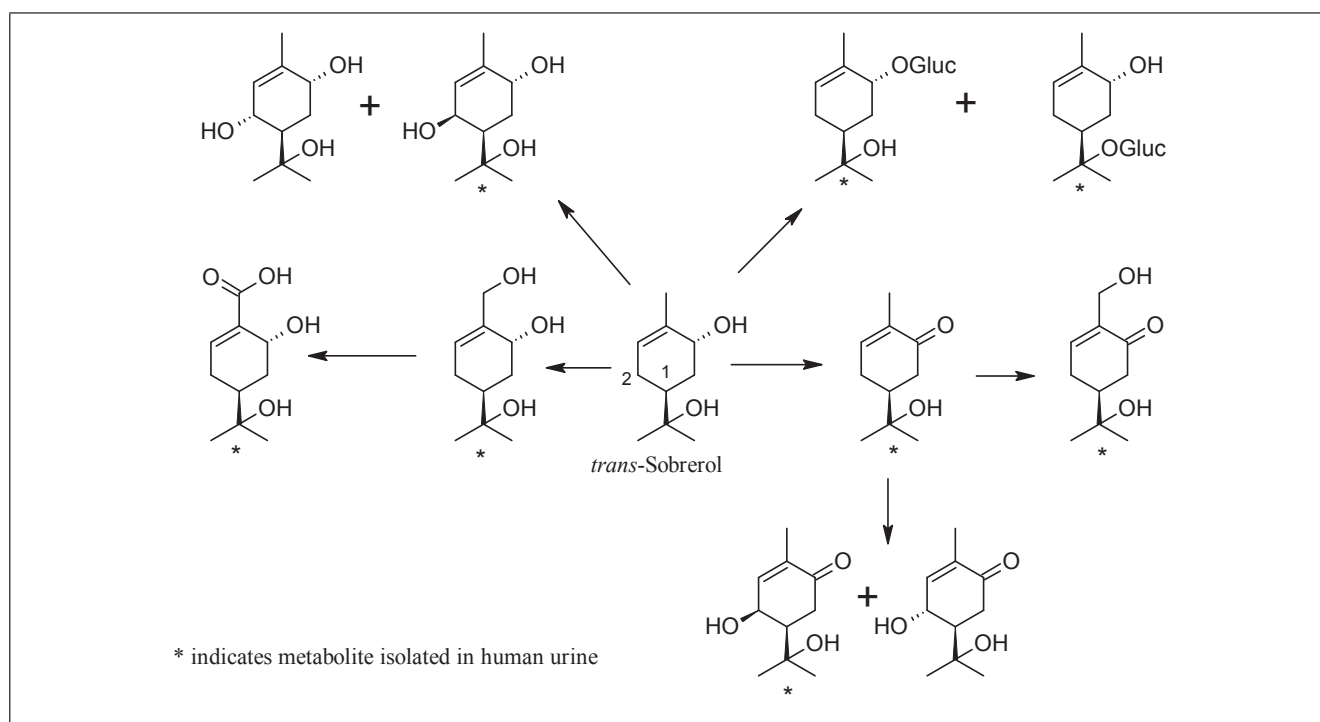
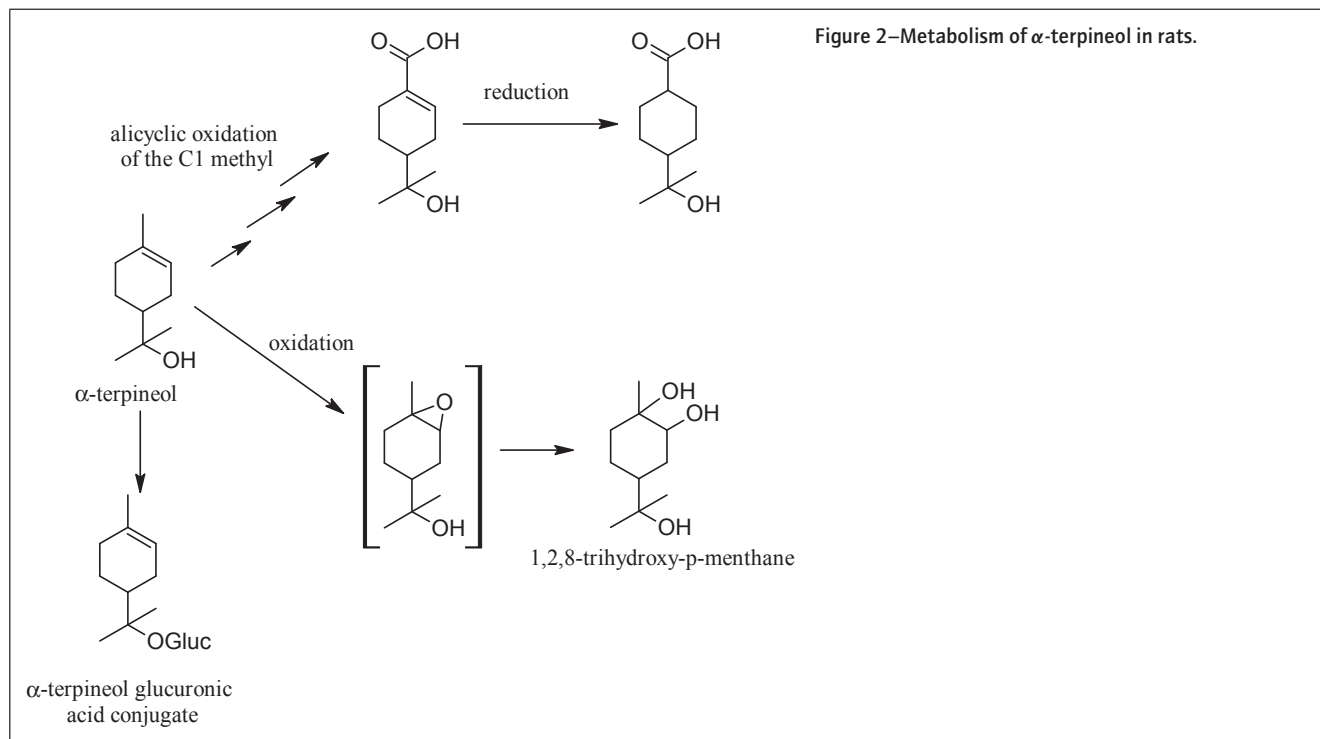


Figure 3–Metabolism of *trans*-sobrerol in rats, dogs, and humans.

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 (Ganzeria 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 Osbourne–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 Osbourne–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 Osbourne–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-acetoxydihydrotheaspirane (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-acetoxydihydrotheaspirane (Nr. 36) and 6-hydroxydihydrotheaspirane (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 acetoxydihydrotheaspirane 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-hydroxydihydrotheaspirane 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.

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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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SCIENTIFIC OPINION

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

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

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

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KEY WORDS

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

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

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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

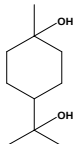
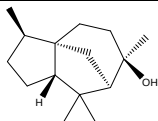
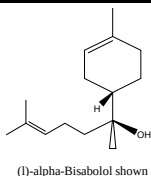
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.041	2-Methylbutan-2-ol		515 75-85-4	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

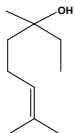
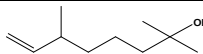
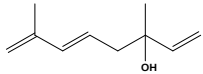
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
								Bisabolol (CAS No.78148-59-1), (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

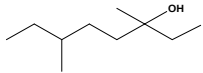
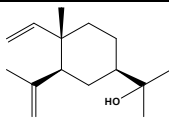
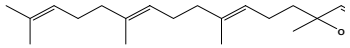
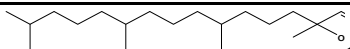
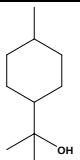
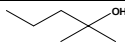
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.147	3,6-Dimethyloctan-3-ol ^(f)		151-19-9	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

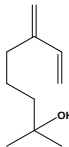
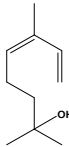
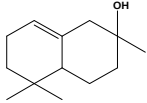
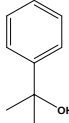
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.184	3-Methylpentan-3-ol		10277 77-74-7	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

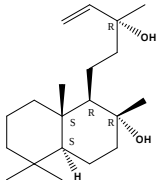
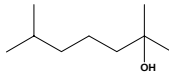
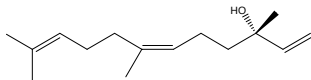
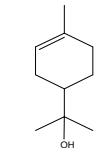
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.206 2029	(-)-Sclareol		4502 10311 515-03-7	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

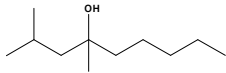
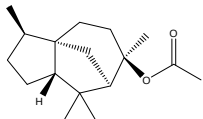
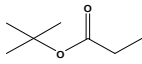
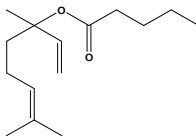
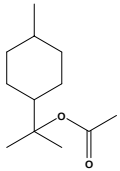
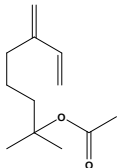
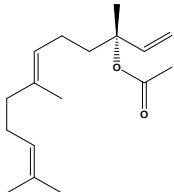
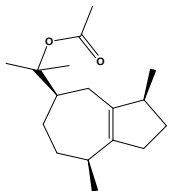
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.253 1850	2,4-Dimethyl-4- Nonanol		4407 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

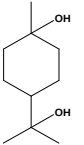
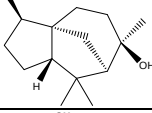
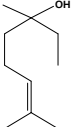
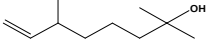
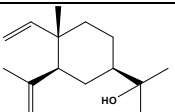
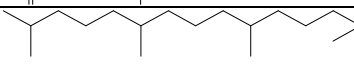
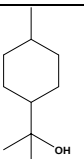
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02.054	p-Menthane-1,8-diol		11	Class I A3: Intake below threshold	d	f	
02.120 2030	(+)-Cedrol		13	Class I A3: Intake below threshold	d	f	
02.140	1,2-Dihydrolinalool		0.044	Class I A3: Intake below threshold	d	f	
02.144	2,6-Dimethyloct-7-en-2-ol		0.0012	Class I A3: Intake below threshold	d	f	
02.149	(-)-alpha-Elemol		1.6	Class I A3: Intake below threshold	d	f	
02.168	Isophytol		0.037	Class I A3: Intake below threshold	d	g	
02.171	p-Menthan-8-ol		0.012	Class I A3: Intake below threshold	d	f	

Table 7: Summary of Safety Evaluation Applying the Procedure

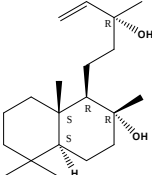
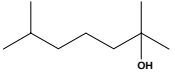
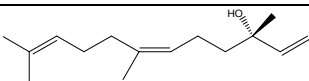
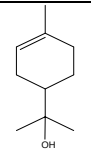
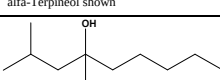
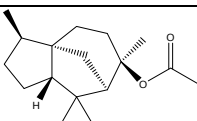
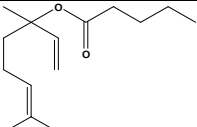
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.206 2029	(-)-Sclareol		0.67	Class I A3: Intake below threshold	d	f	
02.219	2,6-Dimethyl-2-heptanol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

Table 7: Summary of Safety Evaluation Applying the Procedure

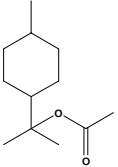
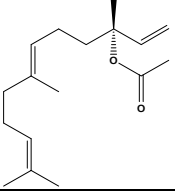
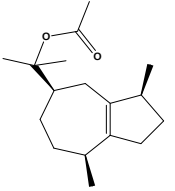
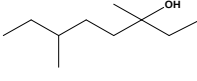
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
09.617	p-Menthan-8-yl acetate		0.012	Class I A3: Intake below threshold	d	f	
09.671	(3S,6Z)-Nerolidyl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

Table 7: Summary of Safety Evaluation Applying the Procedure

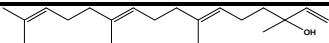
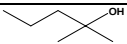
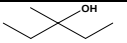
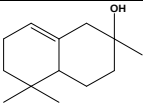
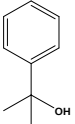
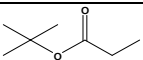
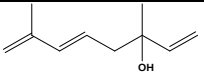
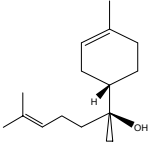
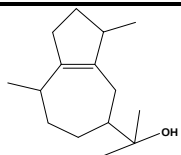
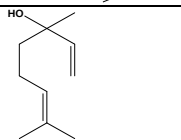
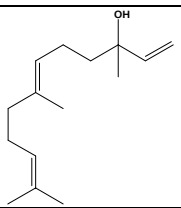
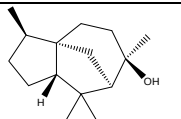
FL-no	EU Register name	Structural formula	MSDI ^a ($\mu\text{g/capita}$ per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.150	(E,E)-Geranyl linalool		0.026	Class II A3: Intake below threshold	d	f	
02.181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

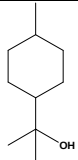
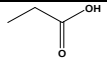
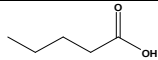
Table 7: Summary of Safety Evaluation Applying the Procedure

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

EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 8: Evaluation Status of Hydrolysis Products of Candidate Esters

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

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

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

SUPPORTING SUBSTANCES SUMMARY

Table 9: Supporting Substances Summary

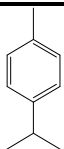
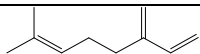
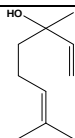
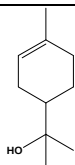
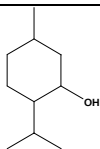
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01.002	1-Isopropyl-4-methylbenzene		2356 620 99-87-6	1325 JECFA specification (JECFA, 2005a).	926	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	
01.008	Myrcene		2762 2197 123-35-3	1327 JECFA specification (JECFA, 2005a)	290	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	EFSA conclusion: B4: Adequate NOAEL exists (EFSA, 2015).
02.013	Linalool		2635 61 78-70-6	356 JECFA specification (JECFA, 1998)	2200	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)
02.014	alpha-Terpineol		3045 62 98-55-5	366 JECFA specification (JECFA, 1998)	2600	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998)	16000	No safety concern (JECFA, 2000a) Category A	ADI: 0-4 (JECFA, 2000a).

Table 9: Supporting Substances Summary

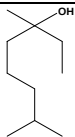
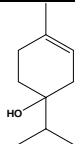
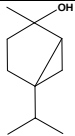
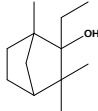
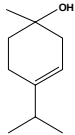
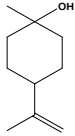
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
						(CoE, 1992)	
02.028	3,7-Dimethyloctan-3-ol		3060 77 78-69-3	357 JECFA specification (JECFA, 1998)	47	No safety concern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

Table 9: Supporting Substances Summary

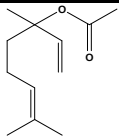
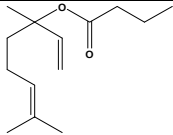
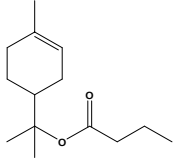
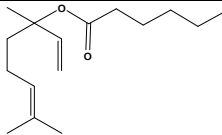
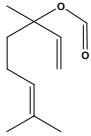
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.013	Linalyl acetate		2636 203 115-95-7	359 JECFA specification (JECFA, 1998)	1700	No safety concern (JECFA, 2000a)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

Table 9: Supporting Substances Summary

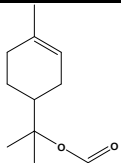
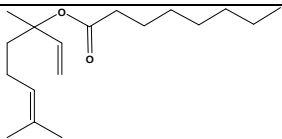
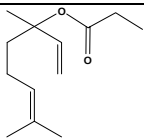
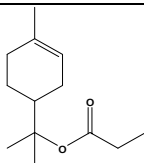
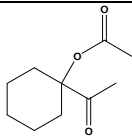
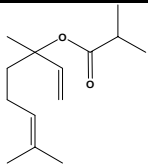
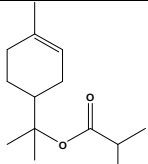
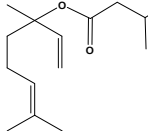
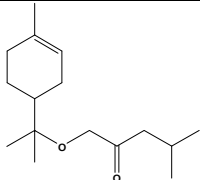
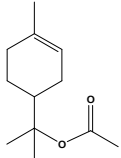
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.081	alpha-Terpinyl formate		3052 348 2153-26-6	367 JECFA specification (JECFA, 2001)	0.12	No safety concern (JECFA,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

Table 9: Supporting Substances Summary

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

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

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

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

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

Appendix B. Procedure for the Safety Evaluation

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

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

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

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

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

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

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

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

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

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

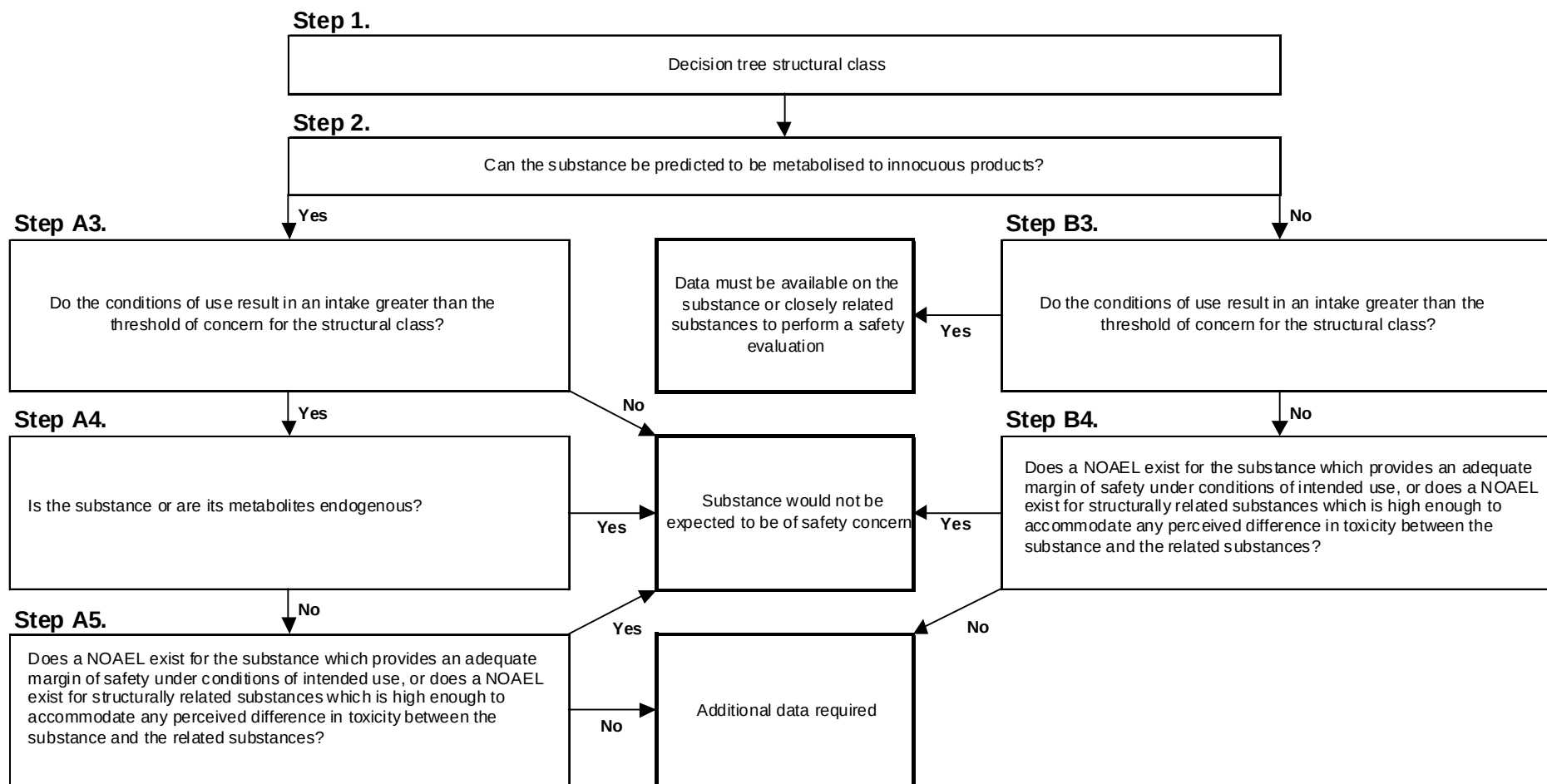


Figure A.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

Appendix C. Use Levels / mTAMDI

C.1 Normal and Maximum Use Levels

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

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

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

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

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

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

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (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
02.171	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.181	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.184	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.185	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.191	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-
02.203	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.206	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.219	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.226	2	-	2	-	-	200	2	2	-	-	-	-	1	-	2	30	2	-
	8	-	80	-	-	500	7	6	-	-	-	-	5	-	5	100	10	-
02.230	20	10	50	-	-	50	-	50	5	-	-	-	50	-	10	60	50	-
	150	50	300	-	-	300	-	300	10	-	-	-	300	-	300	300	300	-
02.253	7	5	10	7	-	10	5	10	2	-	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	-	-	-	25	50	25	50	100	25
09.171	7	5	10	7	7	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	35	50	25	50	10	10	-	-	25	50	25	50	100	2

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

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

C.2 mTAMDI Calculations

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

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

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

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

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

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

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

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

Table 14: Estimated intakes based on the mTAMDI approach

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

Appendix D. Metabolism

D.1 Introduction

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

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

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

D.2 Absorption, Distribution and Elimination

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

D.3 Biotransformation

Ester hydrolysis

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

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

Subgroup 1 - Aliphatic saturated tertiary alcohols and ester

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

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

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

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

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

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

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

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

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

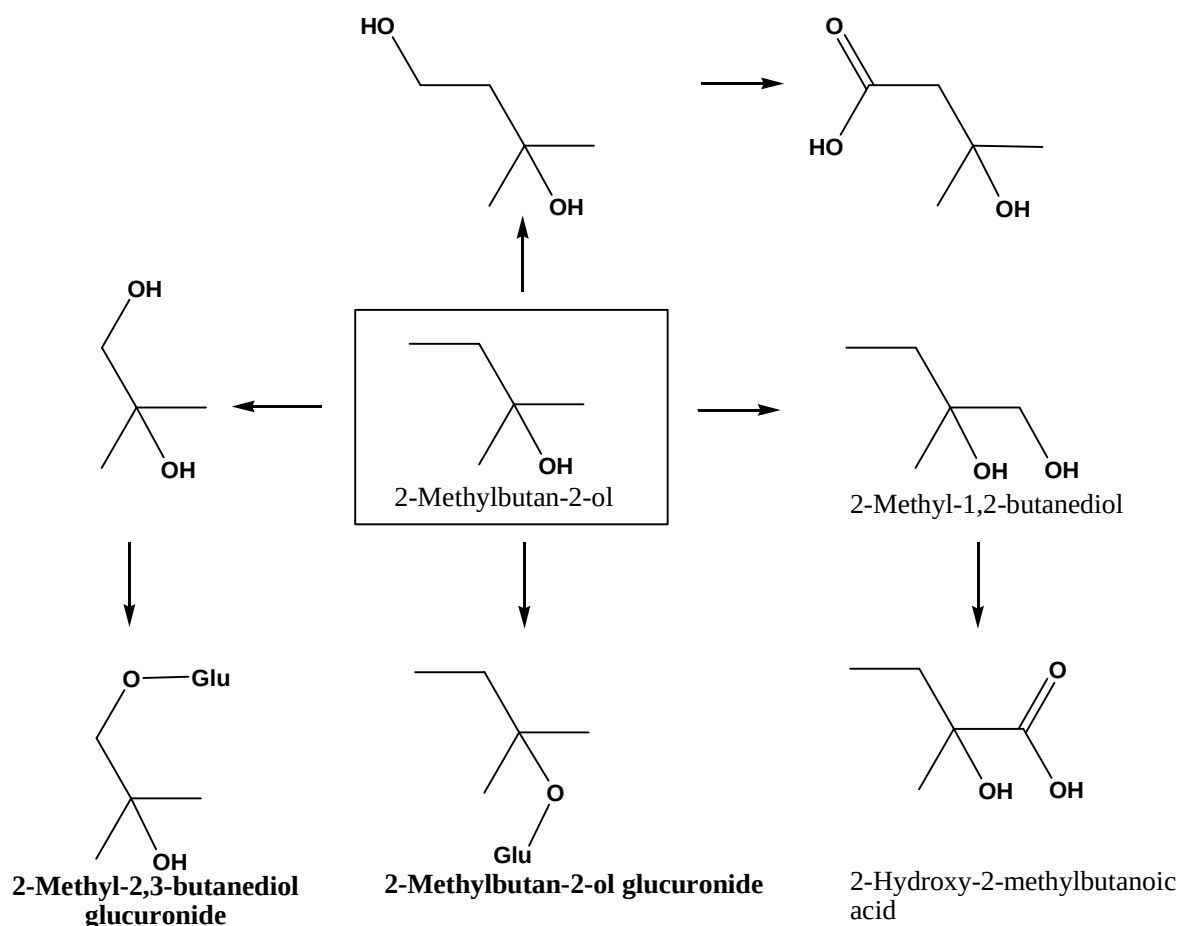


Figure D.1. Metabolism of 2-methylbutan-2-ol (tert-amyl-alcohol) in rats (Amberg et al., 1999). Main metabolism products are in bold.

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

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

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

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

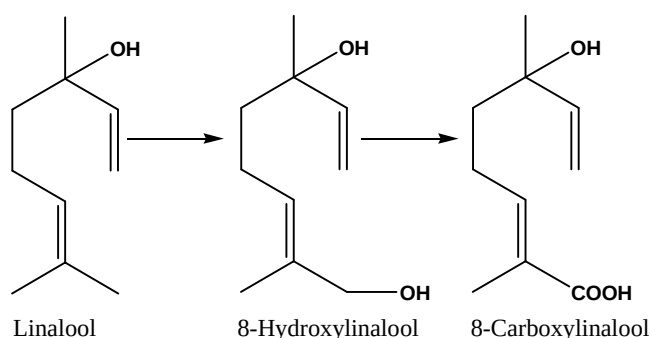


Figure D.2 Metabolism of linalool (Chadha & Madyastha 1984).

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

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

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

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

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

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

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

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

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

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

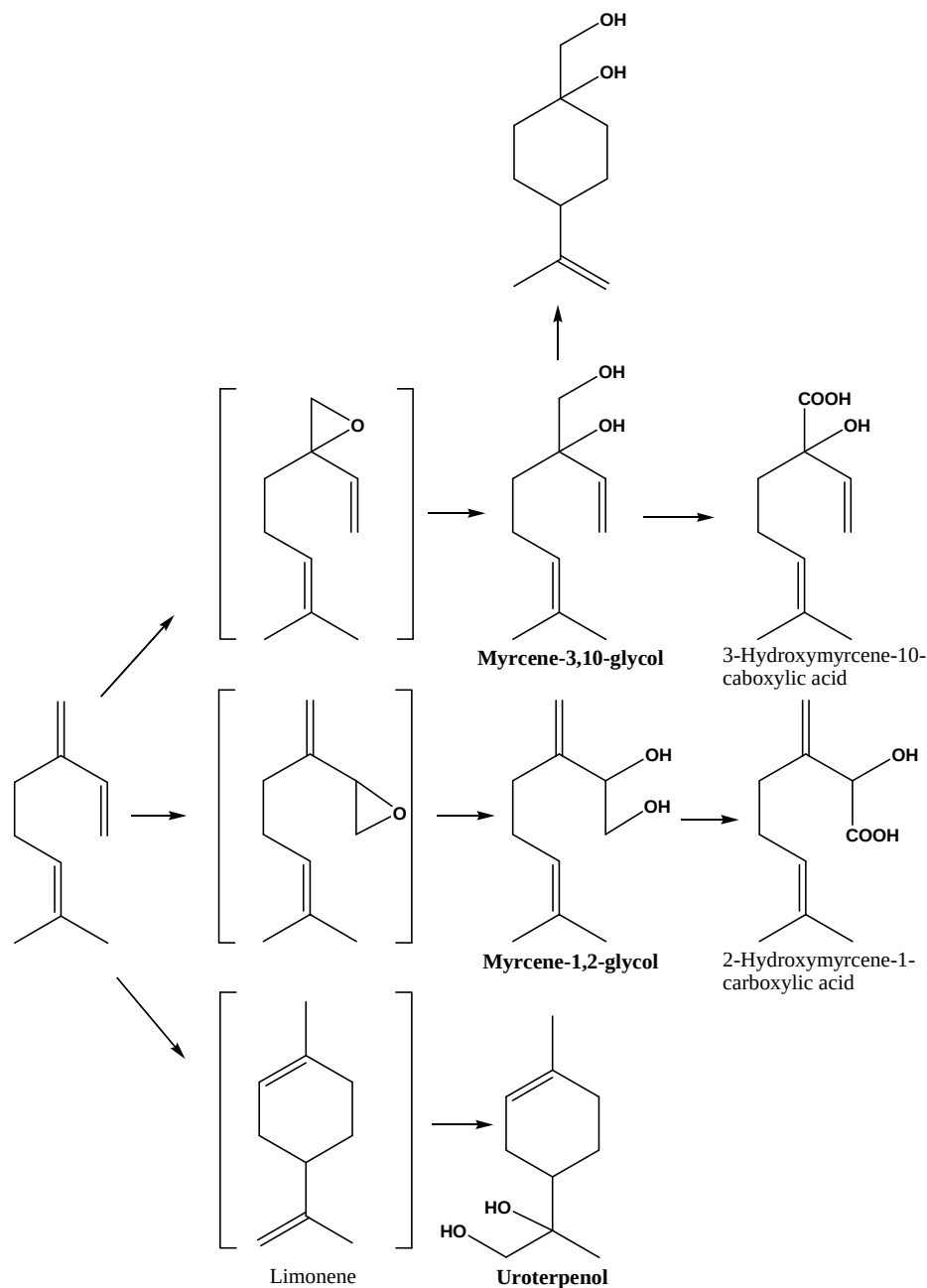


Figure D.3 Metabolism of myrcene (Ishida et al., 1981 and Madyastha & Srivatsan 1987). Intermediate products are in brackets and main metabolism products are in bold.

Subgroup 4: Aliphatic unsaturated tertiary alcohols

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

Subgroup 5: Monocyclic saturated tertiary alcohols and esters

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

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

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

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

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

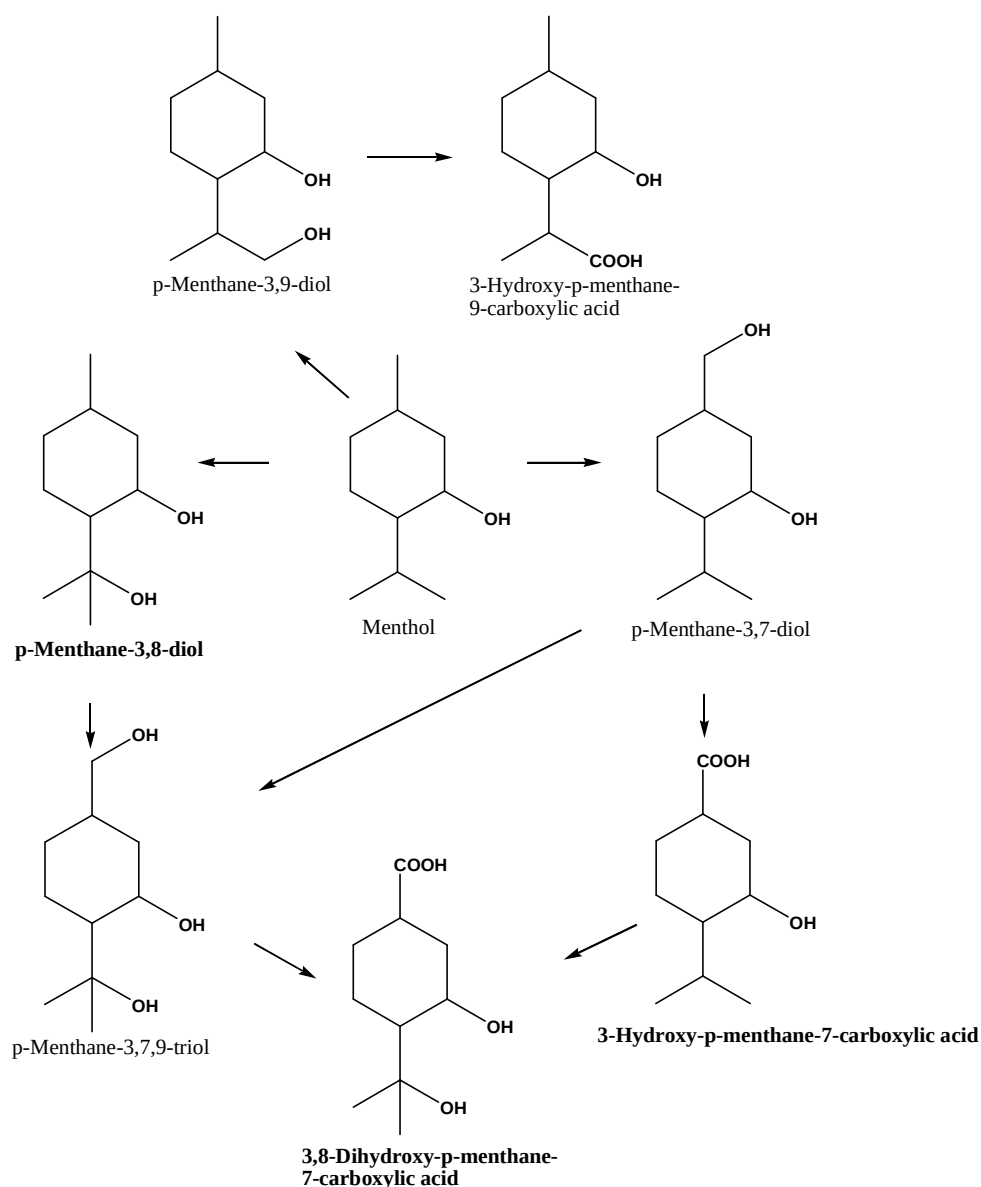


Figure D.4. Metabolism of menthol in rats (Yamaguchi et al., 1994). Main metabolism products are in bold.

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

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

Subgroup 7: Bi- and tricyclic tertiary alcohols and esters

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

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

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

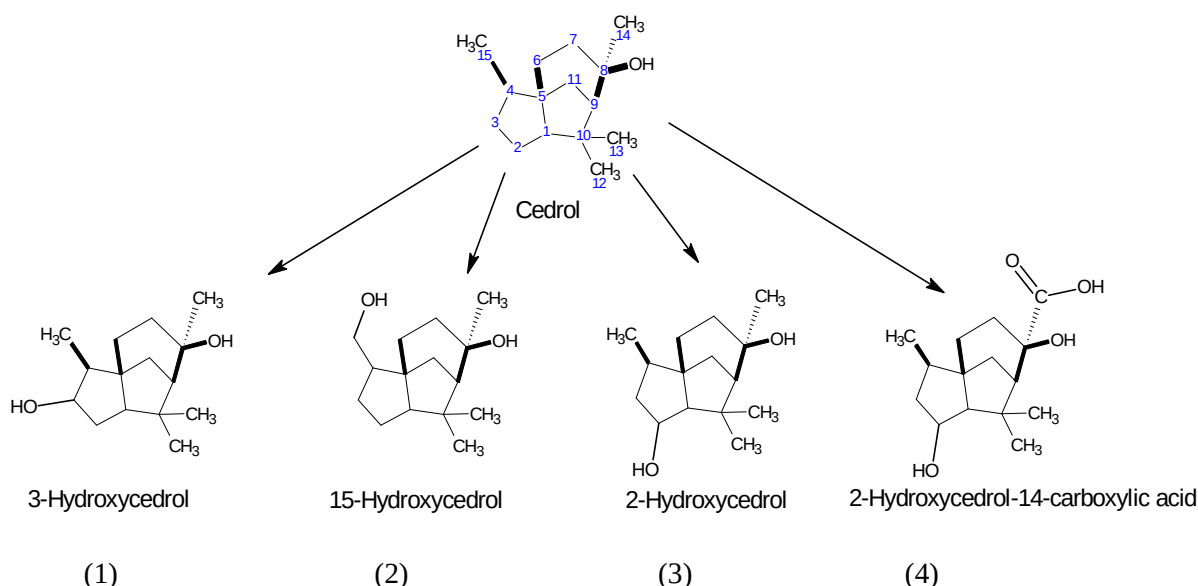


Figure D.5 Metabolism of cedrol in a dog treated with phenobarbital. The carboxylic acid is presumably formed from 2-hydroxycedrol, but this was not further studied (after Trifilieff et al, 1975).

Subgroup 8: Aromatic tertiary alcohols

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

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

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

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

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

Oxidation of terminal double bonds

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

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

D.4 Summary and Conclusion

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

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

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

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

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

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

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

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

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

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

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

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

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

Appendix E. Toxicity

Table 16: Acute Toxicity

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

Table 16: Acute Toxicity

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

Table 16: Acute Toxicity

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

Table 16: Acute Toxicity

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

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

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

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

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

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

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

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

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

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

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

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

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

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

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

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

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

NR Not Reported

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

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

(c): Immunotoxicity study.

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

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

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

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

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

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

Table 18: Developmental and Reproductive Toxicity Studies

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

Table 18: Developmental and Reproductive Toxicity Studies

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

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

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

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

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

Table 19: Genotoxicity (*in vitro*)

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

- NR: Not Reported.
(a): With and without metabolic activation.
(b): With metabolic activation.
(c): Without metabolic activation.
(d): With and without pre-treatment with mitomycin C at 0.15 microM for 21 hours.
(e): With and without presence of beta-glucuronidase.

Table 20: Genotoxicity (*in vivo*)

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

Table 20: Genotoxicity (*in vivo*)

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

Table 20: Genotoxicity (*in vivo*)

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

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

ABBREVIATIONS

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

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



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Short review

RIFM fragrance ingredient safety assessment, 4-Carvomenthenol, CAS Registry Number 562-74-3



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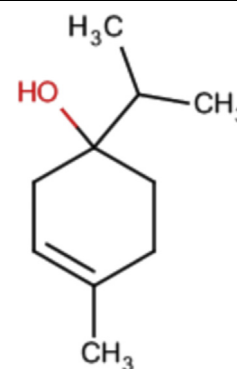
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**Abbreviation list:**

2-Box Model — a RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration

AF— Assessment Factor

BCF— Bioconcentration Factor

Creme RIFM model — The Creme RIFM model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic

(continued on next page)

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(continued)

estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017) compared to a deterministic aggregate approach.

DEREK- Derek nexus is an *in silico* tool used to identify structural alerts

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

The Expert Panel for Fragrance Safety* concludes that this material is safe under the limits described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015) which 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).

*The Expert Panel for Fragrance Safety 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 conditions is supported by existing information.

This material was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, as well as environmental safety. Data show that this material is not genotoxic. Data on the target material and the suitable read across material terpineol (CAS # 8000-41-8) show that this material does not have skin sensitization potential. The local respiratory toxicity endpoint was completed using the TTC (Threshold of Toxicological Concern) for a Cramer Class I material (1.4 mg/day). The repeated dose, developmental and reproductive toxicity endpoints were completed using terpineol (CAS # 8000-41-7) as a suitable read across analogue, which provided a MOE > 100. The phototoxicity/photoallergenicity endpoint was completed based on suitable UV spectra. The environmental endpoint was completed as described in the RIFM Framework.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2000a,b; RIFM, 2015)

Repeated Dose Toxicity: (ECHA REACH Dossier: Terpineol)
NOAEL = 578 mg/kg/day

Developmental Toxicity: NOAEL = 200 mg/kg/day and **Reproductive Toxicity:** NOAEL = 250 mg/kg/day (ECHA REACH Dossier: Terpineol)

Skin Sensitization: Not sensitizing. (ECHA REACH Dossier: Terpineol; RIFM, 1964)

(continued)

Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic (UV Spectra, RIFM DB)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 69% (OECD 301D) (RIFM, 2001a,b)

Bioaccumulation: Screening Level: 65.76 L/kg (EpiSuite ver 4.1)

Ecotoxicity: Critical Ecotoxicity Endpoint: 48 h *Daphnia magna* LC50: 5.18 mg/l (EpiSuite ver 4.1)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-Level: PEC/PNEC (North America and Europe) > 1 (RIFM Framework; Salvito et al., 2002)

Critical Ecotoxicity Endpoint: 48 h *Daphnia magna* LC50: 5.18 mg/l (EpiSuite ver 4.1)

RIFM PNEC is: 0.518 µg/L

• Revised PEC/PNECs (2011 IFRA VoU): North America and Europe < 1

1. Identification

- Chemical Name:** 4-Carvomenthenol
- CAS Registry Number:** 562-74-3
- Synonyms:** 4-Carvomenthenol; 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-; 1-*p*-Menthen-4-ol; 1-Methyl-4-isopropyl-1-cyclohexene-4-ol; Origanol; 4-Terpinenol; シンネール(C = 1 ~ 3) シクロヘキセン-1-オール; テルピネン-4-オール; 1-Isopropyl-4-methylcyclohex-3-en-1-ol; Terpinenol-4 NAT
- Molecular Formula:** C₁₀H₁₈O
- Molecular Weight:** 154.25
- RIFM Number:** 932

2. Physical data

- Boiling Point:** 89 °C @ 6 mm Hg [FMA database], (calculated) 211.85 °C [EPI Suite]
- Flash Point:** 179 °F; CC [FMA database]
- Log Kow:** 3.33 [EPI Suite]
- Melting Point:** 14.86 °C [EPI Suite]
- Water Solubility:** 848 mg/l at 20 ± 2 °C [RIFM, 2000b]; (calculated) 386.6 mg/L [EPI Suite]
- Specific Gravity:** 0.936 [FMA database]
- Vapor Pressure:** 0.02 mm Hg 20 °C [FMA database], 0.0263 mm Hg @ 20 °C [EPI Suite 4.0], 0.0427 mm Hg @ 25 °C [EPI Suite]
- UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L · mol⁻¹ · cm⁻¹)
- Appearance/Organoleptic:** A colorless liquid with a warm-peppery, mildly earthy, musty, and woody odor of moderate tenacity. The taste is rather bitter at concentrations higher than 100 ppm while it becomes quite pleasant, warm, herbaceous, and peppery below 50 ppm.

3. Exposure

- Volume of Use (worldwide band):** 10 to 100 metric tons per year (IFRA, 2011)
- 95th Percentile Concentration in Hydroalcohols:** 0.0051% (RIFM, 2014)
- Inhalation Exposure*:** 0.000019 mg/kg/day or 0.0014 mg/day (RIFM, 2014)
- Total Systemic Exposure**:** 0.00025 mg/kg/day (RIFM, 2014)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM exposure model (Comiskey et al., 2015; Safford et al., 2015 and Safford et al., 2017).

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 4. It is derived from concentration survey data in the Creme RIFM aggregate exposure model and includes exposure via dermal, oral and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015 and Safford et al., 2017).

4. Derivation of systemic absorption

- 1 **Dermal:** Assumed 100%
- 2 **Oral:** Assumed 100%
- 3 **Inhalation:** Assumed 100%

5. Computational toxicology evaluation

- 1 **Cramer Classification:** Class I, Low (Expert Judgment)

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
I*	I	III

*See Appendix below for explanation.

2 Analogs Selected:

- a **Genotoxicity:** None
- b **Repeated Dose Toxicity:** Terpineol (CAS # 8000-41-7)
- c **Developmental and Reproductive Toxicity:** Terpineol (CAS # 8000-41-7)
- d **Skin Sensitization:** Terpineol (CAS # 8000-41-7)
- e **Phototoxicity/Photoallergenicity:** None
- f **Local Respiratory Toxicity:** None
- g **Environmental Toxicity:** None
- 3 **Read-across Justification:** See Appendix below

6. Metabolism

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

7. Natural occurrence (discrete chemical) or composition (NCS)

4-Carvomenthenol is reported to occur in the following foods* and in some natural complex substances (NCS):

Acerola (Malpighia) Alpinia species Angelica (Angelica archangelica L.) Anise (Pimpinella anisum L.) Anise brandy Aannato (Bixa orellana L.) Apple brandy (Calvados) Apple fresh (Malus species) Apple processed (Malus species) Apricot (Prunus armeniaca L.) Ashanti pepper (Piper guineense Schum and Thom) Babaco fruit (Carica pentagona Heilborn) Beans Beerbeli, bael (Aegle marmelos Correa) Black currants (Ribes nigrum L.) Buchu oil Buckwheat Bullock's heart (Annona reticulata L.) Calabash nutmeg (Monodora myristica Dunal) Calamus (sweet flag) (Acorus calamus L.) Camomile Cape gooseberry (Physalis peruviana L.) Capsicum species Caraway (Carum carvi L.) Cardamom (Ellettaria cardamomum Maton.) Carrot (Daucus carota

L.) Celery (Apium graveolens L.) Cherimoya (Annona cherimolia Mill.) Cherry Chicken Chinese quince (Pseudocarya sinensis Schneid) Cinnamomum species Citrus fruits Cloves (Eugenia caryophyllata Thunberg) Cocoa Coriander seed (Coriandrum sativum L.) Cumin seed (Cuminum cyminum L.) Curcuma species Curry (Bergera koenigii L.) Custard apple, atemoya (Annona atemoya) Date (Phoenix dactylifera L.) Dill (Anethum species) Elderberry (Sambucus nigra L.) Eucalyptus oil (Eucalyptus globulus Labill) Fennel (Foeniculum vulg., ssp. capillaceum; var.) Filbert, hazelnut (Corylus avellano) GinGinger (Zingiber species) Grape (Vitis species) Grape brandy Guava and feyoa Guava wine HoneyHop (Humulus lupulus) Juniperus communis Kiwifruit (Actinidia chinensis, syn. A. deliciosa) Laurel (Laurus nobilis L.) Lemon balm (Melissa officinalis L.) Licorice (Glycyrrhiza glabra L.) Litchi (Litchi chinensis Sonn.) Litchi wine Loganberry (Rubus ursinus var. loganobaccus) Lovage (Levisticum officinale Koch) Mace (Myristica fragrans Houttuyn) Malt Mammee apple (Mammea americana L.) Mangifera species Mastic (Pistacia lentiscus) Mentha oils Mountain papaya (C. candamarcensis, C. pubescens) Myrtle (Myrtus communis L.) Nectarine Nutmeg (Myristica fragrans Houttuyn) Ocimum species Olive (Olea europaea) Omija fruit (Schisandra chinensis Baillon) Origanum (Spanish) (Coridothymus cap.(L.) Rchb.) Papaya (Carica papaya L.) Parsley (Petroselinum species) Passion fruit (passiflora species) Peanut (Arachis hypogaea L.) Pecan (Carya illinoensis Koch) Pepino fruit (Solanum muricatum) Pepper (Piper nigrum L.) Pimento (allspice) (Pimenta dioica L. Merr.) Pineapple (Ananas comosus) Pistachio oil (Pistacia vera) Pistacia atlantica Pistacia palaestina (Pistacia terebinthus L.) Plum (Prunus species) Pomegranate juice (Punica granatum L.) Pomegranate wine (Punica granatum L.) Quince, marmelo (Cydonia oblonga Mill.) Raspberry brandy Raspberry, blackberry and boysenberry Red currants (Ribes rubrum L.) Rosemary (Rosmarinus officinalis L.) Salvia species Satureja species Sherry Soybean (Glycine max. L. merr.) Star anise Starfruit (Averrhoa carambola L.) Sweet grass oil (Hierochloa odorata) Sweet marjoram (Origanum majorana L.) Sweetsop, sugar apple (Annona squamosa L.) Syzygium species Tamarind (Tamarindus indica L.) Tarragon (Artemisia dracunculus L.) Tea Tequila (Agave tequilana) Thyme (Thymus species) Tomato (Lycopersicon esculentum Mill.) Turpentine oil (Pistacia terebinthus) Vaccinium species Vanilla Water yam (Dioscorea alata) Wild marjoram (Origanum vulgare L.) Wine Wormwood oil (Artemisia absinthium L.) Xylopi 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

Pre-Registered for 2010; No dossier available as of 04/17/2017.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 4-carvomenthenol does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. 4-Carvomenthenol was tested for genotoxic potential in the BlueScreen assay and was found negative for

both cytotoxicity and genotoxicity indicating a lack of genotoxic concern (RIFM, 2013). Furthermore, the mutagenic potential of 4-carvomenthenol, was tested in a GLP bacterial reverse mutation assay in accordance with OECD TG 471. *S. typhimurium* strains TA1535, TA97a, TA98, TA100 and TA102 were treated with 4-carvomenthenol in DMSO (dimethyl sulfoxide) with and without metabolic activation (S9) at the concentrations of 0.016–1.6 mg/plate for T97, 0.016–5 mg/plate for TA98 and TA102, and 0.016–1.6 mg/plate (without S9) and 0.016–5 mg/plate (with S9) for strains TA 100 and TA1535. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (RIFM, 2000a,b). Under the conditions of the study, the test material was found to have no mutagenic effects.

The clastogenic activity of 4-carvomenthenol was evaluated in an in vitro micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with 4-carvomenthenol in DMSO at concentrations up to 1540 µg/mL in the presence and absence of metabolic activation (S9) at the 4 h and 24 h time points. 4-Carvomenthenol did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either non-activated or S9-activated test systems (RIFM, 2015). Under the conditions of the study, 4-carvomenthenol was considered to be non-clastogenic in the in vitro micronucleus test.

Based on all of the above, 4-carvomenthenol does not present a concern for genetic toxicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 07/01/16.

10.1.2. Repeated dose toxicity

The margin of exposure for 4-carvomenthenol 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 4-carvomenthenol are insufficient for the repeated dose toxicity endpoint. Read across material terpineol (CAS# 8000-41-7; see section 5) has sufficient repeated dose toxicity data. In a GLP/OECD 413 guideline study, CrI:CD(SD) male and female rats (10/sex/group) were exposed to terpineol multiconstituent by snout-only inhalation route at 0.202, 0.572 and 2.23 mg/L (actual levels) for 13 weeks (6 h/day; 5 days/week), corresponding to 0, 52, 148 or 578 mg/kg/day according to standard minute volume and body weight parameters for Sprague-Dawley rats. The MMAD were between 0.52 and 1.6 µm and the respective GSD was between 2.99 and 1.75. A 4 week treatment free recovery group of 10/sex/group of control and high dose group animals was also included. The nasal cavity was identified as a target organ for local effects. Significant reduction in mean group bodyweight gain among males of the high dose group was observed. Examination of recovery phase animals showed no changes in the nasal pharynx respiratory epithelium, suggesting complete recovery after 4 weeks which is therefore not considered adverse. The group mean reticulocyte percentage and the absolute reticulocyte count were significantly lower than control values for males of the high dose group. This alteration was not present among the recovery group animals. In addition, there were no other related hematological alterations reported among treatment or recovery group animals as compared to control. Thus the NOAEL for the repeated dose toxicity endpoint was determined to be 2.23 mg/L, the highest dose tested, equivalent to 578 mg/kg/day according to standard minute volume and body weight parameters for Sprague-Dawley rats (ECHA, REACH Dossier on terpineol). In another study, an OECD 422 gavage combined repeated dose toxicity study with the reproduction/developmental toxicity screening test was conducted in Sprague-Dawley rats. There were 3

treatment groups. The reproductive subgroup (main phase) consisted of 10 males and 10 females/dose (except for control males and at top dose: 5 males/dose) administered terpineol at doses of 0, 60, 250 and 750 mg/kg/day. The toxicity subgroup consisted of 5 females/dose group and 10 males/dose group, administered terpineol at doses of 0, 60, 250 and 750 mg/kg/day. Main phase males and toxicity phase females were dosed daily for a minimum of five consecutive weeks. An additional 10 rats/sex/dose were dosed with the vehicle or 750 mg/kg/day for five weeks and then given two weeks of recovery before termination. The repeated dose toxicity NOAEL was determined to be 750 mg/kg/day, the highest dose tested. Although there were alterations in liver weight, clinical chemistry and histopathological alterations, all the effects were reversible hence not considered adaptive and not adverse (Hall et al., 2012). Histopathological changes associated with hyaline droplets were observed in the kidneys of male rats receiving 250 or 750 mg/kg/day, such changes are commonly associated with administration of volatile hydrocarbons and are of no consequence to human risk assessment (Lehman-McKeeman and Caudill, 1992 and Lehman-McKeeman et al., 1990). In addition, the kidney weights and histopathology among recovery group animals were similar to the control. Testis weight was markedly low in males receiving 750 mg/kg/day and there was also an indication of low epididymal weights at this dose. There were adverse findings related to treatment with test material on the male reproductive parameters reported among the animals of the high dose group. However the effects on the male reproductive system and organs will be discussed in the reproductive toxicity section of the safety assessment (ECHA REACH Dossier: terpineol). In another study, terpineol multiconstituent No. 2 was administered to 10 male Sprague-Dawley rats for 90 days via diet. The test item was dissolved in corn oil, mixed in Sniff powder feed at the dose level of 12000 ppm (equivalent to 622.65 mg/kg bw/day) and fed to male Sprague-Dawley rats (10/dose) daily ad libitum for 13 weeks. The body weights were significantly reduced in rats receiving test item at 12000 ppm. This decrease was associated with a decrease in the food intake throughout the treatment period. There was no other test material related adverse effect reported among the treated males (ECHA, REACH Dossier on terpineol). The most conservative NOAEL of 578 mg/kg/day from the 90 day inhalation toxicity study was selected for the repeated dose toxicity endpoint. Therefore, the 4-carvomenthenol MOE for the repeated dose toxicity endpoint can be calculated by dividing the terpineol NOAEL in mg/kg/day by the total systemic exposure to 4-carvomenthenol, 578/0.00025 or 2,312,000.

In addition, the total systemic exposure to 4-carvomenthenol (0.25 µg/kg/day) is below the TTC (30 µg/kg bw/day) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 11/3/16.

10.1.3. Developmental and reproductive toxicity

The margin of exposure for 4-carvomenthenol is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

10.1.3.1. Risk assessment. An OECD 422 gavage combined repeated dose toxicity study with the reproduction/developmental toxicity screening test was conducted in Sprague-Dawley rats. There were 3 treatment groups. The reproductive subgroup (main phase) consisted of 10 males and 10 females/dose (except for control males and at top dose: 5 males/dose) administered terpineol at doses of 0, 60, 250 and 750 mg/kg/day. The toxicity subgroup consisted of 5

females/dose group and 10 males/dose group, administered terpineol at doses of 0, 60, 250 and 750 mg/kg/day. Main phase males and toxicity phase females were dosed daily for a minimum of five consecutive weeks. An additional 10 rats/sex/dose were dosed with the vehicle or 750 mg/kg/day for five weeks and then given two weeks of recovery before termination. There were no adverse effects towards the development of the fetus up to 250 mg/kg/day. At 750 mg/kg/day, no females became pregnant. It is considered that the testicular and epididymal effects observed in males receiving 750 mg/kg/day would have been sufficient to prevent fertilization. Thus the NOAEL for the developmental toxicity endpoint was determined to be more than 250 mg/kg/day. In another study, terpineol multiconstituent diluted in corn oil was administered by gavage to groups of mated female Sprague-Dawley rats (20 mated females/dose) at the dose levels of 0, 60, 200, 600 mg/kg bw/day from Days 6–19 after mating. The test was conducted according to the OECD 414 protocol. Embryo-fetal growth was slightly reduced by maternal treatment as evidenced by reduced mean male and female fetal weight at 600 mg/kg bw/day. In addition, mean placental weight in this dose group was slightly low with differences attaining statistical significance. Mean placental, litter and fetal weights at 60 or 200 mg/kg/day were unaffected by maternal treatment with terpineol. The incidence of major and minor abnormalities and skeletal variants showed no relationship to maternal treatment with terpineol. Thus the NOAEL for the developmental toxicity was determined to be 200 mg/kg/day (ECHA, REACH dossier on terpineol). The most conservative NOAEL of 200 mg/kg/day was selected for the developmental toxicity endpoint. **Therefore, the terpineol MOE for the developmental toxicity endpoint can be calculated by dividing the terpineol NOAEL by the total systemic exposure to terpineol, 200/0.00025 or 8,000,000.**

In addition, the total systemic exposure to terpineol (0.25 µg/kg/day) is below the TTC (30 µg/kg bw/day) for the developmental toxicity endpoint of a Cramer Class I material at the current level of use.

An OECD 422 gavage combined repeated dose toxicity study with the reproduction/developmental toxicity screening test was conducted in Sprague-Dawley rats. There were 3 treatment groups. There were 3 treatment groups. The reproductive subgroup (main phase) consisted of 10 males and 10 females/dose (except for control males and at top dose: 5 males/dose) administered terpineol at doses of 0, 60, 250 and 750 mg/kg/day. The toxicity subgroup consisted of 5 females/dose group and 10 males/dose group, administered terpineol at doses of 0, 60, 250 and 750 mg/kg/day. Main phase males and toxicity phase females were dosed daily for a minimum of five consecutive weeks. An additional 10 rats/sex/dose were dosed with the vehicle or 750 mg/kg/day for five weeks and then given two weeks of recovery before termination. Testis weight was markedly low in males receiving 750 mg/kg/day and there was also an indication of low epididymal weights at this dose. This effect was also seen in the recovery group males. At 750 mg/kg/day, reduced numbers or complete absence of spermatozoa, accompanied by the presence of degenerate spermatogenic cells in duct(s) were observed in the epididymides and were still present following the 2-week recovery period. Spermatocele granuloma(ta) that were seen in two males receiving 750 mg/kg/day and one receiving 60 mg/kg/day were not seen at the end of the recovery period. The significance of this change in the single male receiving 60 mg/kg/day is uncertain as spermatocele granuloma(ta) can occur spontaneously in rats of this age and considering the absence of other degenerative changes in the testes or epididymides of this animal. Moderate to severe seminiferous tubular atrophy/degeneration was seen in the testes of all animals dosed at 750 mg/kg/

day, accompanied by minimal to moderate spermatid giant cells and minimal to slight seminiferous tubular vacuolation. Similar findings were still evident following the 2-week recovery period but at a lower incidence and severity suggesting a degree of recovery. There were no alterations in the female reproductive cycles or the reproductive organs up to the highest dose tested. Thus the NOAEL for the reproductive toxicity endpoint was determined to be 250 mg/kg/day based on impairment of male fertility at 750 mg/kg/day (ECHA, REACH Dossier on terpineol). In another investigatory study, succeeding the OECD 422 screening test, was performed to compare the toxicity of terpineol to the male reproductive system when administered by dietary or oral gavage routes. Three groups of Crl:CD(SD) male rats (five/dose) were administered terpineol daily by dietary and/or oral gavage routes at the following doses:

Group 1: dietary 7500 ppm + supplementary gavage dose 300 mg/kg/day.

Group 2: dietary 10000 ppm + supplementary gavage dose 150 mg/kg/day, and.

Group 3: 750 mg/kg/day by gavage only.

Necropsy data indicated that decreases in reproductive organ weights and changes to macroscopic appearance were most marked in the animals receiving terpineol multiconstituent at 750 mg/kg/day. Sperm analysis showed that motile sperm with normal morphology were present in 4/5 males of Group 2 and 1/5 males of Group 1. The outliers in each group were at the extreme of achieved overall exposure for the group suggesting that absolute exposure was important, although the route of exposure and consequently potential to exceed threshold levels was of greater significance. Microscopic examination indicated there were relatively fewer changes in the testes and epididymides in the animals which were given terpineol multiconstituent by the dietary route with oral gavage supplementation (Groups 1 and 2), whereas there were significant changes in those which received it solely by oral gavage (Group 3). The results of dietary administration suggest that exposure via the dietary route of administration reduces the testicular and sperm toxicity of the test material compared to dosing by oral gavage. The results of this study, in part, support the hypothesis that a high peak plasma level is necessary to induce the observed toxic effects (ECHA, REACH dossier on terpineol). In another repeated dose oral dietary toxicity study terpineol multiconstituent No. 2 was administered to 10 male Sprague-Dawley rats for 90 days. The test item was dissolved in corn oil, mixed in Sniff powder feed at the dose level of 12000 ppm and fed to male Sprague-Dawley rats (10/dose) daily ad libitum for 13 weeks. Rats in the control group were fed basal diet only without any test item admixtures. Histopathological examination of the testes and the epididymides were carried out. There were no test item-related histological changes observed in the testis and the epididymis. Thus the NOAEL for male reproductive and systemic toxicity was determined to be 12000 ppm (622.65 mg/kg bw/day) or higher the only dose tested (ECHA, REACH dossier on terpineol). The most conservative NOAEL of 250 mg/kg/day was selected for the reproductive toxicity endpoint. **Therefore, the terpineol MOE for the reproductive toxicity endpoint can be calculated by dividing the terpineol NOAEL by the total systemic exposure to terpineol, 250/0.00025 or 1,000,000.**

In addition, the total systemic exposure to terpineol (0.25 µg/kg bw/day) is below the TTC (30 µg/kg bw/day) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 11/3/16.

10.1.4. Skin sensitization

Based on the available data and read across to terpineol (CAS # 8000-41-7), 4-carvomenthenol does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Based on the available data and read across terpineol (CAS # 8000-41-7; see Section 5), 4-carvomenthenol does not present a concern for skin sensitization. The chemical structures of these materials indicate that they would not be expected to react directly with skin proteins (Roberts et al., 2007; Toxtree 2.5.0; OECD toolbox v3.1). However, it should be noted that as cyclic terpenes, these materials could be reasonably anticipated to undergo autooxidation resulting in potentially sensitizing degradation products. In an open epicutaneous test in guinea pigs, no reactions indicative of skin sensitization were observed with 4-carvomenthenol (Klecak, 1985). Similarly, no reactions indicative of sensitization were observed with read across terpineol in guinea pig studies (ECHA Dossier; Klecak, 1979; RIFM, 1982; Ishihara, 1986). Additionally, in human confirmatory studies no reactions indicative of sensitization were observed with 4-carvomenthenol or terpineol (RIFM, 1977; RIFM, 1961; Greif, 1967; RIFM, 1964). Based on weight of evidence from read across, animal and human data 4-carvomenthenol does not present a concern for skin sensitization.

Additional References: RIFM, 1961; Friedrich et al., 2007; Hausen et al., 1999; Klecak, 1979.

Literature Search and Risk Assessment Completed on: 11/15/13.

10.1.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra, 4-carvomenthenol would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available for 4-carvomenthenol in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. Corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity, $1000 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ (Henry et al., 2009). Based on lack of absorbance, 4-carvomenthenol does not present a concern for phototoxicity or photoallergenicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 09/13/16.

10.1.6. Local respiratory toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The material, 4-carvomenthenol, exposure level is below the Cramer Class I TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are limited inhalation data available on 4-carvomenthenol. Based on the Creme RIFM model, the inhalation exposure is 0.0014 mg/day. This exposure is 1000 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: Rice and Coats, 1994a,b; Regnault-Roger and Hamraoui, 1995

Literature Search and Risk Assessment Completed on: 10/2016.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening level risk assessment of 4-carvomenthenol 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). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this Safety Assessment. For the PEC, while the actual regional tonnage is not provided, the range from the most recent IFRA Volume of Use Survey is reported. The PEC is calculated based on the actual tonnage and not the extremes noted for the range. Following the RIFM Environmental Framework, 4-carvomenthenol 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 4-carvomenthenol 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.

10.2.1.2. Risk assessment. Based on current Volume of Use (2011), 4-carvomenthenol does present a risk to the aquatic compartment in the screening level assessment.

10.2.3. Key studies

10.2.3.1. Biodegradation. RIFM, 2001a,b: Biodegradation of 4-carvomenthenol was evaluated according to the OECD 301D method. 3.0 mg/l of the test material was incubated for 28 days. The biodegradation reached the pass level of >60% after 8 days and came to a maximum of 69% after 21 days.

10.2.4. Ecotoxicity

RIFM, 2001a,b: A 48 h acute *Daphnia magna* immobilization test was conducted according to the OECD 202 I method. Under the condition of this study, the EC50 of the test material was 8.2 mg/l after 24 h and 6.3 mg/l after 48 h.

10.2.5. Other available data

4-Carvomenthenol has been pre-registered for REACH with no additional data at this time.

10.2.5.1. Risk assessment refinement. Because 4-carvomenthenol has passed the screening criteria for risk, measured data is included for completeness only and has not been used for PNEC calculations.

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in $\mu\text{g/L}$)

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish)	EC50 (Daphnia)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening Level (Tier 1)	<u>14.49 mg/L</u>			1,000,000	0.0144 µg/L	
ECOSAR Acute Endpoints (Tier 2) Ver 1.11	8.068 mg/L	<u>5.18 mg/L</u>	6.416 mg/L	10,000	0.518 µg/L	Neutral Organics

Exposure information and PEC calculation (following RIFM Framework: [Salvito et al., 2002](#))

Exposure	Europe (EU)	North America (NA)
Log K _{ow} used	3.33	3.33
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	<1	<1

Based on available data, the RQ for this material is <1. No additional assessment is necessary.

The RIFM PNEC is 0.518 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: 11/15/

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:** <http://www.google.com/webhp?tab=ww&ei=KMSoUniQK-arsOS324GwBg&ved=0CBOO1S4>

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment.

This is not an exhaustive list.

Appendix: Read across justification

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 Jmax 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 (v3.4) ([OECD, 2012](#)).
- Developmental toxicity and skin sensitization were estimated using CAESAR (v2.1.6) ([Cassano et al., 2010](#))
- Protein binding were estimated using OECD QSAR Toolbox (v3.4) ([OECD, 2012](#)).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox (v3.4) ([OECD, 2012](#))

	Target material	Read across material
Principal Name	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-562-74-3	Terpineol
CAS No.		8000-41-7
Structure		
Similarity (Tanimoto score) ¹		0.38
Read across endpoint		• Skin sensitization

(continued on next page)

(continued)

	Target material	Read across material
Molecular Formula	C ₁₀ H ₁₈ O	• Repeated dose C ₁₀ H ₁₈ O
Molecular Weight	154.25	154.25
Melting Point (°C, EPISUITE)	14.86	12.36
Boiling Point (°C, EPISUITE)	211.85	214.38
Vapor Pressure (Pa @ 25 °C, EPISUITE)	5.7	2.62
Log Kow (KOWWIN v1.68 in EPISUITE)	3.33	3.33
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPISUITE)	386.6	371.7
J _{max} (mg/cm ² /h, SAM)	1016.613	205.463
Henry's Law (Pa·m ³ /mol, Bond Method, EPISUITE)	1.58E-005	1.58E-005
Repeated dose toxicity		
Repeated Dose (HESS)	• Not categorized	• Not categorized
Reproductive and developmental toxicity		
ER Binding by OECD QSAR Tool Box (3.4)	• Non binder, impaired OH or NH ₂ group	• Non binder, without OH or NH ₂ group
Developmental Toxicity Model by CAESAR v2.1.6	• toxicant (good reliability)	• toxicant (good reliability)
Skin Sensitization		
Protein binding by OASIS v1.1	• No alert found	• No alert found
Protein binding by OECD	• No alert found	• No alert found
Protein binding potency	• Not possible to classify	• Not possible to classify
Protein binding alerts for skin sensitization by OASIS v1.1	• No alert found	• No alert found
Skin Sensitization model (CAESAR) (version 2.1.6)	• Sensitizer (good reliability)	• Sensitizer (good reliability)
Metabolism		
OECD QSAR Toolbox (3.4)	562-74-3 pdf	8000-41-7 pdf
Rat liver S9 metabolism simulator		

Summary

There are insufficient toxicity data on 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) (CAS # 562-74-3). Hence *in-silico* evaluation was conducted by determining suitable read across analogs for this material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, suitable analog terpineol (CAS # 8000-41-7) was identified as a proper read across material with data for its respective toxicity endpoints.

Conclusion/rational

- Terpineol (CAS # 8000-41-7) could be used as structurally similar read across analog for the target material 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) (CAS # 562-74-3) for the skin sensitization, repeated dose, developmental and reproductive endpoints.
 - o The target substance and the read across analog are structurally similar and belong to a class of unsaturated cyclic tertiary terpene alcohols.
 - o The target substance and the read across analog have a cycloalkene (2-(4-methylcyclohex-3-en-1-yl)propan-2-ol) fragment common among them.
 - o The key difference between the target substance and the read across analog is that the target substance has isopropyl branching near the hydroxy group while the read across analog has two methyl groups flanking the hydroxy group. This makes the target substance's hydroxy group more sterically hindered compared to the hydroxy group in the read across analog.

- o The target substance and the read across analog have a Tanimoto score as mentioned in the above table. The Tanimoto score is mainly driven by the cycloalkene branched tertiary alcohol fragment. The differences in the structure which are responsible for Tanimoto score <1 are not relevant from a toxic endpoint perspective.
- o The target substance and the read across analog have similar physical chemical properties. Any differences in the physical chemical properties of the target substance and the read across analog are estimated to be toxicologically insignificant for the skin sensitization, repeated dose, developmental and reproductive endpoints.
- o According to the QSAR OECD Toolbox (V3.4), structural alerts for the skin sensitization, repeated dose, developmental and reproductive endpoints are consistent between the target substance and the read across analog. The CAESAR model v.2.1.6 predicts the target and the read across analog to be sensitizers. Other protein binding alerts for both of the substances are negative. The data described in the skin sensitization section above shows that the read across analog does not pose a concern for skin sensitization endpoint. Therefore this alert will be superseded by the availability of data. In addition, the target and read across analog are predicted to be a toxicant for developmental endpoint with good reliability only by CAESAR model v.2.1.6. The data described in developmental and reproductive section supports that the read across material is safe to use within given margin of exposure and level of use for developmental toxicity endpoint, so this *in-silico* predictions will be superseded.
- o The target substance and the read across analog are expected to be metabolized similarly as shown by metabolism simulator. Due to structural differences and more steric hindrance, the target substance shows less number of metabolic transformations compared to the read across analog, which increases *in-vivo* reactivity of the read across analog.
- o The structural differences between the target substance and the read across analog are deemed to be toxicologically insignificant for the skin sensitization, repeated dose, developmental and reproductive endpoints.

Reason for Cramer classification

Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978).

- Q1.Normal constituent of the body? **No**
 Q2.Contains functional groups associated with enhanced toxicity? **No**
 Q3.Contains elements other than C,H,O,N,divalent S? **No**
 Q5.Simplely branched aliphatic hydrocarbon or a common carbohydrate? **No**
 Q6.Benzene derivative with certain substituents? **No**
 Q7.Heterocyclic? **No**
 Q16.Common terpene (see Cramer et al., 1978 for explanation)? **No**
 Q17.Readily hydrolysed to a common terpene? **No**
 Q19.Open chain? **No**
 Q23.Aromatic? **No**
 Q24.Monocarbocyclic with simple substituents? **No**
 Q25. Cyclopropane, cyclobutane with substituents in Q24 or a mono or bicyclic sulphide or mercaptan? **No**
 Q26.Monocycloalkanone or a bicyclocompound? **No**

Q22.Common component of food? **No**

Q33.Has sufficient number of sulphonate or sulphamate groups? **No** Class High (Class III)

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.fct.2017.07.040>.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.fct.2017.07.040>.

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Safety data sheet

according to Regulation (EC) No. 1907/2006 (REACH), amended by 2020/878/EU



4-Terpinenol ≥95 %

article number: **6309**

Version: **5.0 en**

Replaces version of: 2024-03-01

Version: (4)

date of compilation: 2016-11-17

Revision: 2024-09-17

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier

Identification of the substance

4-Terpinenol ≥95 %

Article number

6309

Registration number (REACH)

It is not required to list the identified uses because the substance is not subject to registration according to REACH (< 1 t/a).

EC number

209-235-5

CAS number

562-74-3

1.2 Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses:

Laboratory chemical
Laboratory and analytical use

Uses advised against:

Do not use for private purposes (household).
Food, drink and animal feedingstuffs.

1.3 Details of the supplier of the safety data sheet

Carl Roth GmbH + Co. KG
Schoemperlenstr. 3-5
D-76185 Karlsruhe
Germany

Telephone: +49 (0) 721 - 56 06 0

Telefax: +49 (0) 721 - 56 06 149

e-mail: sicherheit@carlroth.de

Website: www.carlroth.de

Competent person responsible for the safety data sheet: Department Health, Safety and Environment

e-mail (competent person):

sicherheit@carlroth.de

1.4 Emergency telephone number

Name	Street	Postal code/city	Telephone	Website
National Poisons Information Centre Beaumont Hospital	Beaumont Road	Dublin 9	+353 1 809 2166	https://www.poisons.ie/

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Safety data sheet

according to Regulation (EC) No. 1907/2006 (REACH), amended by 2020/878/EU



4-Terpinenol ≥95 %

article number: **6309**

Classification according to Regulation (EC) No 1272/2008 (CLP)

Section	Hazard class	Cat-egory	Hazard class and category	Hazard statement
3.10	Acute toxicity (oral)	4	Acute Tox. 4	H302
3.2	Skin corrosion/irritation	2	Skin Irrit. 2	H315
3.3	Serious eye damage/eye irritation	2	Eye Irrit. 2	H319
3.8R	Specific target organ toxicity - single exposure (respiratory tract irritation)	3	STOT SE 3	H335

For full text of abbreviations: see SECTION 16

2.2 Label elements

Labelling according to Regulation (EC) No 1272/2008 (CLP)

Signal word

Warning

Pictograms

GHS07



Hazard statements

H302	Harmful if swallowed
H315	Causes skin irritation
H319	Causes serious eye irritation
H335	May cause respiratory irritation

Precautionary statements

Precautionary statements - prevention

P261	Avoid breathing mist/vapours
P280	Wear protective gloves/eye protection

Precautionary statements - response

P302+P352	IF ON SKIN: Wash with plenty of soap and 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

Labelling of packages where the contents do not exceed 125 ml

Signal word: **Warning**

Hazard pictogram(s):



H335	May cause respiratory irritation.
P261	Avoid breathing mist/vapours.

Labelling of packages where the contents do not exceed 10 ml

Signal word:	Not required
Hazard pictogram(s):	Not required
Hazard statements:	Not required

Safety data sheet

according to Regulation (EC) No. 1907/2006 (REACH), amended by 2020/878/EU



4-Terpinenol $\geq 95\%$

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Precautionary statements:

Not required

2.3 Other hazards

This material is combustible, but will not ignite readily.

Results of PBT and vPvB assessment

According to the results of its assessment, this substance is not a PBT or a vPvB.

Endocrine disrupting properties

Does not contain an endocrine disruptor (ED) at a concentration of $\geq 0,1\%$.

SECTION 3: Composition/information on ingredients

3.1 Substances

Name of substance	4-Terpinenol
Molecular formula	$C_{10}H_{18}O$
Molar mass	154,3 g/mol
CAS No	562-74-3
EC No	209-235-5

Substance, Specific Conc. Limits, M-factors, ATE			
Specific Conc. Limits	M-Factors	ATE	Exposure route
-	-	1.300 mg/kg	oral

SECTION 4: First aid measures

4.1 Description of first aid measures



General notes

Take off contaminated clothing.

Following inhalation

Provide fresh air. In all cases of doubt, or when symptoms persist, seek medical advice.

Following skin contact

Rinse skin with water/shower. In case of skin irritation, consult a physician.

Following eye contact

Irrigate copiously with clean, fresh water for at least 10 minutes, holding the eyelids apart. In case of eye irritation consult an ophthalmologist.

Following ingestion

Rinse mouth with water (only if the person is conscious). Call a doctor.

4.2 Most important symptoms and effects, both acute and delayed

Following inhalation: Cough, pain, choking, and breathing difficulties,

Following skin contact: Localised redness, oedema, pruritis and/or pain,

After eye contact: Conjunctival redness of the eyes, Irritation,

Following ingestion: Gastrointestinal complaints, Nausea, Vomiting

4.3 Indication of any immediate medical attention and special treatment needed

none

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SECTION 5: Firefighting measures

5.1 Extinguishing media



Suitable extinguishing media

co-ordinate firefighting measures to the fire surroundings!

water spray, dry extinguishing powder, BC-powder, carbon dioxide (CO₂)

Unsuitable extinguishing media

water jet

5.2 Special hazards arising from the substance or mixture

Combustible.

Hazardous combustion products

In case of fire may be liberated: Carbon monoxide (CO), Carbon dioxide (CO₂)

5.3 Advice for firefighters

In case of fire and/or explosion do not breathe fumes. Fight fire with normal precautions from a reasonable distance. Wear self-contained breathing apparatus.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures



For non-emergency personnel

Avoid contact with skin, eyes and clothes. Do not breathe vapour/spray.

6.2 Environmental precautions

Keep away from drains, surface and ground water. Retain contaminated washing water and dispose of it.

6.3 Methods and material for containment and cleaning up

Advice on how to contain a spill

Covering of drains.

Advice on how to clean up a spill

Absorb with liquid-binding material (sand, diatomaceous earth, acid- or universal binding agents).

Other information relating to spills and releases

Place in appropriate containers for disposal. Ventilate affected area.

6.4 Reference to other sections

Hazardous combustion products: see section 5. Personal protective equipment: see section 8. Incompatible materials: see section 10. Disposal considerations: see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Provision of sufficient ventilation.

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Measures to prevent fire as well as aerosol and dust generation



Keep away from sources of ignition - No smoking.

Advice on general occupational hygiene

Wash hands before breaks and after work. Keep away from food, drink and animal feedingstuffs.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed.

Incompatible substances or mixtures

Observe hints for combined storage. Incompatible materials: see section 10.

Consideration of other advice:

Specific designs for storage rooms or vessels

Recommended storage temperature: 15 – 25 °C

7.3 Specific end use(s)

No information available.

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

National limit values

Occupational exposure limit values (Workplace Exposure Limits)

This information is not available.

8.2 Exposure controls

Individual protection measures (personal protective equipment)

Eye/face protection



Use safety goggle with side protection.

Skin protection



• hand protection

Wear suitable gloves. Chemical protection gloves are suitable, which are tested according to EN 374. For special purposes, it is recommended to check the resistance to chemicals of the protective gloves mentioned above together with the supplier of these gloves. The times are approximate values from measurements at 22 °C and permanent contact. Increased temperatures due to heated substances, body heat etc. and a reduction of the effective layer thickness by stretching can lead to a considerable reduction of the breakthrough time. If in doubt, contact manufacturer. At an approx. 1.5 times larger / smaller layer thickness, the respective breakthrough time is doubled / halved. The data apply only to the pure substance. When transferred to substance mixtures, they may only be considered as a guide.

• type of material

NBR (Nitrile rubber)

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- **material thickness**

>0,3 mm

- **breakthrough times of the glove material**

>480 minutes (permeation: level 6)

- **other protection measures**

Take recovery periods for skin regeneration. Preventive skin protection (barrier creams/ointments) is recommended.

Respiratory protection



Respiratory protection necessary at: Aerosol or mist formation. Type: A (against organic gases and vapours with a boiling point of > 65 °C, colour code: Brown).

Environmental exposure controls

Keep away from drains, surface and ground water.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

Physical state	liquid
Colour	colourless
Odour	characteristic
Melting point/freezing point	not determined
Boiling point or initial boiling point and boiling range	211 – 213 °C at 1.013 hPa
Flammability	this material is combustible, but will not ignite readily
Lower and upper explosion limit	not determined
Flash point	79 °C (c.c.)
Auto-ignition temperature	not determined
Decomposition temperature	not relevant
pH (value)	6 – 7 (20 °C)
Kinematic viscosity	17,8 mm ² /s at 20 °C 4,89 mm ² /s at 40 °C
Dynamic viscosity	16,7 mPa s at 20 °C 4,48 mPa s at 40 °C
<u>Solubility(ies)</u>	
Water solubility	not determined
<u>Partition coefficient</u>	
Partition coefficient n-octanol/water (log value):	this information is not available
Vapour pressure	not determined

Safety data sheet

according to Regulation (EC) No. 1907/2006 (REACH), amended by 2020/878/EU



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Density and/or relative density

Density 0,933 g/cm³ at 20 °C

Relative vapour density Information on this property is not available.

Particle characteristics not relevant (liquid)

Other safety parameters

Oxidising properties none

9.2 Other information

Information with regard to physical hazard classes: hazard classes acc. to GHS (physical hazards): not relevant

Other safety characteristics: There is no additional information.

SECTION 10: Stability and reactivity

10.1 Reactivity

This material is not reactive under normal ambient conditions.

If heated

Vapours may form explosive mixtures with air.

10.2 Chemical stability

The material is stable under normal ambient and anticipated storage and handling conditions of temperature and pressure.

10.3 Possibility of hazardous reactions

Violent reaction with: strong oxidiser

10.4 Conditions to avoid

Keep away from heat.

10.5 Incompatible materials

There is no additional information.

10.6 Hazardous decomposition products

Hazardous combustion products: see section 5.

SECTION 11: Toxicological information

11.1 Information on hazard classes as defined in Regulation (EC) No 1272/2008

Classification according to GHS (1272/2008/EC, CLP)

Acute toxicity

Harmful if swallowed.

Acute toxicity					
Exposure route	Endpoint	Value	Species	Method	Source
oral	LD50	1.300 mg/kg	rat		ECHA
dermal	LD50	>2.500 – <5.000 mg/kg	rabbit		ECHA

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4-Terpinenol $\geq 95\%$

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Skin corrosion/irritation

Causes skin irritation.

Serious eye damage/eye irritation

Causes serious eye irritation.

Respiratory or skin sensitisation

Shall not be classified as a respiratory or skin sensitiser.

Germ cell mutagenicity

Shall not be classified as germ cell mutagenic.

Carcinogenicity

Shall not be classified as carcinogenic.

Reproductive toxicity

Shall not be classified as a reproductive toxicant.

Specific target organ toxicity - single exposure

May cause respiratory irritation.

Specific target organ toxicity - repeated exposure

Shall not be classified as a specific target organ toxicant (repeated exposure).

Aspiration hazard

Shall not be classified as presenting an aspiration hazard.

Symptoms related to the physical, chemical and toxicological characteristics

• If swallowed

gastrointestinal complaints, nausea, vomiting

• If in eyes

Causes serious eye irritation

• If inhaled

Irritation to respiratory tract, cough, Dyspnoea

• If on skin

causes skin irritation, pruritis, localised redness

• Other information

none

11.2 Endocrine disrupting properties

Does not contain an endocrine disruptor (ED) at a concentration of $\geq 0,1\%$.

11.3 Information on other hazards

There is no additional information.

SECTION 12: Ecological information

12.1 Toxicity

Shall not be classified as hazardous to the aquatic environment.

12.2 Persistence and degradability

Theoretical Oxygen Demand: $2,904 \text{ mg/mg}$

Theoretical Carbon Dioxide: $2,853 \text{ mg/mg}$

12.3 Bioaccumulative potential

Data are not available.

12.4 Mobility in soil

Data are not available.

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12.5 Results of PBT and vPvB assessment

Data are not available.

12.6 Endocrine disrupting properties

Does not contain an endocrine disruptor (ED) at a concentration of $\geq 0,1\%$.

12.7 Other adverse effects

Data are not available.

SECTION 13: Disposal considerations

13.1 Waste treatment methods



This material and its container must be disposed of as hazardous waste. Dispose of contents/container in accordance with local/regional/national/international regulations.

Sewage disposal-relevant information

Do not empty into drains.

Waste treatment of containers/packagings

Handle contaminated packages in the same way as the substance itself. Completely emptied packages can be recycled.

13.2 Relevant provisions relating to waste

The allocation of waste identity numbers/waste descriptions must be carried out according to the EEC, specific to the industry and process.

Properties of waste which render it hazardous

HP 4 irritant - skin irritation and eye damage

HP 5 specific target organ toxicity (STOT)/aspiration toxicity

HP 6 acute toxicity

13.3 Remarks

Waste shall be separated into the categories that can be handled separately by the local or national waste management facilities. Please consider the relevant national or regional provisions. Non-contaminated packages may be recycled.

SECTION 14: Transport information

14.1 UN number or ID number	not subject to transport regulations
14.2 UN proper shipping name	not assigned
14.3 Transport hazard class(es)	none
14.4 Packing group	not assigned
14.5 Environmental hazards	non-environmentally hazardous acc. to the dangerous goods regulations
14.6 Special precautions for user	There is no additional information.
14.7 Maritime transport in bulk according to IMO instruments	The cargo is not intended to be carried in bulk.

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14.8 Information for each of the UN Model Regulations

International Maritime Dangerous Goods Code (IMDG) - Additional information

Not subject to IMDG.

International Civil Aviation Organization (ICAO-IATA/DGR) - Additional information

Not subject to ICAO-IATA.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

Relevant provisions of the European Union (EU)

Restrictions according to REACH, Annex XVII

Dangerous substances with restrictions (REACH, Annex XVII)				
Name of substance	Name acc. to inventory	CAS No	Restriction	No
4-Terpinenol	this product meets the criteria for classification in accordance with Regulation No 1272/2008/EC		R3	3

Legend

- R3
1. Shall not be used in:
 - ornamental articles intended to produce light or colour effects by means of different phases, for example in ornamental lamps and ashtrays,
 - tricks and jokes,
 - games for one or more participants, or any article intended to be used as such, even with ornamental aspects,
 2. Articles not complying with paragraph 1 shall not be placed on the market.
 3. Shall not be placed on the market if they contain a colouring agent, unless required for fiscal reasons, or perfume, or both, if they:
 - can be used as fuel in decorative oil lamps for supply to the general public, and
 - present an aspiration hazard and are labelled with H304.
 4. Decorative oil lamps for supply to the general public shall not be placed on the market unless they conform to the European Standard on Decorative oil lamps (EN 14059) adopted by the European Committee for Standardisation (CEN).
 5. Without prejudice to the implementation of other Union provisions relating to the classification, labelling and packaging of substances and mixtures, suppliers shall ensure, before the placing on the market, that the following requirements are met:
 - (a) lamp oils, labelled with H304, intended for supply to the general public are visibly, legibly and indelibly marked as follows: "Keep lamps filled with this liquid out of the reach of children"; and, by 1 December 2010, "Just a sip of lamp oil – or even sucking the wick of lamps – may lead to life-threatening lung damage";
 - (b) grill lighter fluids, labelled with H304, intended for supply to the general public are legibly and indelibly marked by 1 December 2010 as follows: "Just a sip of grill lighter fluid may lead to life threatening lung damage";
 - (c) lamps oils and grill lighters, labelled with H304, intended for supply to the general public are packaged in black opaque containers not exceeding 1 litre by 1 December 2010.;

List of substances subject to authorisation (REACH, Annex XIV)/SVHC - candidate list

not listed

Seveso Directive

2012/18/EU (Seveso III)			
No	Dangerous substance/hazard categories	Qualifying quantity (tonnes) for the application of lower and upper-tier requirements	Notes
	not assigned		

Deco-Paint Directive

VOC content	100 %
VOC content	933 g/l

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Industrial Emissions Directive (IED)

VOC content	100 %
VOC content	933 g/l

Directive on the restriction of the use of certain hazardous substances in electrical and electronic equipment (RoHS)

not listed

Regulation concerning the establishment of a European Pollutant Release and Transfer Register (PRTR)

not listed

Water Framework Directive (WFD)

not listed

Regulation on the marketing and use of explosives precursors

not listed

Regulation on drug precursors

not listed

Regulation on substances that deplete the ozone layer (ODS)

not listed

Regulation concerning the export and import of hazardous chemicals (PIC)

not listed

Regulation on persistent organic pollutants (POP)

not listed

Other information

Directive 94/33/EC on the protection of young people at work. Observe employment restrictions under the Maternity Protection Directive (92/85/EEC) for expectant or nursing mothers.

National inventories

Country	Inventory	Status
AU	AIIC	substance is listed
CA	DSL	substance is listed
CN	IECSC	substance is listed
EU	ECSI	substance is listed
EU	REACH Reg.	substance is listed
JP	CSCL-ENCS	substance is listed
MX	INSQ	substance is listed
NZ	NZIoC	substance is listed
PH	PICCS	substance is listed
TW	TCSI	substance is listed
US	TSCA	substance is listed (ACTIVE)
VN	NCI	substance is listed

Legend

AIIC	Australian Inventory of Industrial Chemicals
CSCL-ENCS	List of Existing and New Chemical Substances (CSCL-ENCS)
DSL	Domestic Substances List (DSL)
ECSI	EC Substance Inventory (EINECS, ELINCS, NLP)
IECSC	Inventory of Existing Chemical Substances Produced or Imported in China
INSQ	National Inventory of Chemical Substances

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Legend

NCI	National Chemical Inventory
NZIoC	New Zealand Inventory of Chemicals
PICCS	Philippine Inventory of Chemicals and Chemical Substances (PICCS)
REACH Reg.	REACH registered substances
TCSI	Taiwan Chemical Substance Inventory
TSCA	Toxic Substance Control Act

15.2 Chemical safety assessment

No Chemical Safety Assessment has been carried out for this substance.

SECTION 16: Other information

Indication of changes (revised safety data sheet)

Section	Former entry (text/value)	Actual entry (text/value)	Safety-relevant
2.2		Labelling of packages where the contents do not exceed 125 ml: change in the listing (table)	yes
2.2		Hazard pictogram(s):	yes
2.2		Hazard pictogram(s):: change in the listing (table)	yes
2.2		Labelling of packages where the contents do not exceed 10 ml	yes
2.2		Signal word: Not required	yes
2.2		Hazard pictogram(s): Not required	yes
2.2		Hazard statements: Not required	yes
2.2		Precautionary statements: Not required	yes
15.1		Dangerous substances with restrictions (REACH, Annex XVII): change in the listing (table)	yes

Abbreviations and acronyms

Abbr.	Descriptions of used abbreviations
ADR	Accord relatif au transport international des marchandises dangereuses par route (Agreement concerning the International Carriage of Dangerous Goods by Road)
ATE	Acute Toxicity Estimate
CAS	Chemical Abstracts Service (service that maintains the most comprehensive list of chemical substances)
CLP	Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
DGR	Dangerous Goods Regulations (see IATA/DGR)
EC No	The EC Inventory (EINECS, ELINCS and the NLP-list) is the source for the seven-digit EC number, an identifier of substances commercially available within the EU (European Union)
ED	Endocrine disruptor
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of Notified Chemical Substances
GHS	"Globally Harmonized System of Classification and Labelling of Chemicals" developed by the United Nations
IATA	International Air Transport Association

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Abbr.	Descriptions of used abbreviations
IATA/DGR	Dangerous Goods Regulations (DGR) for the air transport (IATA)
ICAO	International Civil Aviation Organization
IMDG	International Maritime Dangerous Goods Code
LD50	Lethal Dose 50 %: the LD50 corresponds to the dose of a tested substance causing 50 % lethality during a specified time interval
NLP	No-Longer Polymer
PBT	Persistent, Bioaccumulative and Toxic
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RID	Règlement concernant le transport International ferroviaire des marchandises Dangereuses (Regulations concerning the International carriage of Dangerous goods by Rail)
SVHC	Substance of Very High Concern
VOC	Volatile Organic Compounds
vPvB	Very Persistent and very Bioaccumulative

Key literature references and sources for data

Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures.
Regulation (EC) No. 1907/2006 (REACH), amended by 2020/878/EU.

Agreement concerning the International Carriage of Dangerous Goods by Road (ADR). Regulations concerning the International Carriage of Dangerous Goods by Rail (RID). International Maritime Dangerous Goods Code (IMDG). Dangerous Goods Regulations (DGR) for the air transport (IATA).

List of relevant phrases (code and full text as stated in section 2 and 3)

Code	Text
H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Disclaimer

This information is based upon the present state of our knowledge. This SDS has been compiled and is solely intended for this product.