Toxicological profile for

Ethyl 2-methylbutyrate

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

Ethyl 2-methylbutanoate (PubChem)

1.2. Synonyms

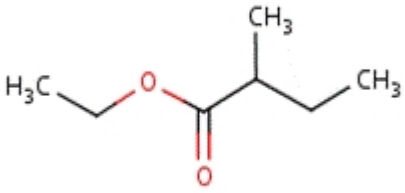
AI3-35155; Butanoic acid, 2-methyl-, ethyl ester; Butyric acid, 2-methyl-, ethyl ester; EINECS 231-225-4; Ethyl 2-methylbutanoate; Ethyl alpha-methylbutyrate; FEMA No. 2443; NSC 1103; Ethyl 2-methylbutyrate; UNII-L1T4AB29DS (ChemIDplus); (±)-Ethyl 2-methylbutanoate (SCCS, 2012)

1.3. Molecular formula

$C_7H_{14}O_2$ (ChemIDplus)

1.4. Structural Formula

(ChemIDplus)



1.5. Molecular weight (g/mol)

130.186 (ChemIDplus)

1.6. CAS registration number

7452-79-1

1.7. Properties

1.7.1. Melting point

(°C): -56.05 (estimated) (EPISuite, 2017); <-20 (GESTIS)

1.7.2. Boiling point

(°C): 132-135.1 (ChemSpider)

1.7.3. Solubility

very slightly soluble in water (Burdock, 2010); 1070 mg/L at 25°C (estimated) (EPISuite, 2017); 4.16 g/L (estimated) (HMDB, 2019) 0.6 g/L at 20°C (GESTIS)

1.7.4. pKa

-7.1 (strongest basic) (estimated) (HMDB, 2019)

1.7.5. Flashpoint

(°C): 26 (ChemSpider)

1.7.6. Flammability limits (vol/vol%)

Highly flammable (PubChem)

1.7.7. (Auto)ignition temperature

(°C): 407 (GESTIS)

1.7.8. Decomposition temperature

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

1.7.9. Stability

“Vapours may form explosive mixtures with air when the substance is heated above its flash point. This may already be possible at elevated ambient temperature” (GESTIS)

1.7.10. Vapor pressure

8.03 mmHg at 25°C (estimated) (EPISuite, 2017); 0.1±0.8 or 7.9±0.2 mmHg at 25°C (estimated) (ChemSpider); 11.73 hPa [8.8 mmHg] at 20°C (GESTIS)

1.7.11. log Kow

2 (GESTIS)

2. General information

2.1. Exposure

To arrive at an estimate of total consumption of a substance, the amount purposefully added to food as an ingredient should be added to the amount consumed of the substance when it is present as a natural constituent in foods. A single point estimate of the total amount of a substance consumed from all food sources is not possible with the data available. However, to obtain a reasonable estimate of consumption, the various consumption estimates of a substance, discussed in the preceding paragraphs, are combined to generate low-intake and high-intake values.

The total lower-intake consumption value for ethyl 2-methylbutyrate was estimated by summing the NAS disappearance per capita consumption with the calculated per capita DINFO consumption, and equal to 0.5587 mg/day or 0.00924 mg/kg/day. The total higher-intake consumption value for ethyl 2-methylbutyrate was estimated by summing the FEMA disappearance per capita consumption with the eaters-only DINFO consumption, and is equal to 11.4244 mg/day or 0.1904 mg/kg/day.

As taken from Burdock Group toxicological Risk Assessment of Ethyl-2-methylbutyrate as a Food Ingredient (2003) available at: http://legacy.library.ucsf.edu/documentStore/i/y/a/iya90g00/Siya90g00.pdf

Maximised Survey-derived Daily Intakes (MSDI-EU): 1900 µg/capita/day (EFSA, 2008).

Reported uses (ppm): (FEMA, 1994)

Food category	Usual	Max		Food category	Usual	Max
Alcoholic beverages	3.98	8.18		Gelatins, puddings	13.68	28.37
Baked goods	24.83	46.70		Hard candy	75.33	113.60
Chewing gum	500	500		Nonalcoholic beverages	10.22	23.79
Frozen dairy	14.84	33.68		Soft candy	14.64	30.56

Used provisionally in beverages at 0.5 ppm and in food at 5 ppm.

FEMA estimated the PADI (possible average daily intake) as 5.488 mg/person.

Consumption: Annual: 10833.33 lb; Individual: 0.009180 mg/kg bw/day.

As taken from Burdock, 2010.

Ethyl 2-methylbutyrate is used as a perfuming ingredient in cosmetics in the EU. As taken from CosIng (undated).

Ethyl 2-methylbutyrate is listed as a fragrance ingredient by IFRA and the USA EPA InertFinder Database (2021).

Ethyl 2-methylbutyrate is listed as an ingredient in auto, inside the home and personal care (<1%) products by the CPID.

“Liquid chromatography-tandem mass spectrometry with electrospray ionization (HPLC-ESI-MS/MS) methods were developed for the simultaneous determination of 42 flavouring compounds and nicotine in liquids for e-cigarettes. The chromatographic separation was performed using an Ace® Ultracore™ SuperC18™ (100×2.1mm, 2.5µm) column in both acidic and alkaline pH conditions to separate all the compounds. A simple "dilute & shoot" approach was used for the sample preparation. The method validation was performed by evaluating key analytical parameters such as linearity, accuracy, selectivity, precision, limit of detection (LOD) and limit of quantification (LOQ). The calibration curves showed good linearity within the specific ranges for the investigated compounds with correlation coefficients greater than 0.990 in each case. The recovery for all the investigated compounds varied from 89% to 110%. The intra- and inter-day precision were within the acceptable limits (±15%) at all tested concentrations. The applicability of the methods was examined by analysing 25 liquid samples from e-cigarettes commercially available on the Polish market.” As taken from Aszyk J et al. 2017. J. Chromatogr. A 1519, 45-54. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28866252

Ethyl 2-methylbutyrate (CAS RNs 7452-79-1) is used as a flavour enhancer for oral use and a fragrance ingredient for topical use 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) J. Anal. Appl. Pyrolysis, 71: 223-311.

Ingredient Name & CAS Number	Mol. Wt (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Ethyl 2-methylbutyrateCAS 7452-79-1	M=130 Bp 133	5	98	Ethyl2methylbutyrate 99.4 Dimethyl butanoate 0.3 Ethyl dimethylbutyrate 0.2 1unidentifiedcompound 0.1	2 0.008 0.005 0.003

2.3. Ingredient(s) from which it originates

Ethyl 2-methylbutyrate (CAS No. 7452-79-1) is a naturally occurring compound found in many fruits, cheeses and beverages (alcoholic and non-alcoholic). It is a colorless liquid with a powerful, green-fruity odor reminiscent of apples. Ethyl 2-methylbutyrate is used in fruit flavor compositions and in perfumes. Ethyl 2-methylbutyrate is approved for food uses by FDA1, FEMA2, JECFA3 and the Council of Europe (CoE). This monograph evaluates the safety-in-use of ethyl 2-methylbutyrate as a food ingredient. As taken from Burdock Group toxicological Risk Assessment of Ethyl-2-methylbutyrate as a Food Ingredient (2003) available at: http://legacy.library.ucsf.edu/documentStore/i/y/a/iya90g00/Siya90g00.pdf

Reported found in nature: the ethyl l-methylbutyrate has been identified in strawberry juice: because of the presence of the asymmetric carbon, the compound should exhibit optically active forms as well as the racemic form: however only the d form and the racemic form are known. Reported found in apple juice, orange and grapefruit juice, bilberry, pineapple, strawberry, cheeses, milk, cognac, rum, whiskey, cider, mango, mountain papaya, spineless monkey orange, Chinese quince and German chamomile oil.

As taken from Burdock, 2010.

Ethyl 2-methylbutyrate is found in bilberry. Ethyl 2-methylbutyrate is found in many fruits, e.g. raw and cooked apple, apricot, orange, grapefruit (HMDB, 2019).

3. Status in legislation and other official guidance

Approved for use in tobacco in Belgium, France, Germany and the UK

Approved for use in food in the EU and USA.

Ethyl 2-methylbutyrate is listed on the US FDA's list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is covered under the US Code of Federal Regulations Title 21 section 172.515 – Synthetic flavoring substances and adjuvants (FDA, 2022a,b).

ADI/TDI:

Ethyl 2-methylbutyrate has been assessed as presenting "no safety concern" at estimated intake levels of 2200 ug/person/day in Europe and 560 ug/person/day in the USA when used as a flavouring agent (JECFA 1998, 1999).

C of E no.: 265 (Burdock, 2010)

FEMA no.: 2443 (Burdock, 2010).

Flavis No.: 09.409.

JECFA No.: 206.

Chemical group number: 01.

Status: No further evaluation needed.

Remarks: (R) or (S) enantiomer not specified by CAS RN in Register.

Reference: JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

As taken from Fl@vouring substances database (Flavis). Accessed April 2020. Available at: http://ec.europa.eu/food/food/chemicalsafety/flavouring/database/dsp_search.cfm

Ethyl 2-methylbutyrate (CAS RN 7452-79-1) is included on the New Zealand Inventory of Chemicals with Approval Code HSR005554 (NZ EPA, 2006) and has been classified by New Zealand's Environmental Protection Authority (NZ EPA CCID).

There is a REACH dossier on ethyl 2-methylbutyrate (ECHA, 2022a).

Ethyl 2-methylbutyrate is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022b).

Ethyl 2-methylbutyrate is listed in the US EPA InertFinder Database (2021) as approved for fragrance use in pesticide products.

Ethyl 2-methylbutyrate is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and in the US EPA 2012 CDR list (Chemical Data Reporting Rule). The Chemical Data Reporting (CDR) Rule requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR. The TSCA inventory and 2012 CDR list are available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

Ethyl ester 2-methylbutanoic acid (CAS RN 7452-79-1) is included on the Safer Chemical Ingredients List (US EPA, 2020).

Ethyl 2-methylbutyrate is authorised for use as a flavouring substance in all categories of flavoured food in the EU under regulation (EU) No. 872/2012 (European Commission, 2012)

Abstract: Chemical group 1 (CG 1) consists of straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes of which 86 are currently authorised for use as flavours in food. The FEEDAP Panel was unable to perform an assessment of ethyl oleate because of its insufficient purity. The following compounds are considered to be safe for all animal species at the use level proposed for feed flavourings: formic acid, acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, oleic acid, decanol, dodecanol, decyl acetate and dodecyl acetate. The remaining substances are considered safe for all animal species at 5 mg/kg complete feed (with a margin of safety between 1 and 120) and at 25 mg/kg complete feed (ethyl acetate and hexyl acetate, with a margin of safety between 2 and 6); and at 1 mg/kg complete feed for pigs and poultry and 1.5 mg/kg complete feed for all other species (ethylacrylate, ethyl hex-3-enoate, ethyl trans-2-butenoate, ethyl isobutyrate, ethyl isovalerate, butyl isovalerate, methyl isovalerate, hexyl isobutyrate, methyl 2-methyl butyrate, pentyl isovalerate, butyl 2-methyl butyrate, hexyl isovalerate, ethyl 2-methyl butyrate, hexyl 2-methyl butyrate and methyl 2-methylvalerate). No safety concern would arise for the consumer from the use of compounds belonging to CG 1 up to the highest safe level in feedingstuffs for all animal species. The FEEDAP Panel considers it prudent to treat all compounds under assessment as irritants to skin, eyes and respiratory tract and as skin sensitizers. No risk for the safety for the environment is foreseen.

Since all 85 compounds are used in food as flavourings, no further demonstration of efficacy is necessary (EFSA, 2013)

Conclusion: No applicable toxicological data are available for the compounds: ethylacrylate, ethyl hex-3-enoate, ethyl trans-2-butenoate, ethyl isobutyrate, ethyl isovalerate, butyl isovalerate, methyl isovalerate, hexyl isobutyrate, methyl 2-methyl butyrate, pentyl isovalerate, butyl 2-methyl butyrate, hexyl isovalerate, ethyl 2-methyl butyrate, hexyl 2-methyl butyrate and methyl 2-methyl valerate. For these Cramer Class I compounds the threshold of toxicological concern (TTC) approach was adopted to derive the maximum safe feed concentration. The calculated safe use levels for these compounds are 1.5 mg/kg complete feed for cattle, salmonids and non food-producing animals and 1 mg/kg complete feed for pigs and poultry (EFSA, 2013)

Regulatory History: Ethyl 2-methylbutyrate has been approved for use in food by PDA, FEMA, JECFA and the CoE (Table 3). The US FDA has approved the direct use of ethyl 2-methylbutyrate in food for use as a synthetic flavoring substance and adjuvant (21 CFR 172.515). The Flavor and Extract Manufacturers' Association has approved ethyl 2-methylbutyrate (FEMA No. 2443) as Generally Recognized As Safe (GRAS) for use in food as a flavoring ingredient (Hall and Oser, 1965). The Council of Europe (CoE No. 265) has approved the use of ethyl 2-methylbutyrate under category B with upper level limits of 0.5 ppm in beverages and 5 ppm in foods (CoE, 2000). The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 206) has reviewed ethyl 2-methylbutyrate as a food additive and has deemed it acceptable for use as a flavouring agent with no safety concern at current levels of intake (JECFA, 2001b). As taken from Burdock Group toxicological Risk Assessment of Ethyl-2-methylbutyrate as a Food Ingredient (2003) available at: http://legacy.library.ucsf.edu/documentStore/i/y/a/iya90g00/Siya90g00.pdf

Ethyl 2-methylbutyrate has been given GRAS (generally recognized as safe) status by FEMA (FEMA no. 2443) (Hall and Oser, 1965).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

It is expected that ethyl 2-methyl butyrate will be readily hydrolysed by esterases to ethanol and 2-methylbutyric acid in the intestinal tract, blood and liver. Both compounds are endogenous and 2-methylbutyric acid is an intermediate in the metabolism of the amino acid isoleucine (JECFA, 1998).

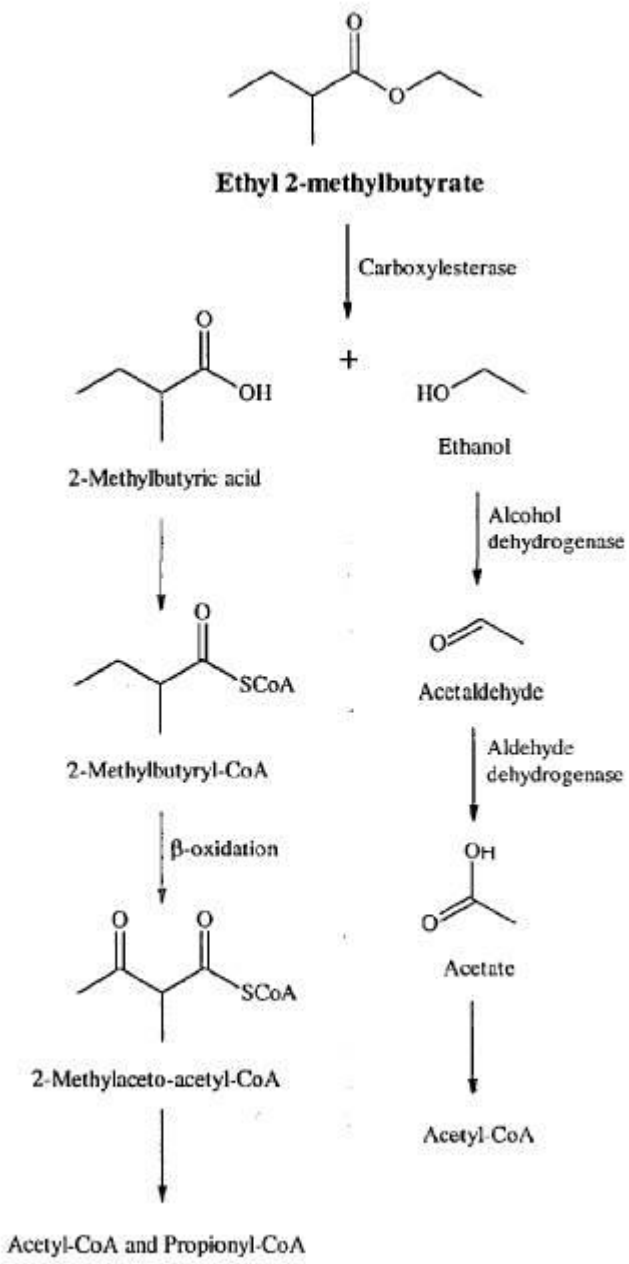
In general, aliphatic esters are rapidly hydrolysed to their component alcohols and carboxylic acids. Hydrolysis is catalysed by classes of enzymes recognized as carboxylesterases or esterases. In mammals, these enzymes occur in most tissues throughout the body (Heymann, 1980; Anders, 1989) but predominate in the hepatocytes (Heymann, 1980). Select isoenzymes exhibit an increase in enzyme binding (lower Km) and maximum velocity (Vmax) as the carbon chain length of either the alcohol or carboxylic acid component of the substrate increases (Heymann, 1980).

4.2. Absorption, distribution and excretion

“There are no known studies specifically evaluating the absorption, distribution,metabolism or elimination of ethyl 2-methylbutyrate. Aliphatic esters are, in general, rapidly hydrolyzed to their component alcohols and carboxylic acids (WHO, 1999). Ethyl 2-methylbutyrate is expected to form 2-methylbutyric acid and ethanol following its hydrolysis. Hydrolysis of esters is catalyzed by the carboxylesterases or generic esterases. These enzymes are ubiquitous in most mammalian tissues are most prominent in hepatocytes (Heymann, 1980).

2-Methylbutyric acid is endogenous in humans as an intermediary product in the metabolism of the amino acid isoleucine (Voet and Voet, 1990). (3-Oxidation and cleavage of 2- methylbutyric acid yields acetyl-CoA and propionyl-CoA which can be completely metabolized to CO2 in the tricarboxylic acid (Kreb's) cycle.

Ethanol is rapidly absorbed from the intestinal tract and distributes into the total body water (Goodman et al., 2001). Primary metabolism of ethanol by alcohol dehydrogenase leads to the formation of acetaldehyde, which is then metabolized further to acetate by acetaldehyde dehydrogenase. Less than 10% of ethanol is excreted unchanged in urine, sweat and breath. Acetate is available to various metabolic processes, including the Kreb's cycle and fatty acid oxidation." As taken from Burdock Group toxicological Risk Assessment of Ethyl-2-methylbutyrate as a Food Ingredient (2003) available at: http://legacy.library.ucsf.edu/documentStore/i/y/a/iya90g00/Siya90g00.pdf



Metabolism of ethyl 2-methylbutyrate

As taken from Burdock Group toxicological Risk Assessment of Ethyl-2-methylbutyrate as a Food Ingredient (2003) available at: http://legacy.library.ucsf.edu/documentStore/i/y/a/iya90g00/Siya90g00.pdf

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

Ethyl 2-methylbutyrate is expected to be of low acute toxicity since other esters of aliphatic acyclic primary alcohols and branched-chain aliphatic acyclic acids have demonstrated oral LD50 values (in rats or rabbits) of >2300 mg/kg bw, with the majority being over 5,000 mg/kg bw (JECFA, 1998).

There are no known studies evaluating the acute toxicity of ethyl 2-methylbutyrate. The acute oral toxicity of ethanol, a primary metabolite of ethyl 2-methylbutyrate, in mice and rats is 3450 and 7060 mg/kg, respectively (Gangolli, 1999). As taken from Burdock Group toxicological Risk Assessment of Ethyl-2-methylbutyrate as a Food Ingredient (2003) available at: http://legacy.library.ucsf.edu/documentStore/i/y/a/iya90g00/Siya90g00.pdf

5.2. Repeated dose toxicity

There are sufficient repeated dose toxicity data on ethyl 2-methylbutrate to support the repeated dose toxicity endpoint. In an OECD 422 combined repeated dose toxicity study with a reproduction/developmental toxicity screening test, groups of 10 Sprague Dawley rats/sex/dose were administered ethyl 2-methylbutyrate via oral gavage at doses of 0, 250, 500, or 1000 mg/kg/day in corn oil. Males were treated for 28–41 days, and females were treated for 40–51 days (maximum of 51 days, males and females). Males were euthanized on day 14 after mating, and females (with offspring) were euthanized on day 5 postpartum. No treatment-related adverse effects were reported for mortality, clinical signs, neurobehavior, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, pathological findings during necropsy, or histopathological examination. The NOAEL for repeated dose toxicity was considered to be 1000 mg/kg/day, the highest dose tested (ECHAREACH Dossier: Ethyl 2-methylbutyrate; ECHA, 2013). A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study. The safety factor has been approved by the Expert Panel for Fragrance Safety*. The derived NOAEL for the repeated dose toxicity data is 1000/3 or 333 mg/kg/day.

As taken from Api AM et al. 2018. Food Chem. Toxicol. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/7452-79-1.pdf

5.3. Reproduction toxicity

There are sufficient reproductive toxicity data on ethyl-2-methylbutyrate to support the reproductive toxicity endpoint. In an OECD 422 combined repeated dose toxicity study with a reproduction/developmental toxicity screening test, groups of 10 Sprague Dawley rats/sex/dose were administered ethyl 2- methylbutyrate via oral gavage at doses of 0, 250, 500, or 1000 mg/kg/day in corn oil. Males were treated for 28–41 days, and females were treated for 40–51 days (maximum of 51 days, males and females). Males were euthanized on day 14 after mating, and females (with offspring) were euthanized on day 5 postpartum. There were no treatment-related effects on mating performance, fertility, conception, gestation length, parturition, survival, litter size, or litter weight. In the F1 generation, no treatment-related effects were reported for mortality, clinical signs, body weight, and bodyweight changes during necropsy. Furthermore, no gross abnormalities were reported in pups. Therefore, the NOAEL for reproductive toxicity was considered to be 1000 mg/kg/day, the highest dose tested (ECHAREACH Dossier: Ethyl 2-methylbutyrate; ECHA, 2013).

As taken from Api AM et al. 2018. Food Chem. Toxicol. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/7452-79-1.pdf

5.4. Mutagenicity

The structurally related ethyl butyrate and ethyl 3-methylbutyrate were negative in the Ames test and in the chromosomal aberration assay, respectively (Ishidate et al. 1984).

Ethyl 2-methylbutyrate 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, 2015b). BlueScreen is a screening assay that assesses genotoxic stress through human derived gene expression. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects on the target material. The mutagenic activity of ethyl 2-methylbutyrate has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation method. Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA102 were treated with ethyl 2-methylbutyrate in dimethyl sulfoxide (DMSO) at concentrations up to 5000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2000b). Under the conditions of the study, ethyl 2-methylbutyrate was not mutagenic in the Ames test. The clastogenic activity of ethyl 2-methylbutyrate 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 ethyl 2-methylbutyrate in DMSO at concentrations up to 1300 µg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h. Ethyl 2-methylbutyrate did not induce binucleated cells with micronuclei when tested up to cytotoxic or the maximum recommended concentrations in either the presence or absence of an S9 activation system (RIFM, 2014). Under the conditions of the study, ethyl 2-methylbutyrate was considered to be non-clastogenic in the in vitro micronucleus test. Based on the available data, ethyl 2-methylbutyrate does not present a concern for genotoxic potential.

As taken from Api AM et al. 2018. Food Chem. Toxicol. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/7452-79-1.pdf

”Electronic nicotine delivery systems (ENDS) are regulated tobacco products and often contain flavor compounds. Given the concern of increased use and the appeal of ENDS by young people, evaluating the potential of flavors to induce DNA damage is important for health hazard identification. In this study, alternative methods were used as prioritization tools to study the genotoxic mode of action (MoA) of 150 flavor compounds. In particular, clastogen-sensitive (γH2AX and p53) and aneugen-sensitive (p-H3 and polyploidy) biomarkers of DNA damage in human TK6 cells were aggregated through a supervised three-pronged ensemble machine learning prediction model to prioritize chemicals based on genotoxicity. In addition, in silico quantitative structure-activity relationship (QSAR) models were used to predict genotoxicity and carcinogenic potential. The in vitro assay identified 25 flavors as positive for genotoxicity: 15 clastogenic, eight aneugenic and two with a mixed MoA (clastogenic and aneugenic). Twenty-three of these 25 flavors predicted to induce DNA damage in vitro are documented in public literature to be in e-liquid or in the aerosols produced by ENDS products with youth-appealing flavors and names. QSAR models predicted 46 (31%) of 150 compounds having at least one positive call for mutagenicity, clastogenicity or rodent carcinogenicity, 49 (33%) compounds were predicted negative for all three endpoints, and remaining compounds had no prediction call. The parallel use of these predictive technologies to elucidate MoAs for potential genetic damage, hold utility as a screening strategy. This study is the first high-content and high-throughput genotoxicity screening study with an emphasis on flavors in ENDS products.” As taken from Hung PH et al. 2020. J. Appl. Toxicol. 40(11), 1566-1587. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32662109/

5.5. Cytotoxicity

Ethyl 2-methylbutyrate 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, 2015b).

As taken from Api AM et al. 2018. Food Chem. Toxicol. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/7452-79-1.pdf

5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

Read-across justification:

Methyl 2-methylbutyrate (CAS # 868-57-5) was used as a read-across analog for the target material, ethyl 2-methylbutyrate (CAS # 7452-79-1) for the skin sensitization endpoint.

○ The target material and the read-across analog are structurally similar and belong to the class of branched saturated esters.

○ The target material and the read-across analog are both short chain esters of 2-methylbutyic acid.

○ The key structural difference between the target material and the read-across analog is that the target material is the ethyl ester of 2-methylbutyric acid, whereas the read-across analog is the methyl ester. This structural difference is toxicologically insignificant.

○ Structural similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score reflects the near identity of these 2-methylbutyrate esters. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.

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

○ According to the OECD QSAR Toolbox v3.4, structural alerts for the toxicological endpoint are consistent between the target material and the read-across analog.

○ The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.

○ The structural alerts for the endpoint evaluated are consistent between the metabolites of the read-across analog and the target material. • Ethyl isobutyrate (CAS # 97-62-1) was used as a read-across analog for the target material, ethyl 2-methylbutyrate (CAS # 7452-79-1) for the skin sensitization endpoint.

○ The target material and the read-across analog are both ethyl esters of branched chain acids.

○ The key structural difference between the target material and the read-across analog is that the target material is the ethyl ester of 2-methylbutyric acid, whereas the read-across analog is the ethyl ester of isobutyric acid. This structural difference is toxicologically insignificant.

○ Structural similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score reflects the near identity of these branched ester structures. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.

○ The structural alerts for the endpoint evaluated are consistent between the metabolites of the read-across analog and the target material

Insufficient skin sensitization studies are available for ethyl 2-methylbutyrate. Based on existing data and read-across materials ethyl isobutyrate (CAS # 97-62-1; see Section V) and methyl 2-

methylbutyrate (CAS # 868-57-5; see Section V), ethyl 2- methylbutyrate does not present a safety concern for skin sensitization under the current, declared levels of use. The chemical structures of these materials indicate that they would not be expected to react with skin proteins (Toxtree 2.6.13; OECD toolbox v3.4). Guinea pig maximization tests with ethyl 2-methylbutyrate, read-across analog ethyl isobutyrate, and read-across analog methyl 2-methylbutyrate did not present reactions indicative of sensitization (RIFM, 1999a; RIFM, 1985; ECHA Dossier: Ethyl isobutyrate, 2017). In human maximization tests, no skin sensitization reactions were observed with read-across materials ethyl isobutyrate and methyl 2-methylbutyrate (RIFM, 1982; RIFM, 1975). Based on weight of evidence from structural analysis, animal and human studies, and read-across analogs, ethyl isobutyrate and methyl 2-methylbutyrate, ethyl 2-methylbutyrate does not present a safety concern for skin sensitization under the current, declared levels of use.

“The phototoxicity/photoallergenicity endpoint was evaluated based on UV spectra; ethyl 2-methylbutyrate is not expected to be phototoxic/photoallergenic.”

As taken from Api AM et al. 2018. Food Chem. Toxicol. Epub ahead of print. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/7452-79-1.pdf

5.8. All other relevant types of toxicity

There are no known studies evaluating the effects of ethyl 2-methylbutyrate in humans. The pharmacological and toxicological effects of ethanol, a primary metabolite of ethyl 2-methylbutyrate, are well documented in the literature. The first signs of alcohol intoxication become evident in humans when the blood concentration reaches 20 - 30 mg/dl and 50% of people are grossly intoxicated by a concentration of 150 mg/dl (Goodman et al., 2001). The average blood concentration in fatal cases of acute ethanol poisoning is 400 mg/dl. The chronic consumption of large amounts of ethanol (> 20 - 30 g/day) is associated with an increased risk for a variety of deleterious cardiovascular, gastrointestinal, nutritional, hematological and immunological effects. As taken from Burdock Group toxicological Risk Assessment of Ethyl-2-methylbutyrate as a Food Ingredient (2003) available at: http://legacy.library.ucsf.edu/documentStore/i/y/a/iya90g00/Siya90g00.pdf

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing ethyl-2-methylbutyrate was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of ethyl-2-methylbutyrate when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	163	JTI KB study reports
In vitro cytotoxicity	163	JTI KB study reports

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

No data available to us at this time.

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

No data available to us at this time.

7. Addiction

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

8. Burnt ingredient toxicity

Tobacco smoke condensates from cigarettes containing Ethyl-2-methylbutyrate and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of Ethyl 2-methylbutyrate. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	1.3 65 60	JTI KB Study Report(s)
	12	Baker et al., 2004a
	9	Carmines, 2002 & Rustemeier et al, 2002
	6	Roemer et al, 2014
In vitro genotoxicity	1.34 60	JTI KB Study Report(s)
	10	fGLH Study Report (2010)
	9	Carmines, 2002 & Roemer et al., 2002
	12	Baker et al., 2004c
	6	Roemer et al, 2014
In vitro cytotoxicity	1.3 4 60	JTI KB Study Report(s)
	12	Baker et al., 2004c
	9	Carmines, 2002 & Roemer et al., 2002
	10	fGLH Study Report (2010)
	6	Roemer et al, 2014
Inhalation study	1.3	JTI KB Study Report(s)

	4 60	
	9	Carmines, 2002 & Vanscheeuwijck et al, 2002
	12	Baker et al., 2004c
	6	Schramke et al, 2014
Skin painting	1.3	JTI KB Study Report(s)
In vivo genotoxicity	6 60	Schramke et al, 2014 JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing ethyl-2-methylbutyrate was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of ethyl-2-methylbutyrate when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	163	JTI KB study reports
In vitro cytotoxicity	163	JTI KB study reports

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Ethyl-2-methylbutyrate was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	1,200	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	1,200	Logic (2019a) Labstat International Inc. (2022)
In vitro cytotoxicity	1,200	Logic (2019a) Labstat International Inc. (2022)
In vivo genotoxicity	250	Logic (2019a)
Inhalation study	250	Logic (2019a)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing Ethyl 2-methylbutyrate was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to

those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	Tested level	Reference
Aerosol chemistry	0.0309 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	0.0309 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro cytotoxicity	0.0309 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	0.0309 mg/(tobacco portion; 310 mg)	Logic (2019b)
Inhalation study	0.0309 mg/(tobacco portion; 310 mg)	Logic (2019b)

Aerosol from heated tobacco stick(s) containing Ethyl-2-methylbutyrate 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	1.45	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	1.45	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	1.45	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List state that butanoic acid, 2-methyl-, ethyl ester is persistent in the environment:

Media of concern leading to Categorization	Air
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.884
TOPKAT probability of biodegradation	1
EPI Predicted hydrolysis half-life (days)	5.52E+003
EPI Predicted Ozone reaction half-life (days)	999

EPI Predicted Atmospheric Oxidation half-life (days)	2.753

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

EPISuite provides the following information:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]: Bond Method :	5.45E-004 atm-m3/mole (5.52E+001 Pa-m3/mole)	
Group Method:	6.89E-004 atm-m3/mole (6.99E+001 Pa-m3/mole)	
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.286E-003 atm-m3/mole (1.303E+002 Pa-m3/mole)VP: 8.03 mm Hg (source: MPBPVP)WS: 1.07E+003 mg/L (source: WSKOWWIN)	

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]: Log Kow used:	2.26 (KowWin est)
Log Kaw used:	-1.652 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	3.912
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):	0.8597 0.9947 3.0517 (weeks) 3.8889 (days) 0.7180 0.8840 0.6553
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01): Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]: Vapor pressure (liquid/subcooled):	988 Pa (7.41 mm Hg)
Log Koa (Koawin est):	3.912
Kp (particle/gas partition coef. (m3/ug)):Mackay model: Octanol/air (Koa) model:	3.04E-009 2E-009
Fraction sorbed to airborne particulates (phi): Junge-Pankow model:	1.1E-007
Mackay model:	2.4E-007
Octanol/air (Koa) model:	1.6E-007
	3.8848 E-12 cm3/molecule-sec

Atmospheric Oxidation (25 deg C) [AopWin v1.92]: Hydroxyl Radicals Reaction: OVERALL OH Rate Constant:	
Half-Life:	2.753 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life:	33.040 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 1.76E-007 (Junge-Pankow, Mackay avg) 1.6E-007 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	
Soil Adsorption Coefficient (KOCWIN v2.00): Koc :	31.77 L/kg (MCI method)128.7 L/kg (Kow method)
Log Koc:	1.502 (MCI method)2.110 (Kow method)
Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]: Total Kb for pH > 8 at 25 deg C :	1.453E-002 L/mol-sec
Kb Half-Life at pH 8:	1.512 years
Kb Half-Life at pH 7:	15.118 years

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

Volatilization from Water: Henry LC: 0.000689 atm-m3/mole (estimated by Group SAR Method) Half-Life from Model River:	2.134 hours
Half-Life from Model Lake:	119 hours (4.956 days)
Removal In Wastewater Treatment: Total removal:	24.75 percent
Total biodegradation:	0.08 percent
Total sludge adsorption:	2.06 percent
Total to Air:	22.61 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount(percent)	Half-Life(hr)	Emissions(kg/hr)
Air	17.4	66.1	1000
Water	38.4	360	1000
Soil	44.1	720	1000
Sediment	0.121	3.24e+003	0

Persistence Time: 196 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that butanoic acid, 2-methyl-, ethyl ester is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	40
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	40
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	14.304
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	53.3624
Toxicity to fish (LC50 in mg/l) as predicted by Aster	16.033609
Toxicity to fish (LC50 in mg/l) as predicted by PNN	20.80819
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	59.975
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.83E+001

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

ECOSAR version 1.11 provides the following aquatic toxicity data for CAS RN 7452-79-1:

Values used to Generate ECOSAR Profile Log Kow: 2.264 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 1070 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Esters

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Esters :	Fish	96-hr	LC50	12.204
Esters :	Daphnid	48-hr	LC50	24.662
Esters :	Green Algae	96-hr	EC50	10.066
Esters :	Fish		ChV	0.868
Esters :	Daphnid		ChV	15.630

Esters :	Green Algae		ChV	2.811
Esters :	Fish (SW)	96-hr	LC50	18.267
Esters :	Mysid	96-hr	LC50	15.775
Esters :	Fish (SW)		ChV	2.746
Esters :	Mysid (SW)		ChV	677.816
Neutral Organic SAR :	Fish	96-hr	LC50	61.955
(Baseline Toxicity) :	Daphnid	48-hr	LC50	36.046
	Green Algae	96-hr	EC50	29.698
	Fish		ChV	6.232
	Daphnid		ChV	3.762
	Green Algae		ChV	8.211

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR version 1.11 provides the following terrestrial toxicity data for CAS RN 7452-79-1:

Values used to Generate ECOSAR Profile

Log Kow: 2.264 (EPISuite Kowwin v1.68 Estimate) Wat Sol: 1070 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	1395.273 *

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

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that butanoic acid, 2-methyl-, ethyl ester is not bioaccumulative in the environment:

Log Kow predicted by KowWin	2.26
Log BAF T2MTL predicted by Gobas	1.09565521072471

Log BCF 5% T2LTL predicted by Gobas	1.00978406028256
Log BCF Max predicted by OASIS	1.8508876067309
Log BCF predicted by BCFWIN	1.043

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

EPISuite provides the following information:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	1.161 (BCF = 14.48 L/kg wet-wt)
Log Biotransformation Half-life (HL):	0.932 (BCF = 8.558)
Log BCF Arnot-Gobas method (upper trophic):	0.612 (BCF = 4.096)
Log BAF Arnot-Gobas method (upper trophic):	0.932 (BAF = 8.558)
log Kow used:	2.26 (estimated)

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12. Other information

No data available to us at this time.

13. Last audited

June 2022

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives

ETHYL 2-METHYLBUTYRATE

COE No.:	265
FEMA No.:	2443
JECFA No.:	206
Chemical names:	ETHYL-2-METHYLBUTANOATE
Functional class:	FLAVOURING AGENT
Latest evaluation:	1997
ADI:	ACCEPTABLE
Comments:	No safety concern at current levels of intake when used as a flavouring agent
Report:	TRS 884-JECFA 49/52
Specifications:	COMPENDIUM ADDENDUM 8/FNP 52 Add.8/136 (2000)
Tox monograph:	FAS 40-JECFA 49/267
Previous status:	1997, COMPENDIUM ADDENDUM 5/FNP 52 Add.5/228. N

12 Nov 01

See Also:
Toxicological Abbreviations

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization or of the Food and Agriculture Organization of the United Nations

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Forty-ninth report of the
Joint FAO/WHO Expert Committee on
Food Additives

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Rome, 17–26 June 1997

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 40, 1998.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, Addendum 5. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.

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 Organisation, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Rome from 17 to 26 June 1997. The meeting was opened by Dr H. de Haen, Assistant Director-General, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr de Haen emphasized the importance of the work of the Committee and its impact on international trade and stressed the necessity for transparency in its deliberations. Dr de Haen highlighted the significance of accurate, comprehensive and concise reporting of the proceedings and deliberations of the Committee's meetings. He also stated that the scientific committees that advise the Codex Alimentarius Commission must have adequate expertise and experience to enable them to perform accurate and scientifically sound evaluations. FAO and WHO make every effort to ensure that the process for the selection of Committee members is open and transparent, and that this process results in the selection of highly qualified scientists in the fields of competence needed to make important decisions that affect the safety of the world's food supply.

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 48 previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the forty-sixth meeting (Annex 1, reference *122*).

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, food ingredients, flavouring agents and contaminants (sections 3, 4 and 5 and Annex 2);
— to review and prepare specifications for selected food additives and flavouring agents (sections 3, 4 and 6 and Annex 2);
— to assess the risks associated with aflatoxin contamination (section 5); and
— to consider approaches to evaluate the intake of food additives in the light of the recommendations of the Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants (*2*) (section 2.4.2).

2.1 Modification of the agenda

The flavouring agents isoamyl alcohol, isoamyl formate and isoamyl acetate were removed from the agenda for toxicological evaluation because they had been evaluated at the forty-sixth meeting of the Committee (Annex 1, reference *122*). The flavouring agent geranyl 2-ethylbutanoate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 2-ethylbutanoate) was on the agenda under the esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids; however, it was evaluated with the esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids. The flavouring agent 4-hydroxy-3-methyloctanoic acid γ-lactone was added for toxicological evaluation under the aliphatic lactones.

Sixty flavouring agents included on the agenda under the saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids and the aliphatic lactones were not considered for specifications at the present meeting. The specifications for these substances will be reviewed at a future meeting of the Committee at which food additives and contaminants are considered.

The Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants (*2*) reviewed the specifications of identity and purity of selected food additives that had been prepared at the forty-sixth meeting of the Expert Committee (Annex 1, reference *122*). The Codex Committee referred back to the Expert Committee the specifications of nine compounds: citric acid, propylene glycol, allyl cyclohexane propionate (2-propenyl cyclohexane propanoate), ethyl nonanoate, ethyl octanoate, isoamyl acetate (3-methylbutyl acetate), isoamyl butyrate (3-methylbutyl butanoate), isoamyl isobutyrate (3-methylbutyl 2-methylpropanoate) and isoamyl isovalerate (3-methylbutyl 3-methylbutanoate); these were added to the agenda of the present meeting.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, food ingredients, flavouring agents 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* and *122*), 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 *Procedure for the Safety Evaluation of Flavouring Agents*

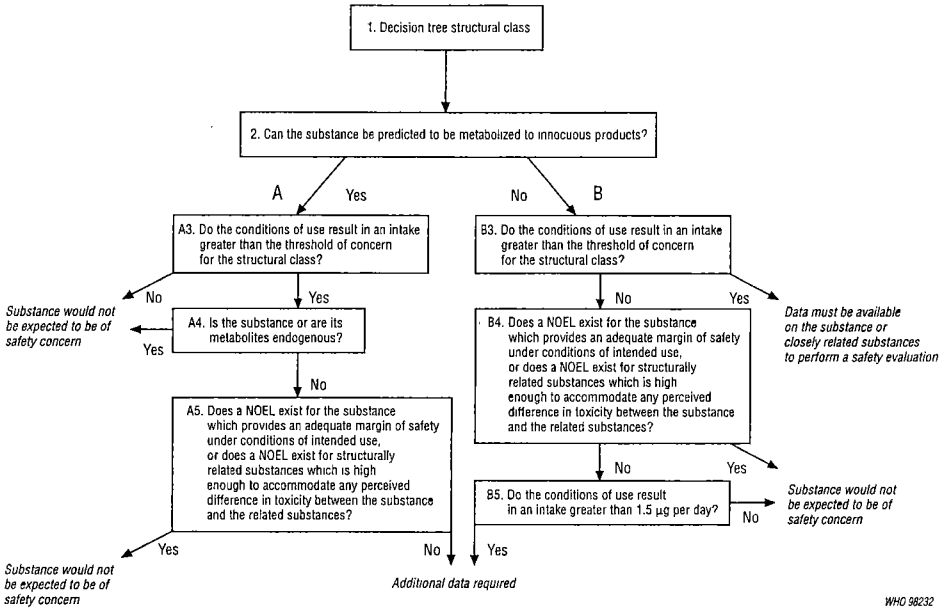
At its forty-sixth meeting (Annex 1, reference *116*), the Committee evaluated three groups of flavouring agents using a procedure based on that reviewed at its forty-fourth meeting (Annex 1, reference *122*). At the forty-sixth meeting, the Committee did not fully discuss the application of the last step on the right-hand side of the Procedure ("Do the conditions of use result in an intake greater than 1.5 μg per day?") to flavouring agents and this step was not considered. At that time, the Committee recommended that this step be considered at a future meeting at which food additives and contaminants were evaluated.

At its present meeting, the Committee considered the numbering of the various steps in the Procedure for the Safety Evaluation of Flavouring Agents. Concern was expressed about the numbering system used in the Procedure at the forty-sixth meeting, which implied that the two sides of the Procedure were parallel. To avoid this confusion, the Committee decided to prefix the steps on the left- and right-hand sides of the Procedure with the letters "A" and "B" respectively.

At its present meeting, the Committee also considered a paper that will be published in the WHO Food Additives Series (*3*) that provided further information relating to the derivation of the value of 1.5 μg per person per day. The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 μg per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the conditions of use result in an intake greater than 1.5 μg per day?") (Fig. 1). The Committee

Figure 1

Procedure for the Safety Evaluation of Flavouring Agents adopted by the Committee at its present meeting



recognized that flavouring agents with unresolved toxicity problems could be evaluated by steps B3, B4 and B5 of the Procedure, but reaffirmed its view, expressed at the forty-sixth meeting, that as with any scheme, its application calls for judgement, and it should not replace expert opinion; the Committee therefore reserved the right to use alternative approaches when data on specific flavouring agents warranted such action.

During its present meeting, the Committee used the Procedure for the Safety Evaluation of Flavouring Agents given in Fig. 1.

In applying the Procedure at the present meeting, a number of general issues were raised.

Consideration of flavouring agents as groups

The flavouring agents were evaluated by the Committee in groups of structurally and/or metabolically related substances. Systematic changes in structure are the basis for understanding the effect of structure on the chemical and biological properties of a substance (Annex 1, reference *117*, Annex 5). When the Procedure is being used, the evaluation of flavouring agents in groups facilitates the consideration of common pathways of metabolism which apply to

members of a group. For substances which share common metabolites, consideration should be given to the total intake from all related substances. The total intake of such related flavouring agents, either from within the same group or across groups, should be evaluated where assessment of the intake data and toxicological profile warrant a combined appraisal. Total intakes should be considered for substances which produce the same potentially toxic metabolite (for example the esters of allyl alcohol considered at the forty-sixth meeting of the Committee (Annex 1, reference *122*)). The Committee concluded that flavouring agents which produce metabolites that are innocuous and endogenous would not be of safety concern, providing that the total intake from all related substances was judged not to give rise to perturbations outside the normal physiological range. Evaluations of such theoretical combined intakes should also take into account, where possible, the likelihood of the different flavouring agents being consumed together.

Natural occurrence

Some substances used as flavouring agents also occur as natural constituents of food; the influence of natural occurrence on the application of the Procedure was considered at the forty-fourth meeting of the Committee (Annex 1, reference *117*, Annex 5). Intakes from use as flavouring agents should be considered relative to the intakes from natural sources. This is a complex issue and interpretation of data on natural occurrence is dependent on:

— the nature of the flavouring substance and its structural class (step 1);
— the natural source of the substance;
— the intake from natural sources; and
— the proportion of the total intake accounted for by its use as a flavouring agent.

As a consequence the intake of such naturally occurring substances should be considered on a case-by-case basis.

Data on intakes of flavouring agents

The only estimates of intake of flavouring agents available to the Committee were derived from surveys in Europe and the USA. The Committee would welcome additional information on intakes of flavouring agents from other geographical regions. The Committee noted that the evaluations performed to date using the Procedure have been based on intake estimates available at the meeting at which the flavouring agents were considered and that changes in intake might warrant re-evaluation of a flavouring agent. The Committee

recommended that information on intakes be updated periodically to ensure the validity of the safety evaluations, particularly for flavouring agents for which the annual volume of production is variable.

The Committee considered that the estimation of intakes based on production data is a practical and realistic approach. Further consideration of intake should be given to flavouring agents for which there are high reported levels of use in some foods or beverages, but low intakes when calculated from production data. This would be particularly important for flavouring agents with intakes calculated to be only slightly below one of the threshold criteria in the evaluation process (i.e. steps A3, B3 or B5). However, the Committee recognized that estimation of intakes of flavouring agents based on reported levels of use would require detailed information on the specific products in which the flavouring agent is used, the actual levels of use and the intake of the individual food products containing the flavouring agent by consumers of both average and above average quantities of these foods.

2.2.2 *Role of the Committee in the risk analysis process of the Codex Alimentarius Commission*

The Committee welcomed the acknowledgement by the Codex Alimentarius Commission of the continuing need for the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues to provide risk assessments to the Codex Alimentarius Commission (*4*). The Committee wished to emphasize the essential role of the expert scientific judgements provided by scientific committees as the basis for risk assessment. Such expertise will be of increasing importance for future developments in understanding the scientific basis of risk assessment, such as mechanisms of toxicity, differences between test species and humans, and human diversity and genetic variability. The Committee will continue to welcome developments in methods of risk assessment which will provide a more sound scientific basis to the advice that it provides to the Codex Alimentarius Commission.

2.3 Principles governing the establishment and revision of specifications

2.3.1 *Significance of identity and purity requirements*

The Committee noted that the specifications monographs contain requirements under two subheadings, "identity tests" and "purity tests", and it emphasized that substances should meet all require-

ments listed under these headings. The meanings of "identification" and "purity" are both clearly defined in FAO Food and Nutrition Paper No. 5, Rev. 2 (Annex 1, reference *100*).

2.3.2 *Limits for arsenic, lead and other heavy metals*

In keeping with its previously stated intentions (Annex 1, references *116* and *122*) regarding limits for arsenic, lead and other heavy metals, the Committee considered four issues:

— the lack of specificity in the current limit test for heavy metals (expressed as lead) and the potential loss of metals during the dry ashing procedure, both of which compromise the validity of the test;
— the need to replace the general test for heavy metals with tests for specific metals, particularly for lead, cadmium, mercury and arsenic;
— the need for more specific and sensitive analytical methods, such as atomic absorption spectroscopy (including the graphite furnace technique) and for improved methods of sample preparation; and
— the need to consider the amount of a food additive consumed and its potential for contamination by specific heavy metals.

In order to address these issues in greater depth the Committee will seek information on the specific content of heavy metals in food additives as well as on methods of analysis. At the present meeting, the Committee decided to delete specifications for limits for heavy metals (expressed as lead) from specifications monographs where a specification for lead was available.

2.3.3 *Analytical methods*

Alternative methods

The Committee's policy has been to describe only one analytical method for an analyte with a limit in the specifications. However, the Committee has now concluded that in some instances the inclusion of alternative methods may be desirable for reasons of economy or simplicity. For example, the use of nuclear magnetic resonance spectroscopy has been presented as an alternative to gas chromatography for the method of assay in the specifications monograph on salatrim, because of the comparative simplicity of the sample preparation and instrumental analysis.

Methods of determining residual solvents and other volatile substances

The Committee observed that a number of specifications require analysis for volatile substances such as residual solvents and reaction

by-products. However, many of the analytical methods are out of date. The Committee wishes to encourage the use of improved techniques such as headspace gas chromatography, and in the future will expect descriptions of such methods to be supplied as part of information for revised or new specifications.

References to standard methods

During the revision of existing specifications the Committee noted that several references have been made to published standard analytical procedures (e.g. American Society for Testing and Materials (ASTM) methods) instead of providing full descriptions of the methods. One such reference was also included in a new monograph prepared at this meeting. The Committee concluded that in future such references should be replaced by a complete description of the methods.

2.3.4 *Enzymes derived from genetically modified organisms*

The Committee designated as "tentative" new specifications for two enzyme preparations derived from genetically modified organisms. This decision was made because 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*, section 2.3.4), referred to in the specifications, is itself tentative. The Committee decided to review Appendix B at its next meeting in 1998 and subsequently to re-evaluate the specifications for enzyme preparations which refer to Appendix B.

The Committee requested comments on Appendix B, to be reviewed in 1998.

2.3.5 *Flavouring agents*

The Committee was asked to consider the specifications for 224 flavouring agents at the present meeting, of which 60 were transferred to the agenda for a future meeting. It decided to modify the tabular format for the specifications for flavouring agents adopted at the forty-sixth meeting (Annex 1, reference *124*). Special methods of analysis and spectra will continue to be included as appendices to the table of flavouring agents. Where a substance has a function in addition to that of a flavouring agent, a standard specification format will be included.

The common names, chemical names and synonyms will be used to index the substances. A new identification number was allocated to

each substance for the convenience of the Committee and an identification number will also be allocated to each substance evaluated in the future. The Committee proposed to develop a classification by chemical group, for ease of future safety evaluations.

2.4 Principles governing intake assessments of food additives and contaminants

The Joint FAO/WHO Expert Consultation on the Application of Risk Analysis to Food Standards Issues (*4*) recognized that assessments of the intake of food additives, contaminants and residues of pesticides and veterinary drugs should be considered an integral part of the risk assessment process for these substances. At its present meeting, the Committee also recognized the importance of assessments of dietary intake in characterizing any potential risks posed by food additives and contaminants.

2.4.1 *Methods for assessing dietary intake*

The Committee agreed with the conclusion of the FAO/WHO Consultation on Food Consumption and Exposure Assessment to Chemicals (*5*) that, in principle, the estimation of potential dietary intakes is the same for all food chemicals. However, because of differences in their occurrence in the food supply, assessments of intake of food additives and contaminants may be based on different types of data on consumption of food and on occurrence of chemicals in food. The procedures developed for pesticide residues are applicable to assessments of the dietary intake of contaminants which also occur in raw agricultural commodities (*6*). These procedures were employed in the assessment of the intake of aflatoxins (section 5).

In most cases, assessments of the intake of food additives are conducted using data on consumption of processed foods. For certain food additives, "poundage" data may be used to estimate per capita intake. Five main approaches for assessing dietary intake of food additives have been used by countries at the national level (*7*, *8*). These approaches usually overestimate chronic (long-term) daily intake, and this could compensate for potential differences in intake between population subgroups and for day-to-day fluctuations in individuals. The five approaches are summarized below.

The budget method

This method takes into account the physiological requirements for energy and fluid in estimating consumption of foods in solid and liquid form. The Committee emphasized that the budget method is

not a procedure for estimating food additive intake *per se*. However, because of its simplicity it is generally accepted as an appropriate screening tool at the international or national level for the identification of food additives that require further assessment.

The "poundage" approach
This approach uses statistics on usage of food additives, adjusted for imports, exports and non-food uses.

The food balance sheet/household survey approach
This method is based on food available for consumption either at a national or household level. Food balance sheets are adjusted for the proportion of each raw commodity that is processed and likely to contain the food additive being studied.

The model diet approach
This method is used to construct a model diet from information on food consumption for a selected population subgroup.

Individual dietary records
These methods use data from representative national surveys of individual food consumption.

These approaches are listed in the order of their increasing ability to accurately predict dietary intake. However, increased accuracy requires more comprehensive data on food consumption and use of food additives as well as greater resources for the assessment of dietary intake. Other approaches for assessing intake such as duplicate diet studies and total diet studies may also be used.

2.4.2 *Assessment of dietary intake of specific additives*

The Committee was requested at the Twenty-ninth Session of the Codex Committee on Food Additives and Contaminants (*2*) to perform assessments of the intake of five food additives: benzoic acid and its salts, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and sulfites. These food additives have been identified for priority assessment by the Codex Committee because initial assessments indicate that intakes of these food additives may approach or exceed the ADIs established by the Expert Committee.

Information on national assessments of the intake of these five food additives will be requested from countries based on procedures developed by the Committee. The Committee recommended that an evaluation of intake assessments from various countries should be

undertaken at the next meeting of the Committee at which food additives and contaminants are considered. The likely intakes of these substances on a global basis will be assessed to respond to the request of the Codex Committee to provide guidance on the potential for the intakes of these food additives to exceed their ADIs.

3. Specific food additives and food ingredients

The Committee evaluated three food additives and one food ingredient for the first time and re-evaluated several food additives and aflatoxins considered at previous meetings. In addition, the Committee evaluated a large number of flavouring agents using the Procedure for the Safety Evaluation of Flavouring Agents (section 2.2.1). Information on the evaluations and on specifications is summarized in Annex 2.

3.1 Antioxidant: *tert*-butylhydroquinone (TBHQ)

tert-Butylhydroquinone (TBHQ) was previously evaluated by the Committee at its nineteenth, twenty-first, thirtieth, thirty-seventh and forty-fourth meetings (Annex 1, references *38*, *44*, *73*, *94* and *116*). At the forty-fourth meeting, the previously established temporary ADI of 0–0.2 mg/kg of body weight was extended, pending results from long-term toxicity studies in rodents. This ADI was derived from a no-observed-effect level (NOEL) of 1500 mg/kg in the diet (equivalent to 37.5 mg/kg of body weight per day) in a 117-week feeding study in dogs on the basis of haematological changes observed at the next highest dose level of 5000 mg/kg in the diet. At its present meeting, the Committee reviewed the results of the long-term toxicity studies in mice and rats. In addition, new information relating to the metabolism of TBHQ, its effects on the induction of enzymes, and its short-term and reproductive toxicity in rodents was available for review. The results from the long-term study in dogs and the genotoxicity studies relating to the clastogenic potential of TBHQ were also re-evaluated.

In studies reviewed at earlier meetings of the Committee (Annex 1, references *39* and *117*), TBHQ was shown to be extensively absorbed and rapidly excreted following ingestion by rats, dogs and humans. The major urinary metabolites in all three species were the *O*-sulfate and *O*-glucuronide conjugates, with the former predominating. In numerous *in vitro* studies, TBHQ was shown to induce the activity of phase II enzymes, including glucuronosyl transferase and glutathione transferase, by a mechanism independent of the *Ah* receptor.

Induction of hepatic glutathione transferase activity was also demonstrated following short-term administration of TBHQ in the diet of female mice.

TBHQ also undergoes redox cycling with the corresponding quinone, accompanied by the production of reactive oxygen species. In a study reviewed at the present meeting, three glutathione conjugates of TBHQ were identified in the bile of male rats and sulfur-containing metabolites of TBHQ were detected in the urine. In other studies, glutathione conjugates of TBHQ demonstrated higher redox cycling activity than unconjugated TBHQ, and were toxic to the kidney and bladder when administered intravenously to male rats.

In a new 13-week feeding study conducted in mice, significant treatment-related effects were noted in both sexes. These effects were decreased body-weight gain and hyperplasia of the mucosal epithelium of the forestomach. The latter effect was noted only at very high doses of TBHQ; 20 g/kg in the diet (equal to 4000 mg/kg of body weight per day) and above. The NOEL was 870 mg/kg of body weight per day. In a 13-week feeding study conducted in rats continuously exposed to TBHQ, starting *in utero*, treatment-related haemosiderin pigmentation of the spleen was noted in both sexes. In addition, there was a treatment-related increase in atrophy of the red pulp of the spleen in female rats receiving 2.5 and 5 g/kg in the diet. Bone marrow and haematological parameters were not altered at these doses. Hyperplasia of the forestomach was not observed in this study, even at the highest dose tested; 10 g/kg in the diet (equal to 800 mg/kg of body weight per day), although it has been noted in another study in adult rats following short-term administration of TBHQ at 20 g/kg in the diet. Treatment with TBHQ had no effect on the estrous cycle or on the histological appearance of the reproductive organs. Because pigmentation of the spleen was noted in female rats at the lowest dose tested, a NOEL could not be established, but the lowest-observed-effect level (LOEL) was 190 mg/kg of body weight per day. Irritation and hyperplasia observed in the nasal epithelium of both rats and mice and on the skin of mice were considered to be the consequence of direct contact with TBHQ from the diet.

The results of two recently conducted carcinogenicity studies were reviewed. In female mice, TBHQ induced an increase in the incidence of hyperplasia of the thyroid follicular cells at all dose levels. A non-significant increase in follicular cell adenomas was reported at the highest dose of 5 g/kg in the diet (equal to 600 mg/kg of body weight per day) but the incidence was within the range of historical controls. No follicular cell carcinomas were observed. Decreased body-weight

gains were also observed at the highest dose in both sexes. Since the Committee was aware that hydroquinone (the unsubstituted parent compound) induces thyrotoxicity in mice, but not rats (*9*), it considered that the hyperplasia of the follicular cells observed with TBHQ in this study might be a toxicologically significant effect. Consequently, it concluded that a NOEL could not be identified in this study and that the lowest dose of 1.25 g/kg in the diet, equal to 130 mg/kg of body weight per day, represented the LOEL. In the study in rats, toxicologically significant effects were noted only at the highest dose tested, 5 g/kg in the diet (equal to 220 mg/kg of body weight per day); these effects were an increase in the incidence of hyperplasia of transitional cells and suppurative inflammation of the kidneys of male rats and haemosiderin pigmentation of the spleen of the females. The Committee considered that 2500 mg/kg in the diet, equal to 110 mg/kg of body weight per day, represented the NOEL and that TBHQ was not carcinogenic in mice or rats.

The 117-week study on dogs in which the temporary ADI had been based was re-evaluated in the light of supplementary information requested from its authors. On the basis of actual intake data, nominal levels of 500, 1580 and 5000 mg/kg in the diet were equal to doses of 21, 72 and 260 mg/kg of body weight per day. In dogs of both sexes given the highest dose, statistically significant reductions in haemoglobin concentrations and erythrocyte volume fractions were observed at several sampling intervals throughout the study, although the values were within the ranges in historical controls. Red blood cell counts were also significantly decreased in male and female dogs in the highest-dose group at week 112, the only time at which measurement of this parameter was reported. Increases in the reticulocyte count (as a percentage of red blood cells) and the presence of immature red blood cell forms in the peripheral blood of animals from all treated groups, reported to occur late in the study, were not dose-related nor were they accompanied by changes in red blood cell parameters at 500 and 1580 mg/kg in the diet. On the basis of this re-evaluation, the Committee confirmed that the NOEL for long-term toxicity in dogs was 1580 mg/kg in the diet, equal to 72 mg/kg of body weight per day.

In view of the conflicting results of the genotoxicity assays reviewed at previous meetings of the Committee, many of the studies of TBHQ were re-evaluated at the present meeting with respect to the validity of the protocol and interpretation of data. The conclusions of a number of the studies could no longer be supported. The results of the well conducted studies indicated that TBHQ was clastogenic *in vitro* in the absence or presence of metabolic activation, but did not induce

the formation of micronuclei *in vivo*. In sister chromatid exchange assays, TBHQ was positive in mice *in vivo* and in an *in vitro* system. The results from several studies suggested that damage to DNA resulting from exposure to TBHQ, including chromosome loss and breakage, was secondary to the production of reactive oxygen species. In the light of this information, and the fact that TBHQ was not carcinogenic in rats or mice, the Committee concluded that TBHQ was unlikely to be genotoxic *in vivo* under conditions of use as an antioxidant, and that further genotoxicity studies were unnecessary.

The results of four reproductive toxicity studies in rats were evaluated. Taken together, the results of these studies indicated an adverse effect of TBHQ on the survival and/or body weight of pups at levels of 5 g/kg in the diet or higher. The effect on pup body weight occurred late in the lactation period. The NOEL was 2.5 g/kg in the diet, equivalent to 125 mg/kg of body weight per day.

On the basis of the data reviewed at the present meeting, the Committee concluded that TBHQ was not carcinogenic in rats or mice. After reviewing the long-term toxicity studies in mice, rats and dogs and the reproductive toxicity studies in rats, the Committee concluded that the most sensitive species was the dog. The Committee allocated an ADI of 0–0.7 mg/kg of body weight for TBHQ, based on the NOEL of 72 mg/kg of body weight per day and a safety factor of 100. The NOEL was rounded to one significant figure, as is the usual practice.

A toxicological monograph incorporating new information and relevant information from the earlier toxicological monographs and monograph addenda was prepared. The existing specifications were revised, with minor changes.

3.2 Emulsifiers

3.2.1 *Microcrystalline cellulose*

Microcrystalline cellulose was evaluated at the fifteenth, seventeenth and nineteenth meetings of the Committee (Annex 1, references *26*, *32* and *38*). At the nineteenth meeting, an ADI "not specified" was allocated. In the light of concern about possible persorption and consequential adverse effects of fine particles, the substance was re-evaluated at the present meeting.

In early studies persorption of microcrystalline cellulose was reported in various species including rats. A recent study in which a special

preparation of fine particle size of microcrystalline cellulose (median diameter 6 μm) was administered orally to rats (5 g/kg of body weight per day) for 90 days has failed to confirm the earlier observations. In this study precautions were taken to ensure that there was no cross-contamination of the tissues with fine particulate matter at autopsy.

In various acute toxicity studies in animals given microcrystalline cellulose parenterally there have been signs consistent with a tissue response to foreign particles. Similarly microcrystalline cellulose has been associated with the formation of granulomas in human lung when it has been injected intravenously by drug abusers. No such lesions have been described as a consequence of oral ingestion of microcrystalline cellulose by rats or humans.

In 90-day toxicity tests during which microcrystalline cellulose was administered to rats at concentrations of 25 g/kg to 500 g/kg in the diet, increased consumption of food to compensate for the low energy content of this material was observed. Although this may have some adverse effects on mineral absorption there was, in general, no compound-related systemic toxicity. The NOEL was 50 g/kg in the diet, equal to 3.8 g/kg of body weight per day.

A 2-year study in rats, which were fed microcrystalline cellulose in the diet, was brought to the attention of the Committee. Despite a lack of evidence of toxic effects, the Committee considered that the execution and reporting of the study were not adequate to identify a NOEL. *In vitro* and *in vivo* genotoxicity studies were negative.

In a 3-generation reproductive toxicity study in rats that had been reviewed by the Committee at its fifteenth meeting (Annex 1, reference *27*), there were some effects in animals given microcrystalline cellulose at 300 g/kg in the diet; these were considered to be a consequence of the quantity of material reducing the energy density of the diet. In recent embryotoxicity and teratogenicity studies in rats, there was no evidence of treatment-related effects at levels of up to 50 g/kg in the diet (equal to 4.6 g/kg of body weight per day), given on days 6 to 15 of pregnancy.

In some studies in humans there have been reports of alterations in gastrointestinal function following ingestion of microcrystalline cellulose. The changes do not appear to be related to systemic toxicity. The Committee concluded that the toxicological data from humans and animals provided no evidence that the ingestion of microcrystalline cellulose can cause toxic effects in humans when used in foods according to good manufacturing practice.

It is recognized that small particles of other materials may be persorbed and that the extent of persorption is greater with very small particles (<1 μm in diameter). Despite the absence of any demonstrated persorption of microcrystalline cellulose in the recent study in rats, the Committee, as a precautionary measure, revised the specifications for microcrystalline cellulose at the present meeting to limit the content of particles less than 5 μm in diameter. The Committee retained the ADI "not specified" for microcrystalline cellulose conforming to these specifications.

A toxicological monograph that incorporated the updated earlier monograph and summaries of studies reviewed for the first time at the present meeting was prepared. The existing specifications were revised and the requirement for particle size was changed from "greater than 5 μm" to "not more than 10% of the material has a particle size of less than 5 μm".

3.2.2 *Sucrose esters of fatty acids and sucroglycerides*

Sucrose esters of fatty acids and sucroglycerides were previously reviewed by the Committee at its thirteenth, seventeenth, twentieth, twenty-fourth, thirty-fifth, thirty-ninth and forty-fourth meetings (Annex 1, references *19*, *32*, *41*, *53*, *88*, *101* and *116*). At the forty-fourth meeting, the NOEL from a new long-term toxicity/carcinogenicity study in rats was used as the basis for the ADI. The sucrose ester formulations used in this study contained no monoglycerides or diglycerides and the highest dose tested was 50 g/kg in the diet, equal to 1970 mg/kg of body weight per day. Because the results from a tolerance study in humans raised some concerns about potential laxative effects and related abdominal symptoms, a temporary group ADI of 0–20 mg/kg of body weight was established for the sucrose ester content of sucrose esters of fatty acids and sucroglycerides, based on the long-term toxicity study in rats and a safety factor of 100. The results of a well designed and conducted tolerance study in humans were requested for review in 1997.

In the previous study in humans, single doses of 1.5–3.0 g, or divided doses of 3.0–4.5 g per day for 5–7 days, induced laxation and related abdominal symptoms. The results from a new study in humans indicated an absence of effects of sucrose esters of fatty acids on the frequency and appearance of faeces and related abdominal symptoms in men and women ingesting a divided daily dose of 1.5 g of sucrose esters of fatty acids (equal to 27 and 29 mg/kg of body weight per day, respectively) for 5 days. Even though the deficiencies in design noted in the earlier study, i.e. the small number of subjects and the lack of

proper controls, were corrected in the new study only a single dose level was used, which was below that previously associated with gastrointestinal disturbances. Consequently, it was not possible to confirm that the effects observed in the previous study were the result of treatment with sucrose esters of fatty acids.

The Committee noted that no systemic effects were observed in a well conducted long-term toxicity study in rats up to the highest dose tested, 1970 mg/kg of body weight per day. Consequently, a group ADI of 0–30 mg/kg of body weight for sucrose esters of fatty acids and sucroglycerides was allocated on the basis of the new study in humans without the application of a safety factor.

An addendum to the toxicological monograph was prepared. The existing specifications for sucrose esters of fatty acids and sucroglycerides were revised, with minor changes.

3.3 Enzyme preparations

3.3.1 *α-Acetolactate decarboxylase*

α-Acetolactate decarboxylase is an enzyme produced by submerged fermentation of *Bacillus subtilis* carrying the gene coding for α-acetolactate decarboxylase from *B. brevis*. It is used as a processing aid in the brewing and alcohol industry to avoid formation of the unpleasant tasting α-diacetyl from α-acetolactate during fermentation.

α-Acetolactate decarboxylase expressed in *B. subtilis* has not been previously evaluated by the Committee.

Data reviewed by the Committee included information on the pathogenicity of the source and donor organisms as well as short-term toxicity and genotoxicity studies on the enzyme preparation.

Two forms of α-acetolactate decarboxylase have been used in the toxicity studies, namely, an unstabilized form and a glutaraldehyde-stabilized form, which is the form used in the final commercial product.

The available data indicate that both the source organism, *B. subtilis*, and the donor organism, *B. brevis*, are considered to be non-pathogenic species. *B. subtilis* was grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation. The vector, pUB110, is a plasmid commonly used in the construction of recombinant microorganisms in the production of enzymes and was not considered to be of toxicological concern.

The pathogenicity of four strains of *B. subtilis* involved either in the construction of the recombinant strain or in the production of α-acetolactate decarboxylase was tested in a study in mice given a single dose by the intraperitoneal route. There were no clinical symptoms related to treatment, and no pathological changes were noted at the end of the study that could be associated with treatment.

The Committee considered the recombinant DNA procedures used, and concluded that the recombinant strain of *B. subtilis* should be regarded as a safe source of α-acetolactate decarboxylase.

Administration of α-acetolactate decarboxylase in the diet to rats in a 14-day and a 13-week study was not associated with any signs of toxicity at dietary levels equivalent to 2500 mg/kg in the feed (14-day study) or 500 mg/kg in the feed (13-week study) for either the unstabilized or stabilized enzyme. No long-term toxicity studies were available. In genotoxicity studies, negative results were obtained with both unstabilized and stabilized α-acetolactate decarboxylase in *in vitro* gene mutation assays in bacteria and mammalian cells and in a chromosomal aberration assay in human lymphocytes.

On the basis of the toxicological data, the Committee concluded that α-acetolactate decarboxylase is an enzyme of low toxicity and that no further studies are required to assess its safety.

The Committee established a temporary ADI "not specified" for α-acetolactate decarboxylase from this recombinant strain of *B. subtilis* when the preparation is used in accordance with good manufacturing practice. A temporary ADI was allocated, pending consideration of the "tentative" qualification of the specifications.

A toxicological monograph was prepared. New specifications were prepared and designated as "tentative" because of the tentative qualification of 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*, section 2.3.4).

3.3.2 *Maltogenic amylase*

The enzyme under evaluation is a maltogenic amylase produced by submerged fermentation of a non-pathogenic and non-toxicogenic strain of *Bacillus subtilis* which contains the *amy*M gene from *B. stearothermophilus* coding for maltogenic amylase.

Maltogenic amylase expressed in *B. subtilis* has not been previously evaluated by the Committee.

Formulations of maltogenic amylase are used in the baking and starch industry. It is an exo-acting maltogenic amylase enzyme (EC 3.2.1.133, glucan 1,4-α-maltohydrolase), which catalyses the hydrolysis of 1,4-α-glucosidic linkages in amylose, amylopectin and related glucose polymers. Maltose units are successively removed from the non-reducing end of the polymer chain until the molecule is degraded or, in the case of amylopectin, until a branch point is reached. The Committee noted that the human intake of this recombinant maltogenic amylase resulting from its intended use in the baking and starch industry would be low and that the material consumed would not be the active maltogenic amylase but a heated, denatured material.

The data reviewed included the genetic modification procedures employed, characterization of the producing organisms, the fermentation process, acute and short-term toxicity studies in animals, and genotoxicity studies.

The Committee noted that well documented non-pathogenic and non-toxicogenic strains of microorganisms (*Bacillus subtilis*, *Escherichia coli* K12 and *B. stearothermophilus*) had been used in the genetic modification procedures. The final vector used (pUB110) is well characterized and has been used for several years as a cloning vehicle for *B. subtilis*. The plasmid construct pDN1413, containing the *amy*M gene, was introduced into *B. subtilis* (a derivative of strain 168) using standard transformation procedures. Although the plasmid pDN1413 carries the gene for kanamycin resistance, it is unlikely that this gene can be transferred, since it is well integrated into the host genome and no plasmid DNA could be detected in the end-product. The entire DNA sequence of pDN1413 was determined, which confirmed that genes coding for shiga-like toxins are not present.

B. subtilis was grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation.

From the evaluation of the recombinant DNA procedures being employed, the Committee concluded that the final construct should be regarded as a safe source of maltogenic amylase.

The product tested in the toxicological studies was a concentrated material with an enzyme activity of 35900 units/g. It was produced according to the standard production process except that the formulation/standardization step was omitted and the product was lyophilized.

In a 90-day study in which the lyophilized test compound was administered in the diet of rats, the highest dose, 50 g/kg in the diet, caused a significant reduction in body-weight gain accompanied by a slight decrease in food consumption in both males and females. A significant decrease in thyroid weights was also seen in both males and females. At the next dose, 15 mg/kg in the diet, no statistically significant treatment-related findings were observed. The NOEL in this study was 15 g/kg in the diet, equal to 1200 mg/kg of body weight per day.

The test compound had no effects in *in vitro* gene mutation studies in bacteria or mammalian cells, and the results of chromosomal aberration tests *in vivo* and *in vitro* were consistently negative.

The test compound did not cause skin or eye irritation in rabbits and did not produce skin sensitization in a delayed-contact hypersensitivity assay in guinea-pigs.

The Committee allocated a temporary ADI "not specified" to maltogenic amylase derived from this recombinant strain, pending deletion of the "tentative" qualification of the specifications.

A toxicological monograph was prepared. New specifications were prepared and designated as "tentative" because of the tentative qualification of 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*, section 2.3.4).

3.4 Flavouring agent: *trans*-anethole

trans-Anethole was previously reviewed by the Committee at its eleventh, twenty-third, twenty-seventh, twenty-eighth, thirty-first, thirty-third, thirty-seventh and thirty-ninth meetings (Annex 1, references *14*, *50*, *62*, *66*, *77*, *83*, *94* and *101*). At the thirty-seventh meeting, a temporary ADI of 0–0.6 mg/kg of body weight was allocated on the basis of the results of a long-term toxicity/carcinogenicity study in Sprague–Dawley rats. In this study, a dose-related increase in the incidence of non-neoplastic proliferative lesions in the liver was observed in both males and females at all doses. In addition, a clear increase in the incidence of hepatocellular adenomas and carcinomas was observed in female rats receiving *trans*-anethole at the highest level, 10 g/kg in the diet (equal to 550 mg/kg of body weight per day). In male animals a slight increase in the incidence of hepatocellular adenomas but not carcinomas was observed at 10 g/kg in the diet (equal to 400 mg/kg of body weight per day).

At the thirty-ninth meeting, the Committee was informed that comparative metabolic studies in mice and rats, studies on the effects of long-term dietary administration of *trans*-anethole on hepatic enzyme induction and cell proliferation in these species and on enzyme induction in humans, and *in vitro* cytotoxicity and genotoxicity studies were in progress. The temporary ADI was extended to 1997, pending the completion of these studies and the results of a long-term study in mice at appropriate levels to establish a no-effect level.

Not all of the requested studies were available for review at the present meeting. The Committee was informed that further studies would be available by 1998.

The Committee extended the previously allocated temporary ADI of 0–0.6 mg/kg of body weight until 1998, pending the submission of the results of the remaining studies.

No toxicological monograph was prepared. The specifications were revised and, as *trans*-anethole is used only as a flavouring agent, they were transferred to the list of flavouring agents in the *Compendium of food additive specifications, Addendum 5* (FAO Food and Nutrition Paper, No. 52, Add. 5, 1997).

3.5 Glazing agent: hydrogenated poly-1-decene

Hydrogenated poly-1-decene has not been previously evaluated by the Committee. It is used as a glazing and releasing agent.

The Committee considered that the data available from a 28-day range-finding study and a 90-day study in which hydrogenated poly-1-decene was administered in the feed of rats were inadequate to support the use of this product as a food additive. Considering the potentially high intake from its use, the Committee concluded that adequate data are required to establish that oily coats observed in rats fed hydrogenated poly-1-decene are not the result of systemic absorption. In addition, data in humans that clearly demonstrate the lack of absorption of this substance should be provided. In the absence of these data, long-term toxicity and reproductive toxicity studies and information on the metabolism, distribution and excretion of hydrogenated poly-1-decene would be required.

A toxicological monograph was not prepared. New specifications were prepared.

3.6 Sweetening agent: maltitol syrup

At the present meeting, the Committee reviewed a request for the amendment of the specifications for maltitol syrup in the context of its

toxicological implications. The current specifications (Annex 1, reference *124*) to which the ADI "not specified" applies (Annex 1, references *83* and *107*) require that maltitol syrup has a maltitol content of no less than 50%, a sorbitol content of no more than 8%, a maltotriitol content of no more than 25% and a content of hydrogenated polysaccharides containing more than three glucose or glucitol units of no more than 30%. An ADI "not specified" was allocated to maltitol syrup produced from glucose syrups that meet these specifications at the thirty-third meeting of the Committee (Annex 1, reference *83*) and confirmed at the forty-first meeting (Annex 1, reference *107*).

The proposed amendment would support the use of a broader range of starch hydrogenation products than are currently permitted. By deletion of the specification tests for hydrogenated saccharides other than maltitol, while still requiring a maltitol content of no less than 50.0% and a total polyol content of no less than 99.0%, the content of any of these components in malititol syrup (sorbitol, maltotriitol and higher-order polyols) could theoretically be as high as 49%. Since an ADI "not specified" has been established for both sorbitol and maltitol, the toxicological review concentrated on the consequences of high concentrations of the higher-order hydrogenated saccharides.

The results of metabolic studies in rats and humans indicated that the higher-order polyol components in hydrogenated starch hydrolysates of differing composition were efficiently hydrolysed in the gastrointestinal tract to glucose and a small amount of maltitol. Maltitol was hydrolysed less readily by endogenous enzymes and a considerable portion undergoes fermentation in the lower gastrointestinal tract. The small amount that is absorbed is rapidly excreted unchanged in the urine.

Studies in animals treated with maltitol syrup composed of up to 41% higher-order polyols were reviewed at the twenty-ninth meeting of the Committee (Annex 1, reference *70*). At its present meeting, the Committee reviewed the toxic potential of two materials that contain more than 49% of the hydrogenated polysaccharides, the first containing 10% sorbitol, 8% maltitol and 82% higher-order polyols and the second containing 100% hydrogenated dextrin, were evaluated in studies in which they were fed to animals, and the mutagenic potential of hydrogenated dextrin was also examined in bacterial assays. A study in rats showed that ingestion of up to 5.2 g/kg of body weight per day of hydrogenated dextrin for 13 weeks did not result in any treatment-related effects. No treatment-related toxicity was seen in

rats or dogs when the material containing 10% sorbitol, 8% maltitol and 82% higher-order polyols was administered in the diet at dosages of up to 18 and 43 g/kg of body weight per day, respectively, for 90 days. Hydrogenated dextrin was not mutagenic in either *Salmonella typhimurium* or *Escherichia coli* strains in the absence or presence of rat S9 microsomal fraction.

On the basis of the above considerations, the Committee confirmed the previous ADI "not specified" and concluded that it could be applied to maltitol syrup meeting the revised specifications.

An addendum to the toxicological monograph was prepared. In reviewing the specifications for maltitol syrup, the Committee considered the specifications for "hydrogenated saccharides other than maltitol" to be unnecessary and deleted the reference and respective purity test.

3.7 Miscellaneous substance: salatrim (short- and long-chain acyltriglyceride molecules)

Salatrim has not been previously reviewed by the Committee. Salatrim is a family of structured triglycerides containing, on average, one or two long-chain fatty acid moieties (usually stearic acid), the remainder being short-chain fatty acids. It is intended for use as a reduced-calorie replacement for conventional fats and oils.

The Committee evaluated studies on the caloric value of salatrim, being aware that short-chain fatty acids supply fewer kilocalories per gram than long-chain fatty acids. However, the claim of poor absorption of stearic acid from salatrim has not been proven for humans. Because there is no specific formulation for salatrim, it is not possible to assign a single caloric value to this product. The Committee noted that the specifications for salatrim that were elaborated at the present meeting permit formulations that include up to 0.87 g of stearate per g of fat. The biological data available do not provide information on materials with such compositions. If future studies determine that stearic acid is poorly absorbed from such formulations, the Committee considered that the consequences of this will need to be determined.

In evaluating the safety of salatrim, the Committee considered various studies. An *in vitro* study with porcine pancreatic lipase demonstrated that a wide range of the salatrim triacylglycerides are hydrolysed rapidly. In rats, the *in vivo* metabolism of a specific salatrim formulation indicated that it was metabolized in an analogous manner to triolein.

Salatrim products do not contain any structural features suggestive of potential mutagenicity, and no evidence of genotoxicity was observed in an adequate range of *in vitro* or *in vivo* studies.

Five 90-day studies in rats, each using a different salatrim formulation administered at concentrations of up to 100 g/kg in the diet, showed no toxicologically significant effects. A 28-day study in minipigs of a specific salatrim formulation was carried out using dose levels of 30, 60 and 100 g/kg in the diet, and also showed no toxicologically significant effects. These studies were not designed to detect potential nutritional effects, and the study in minipigs was of insufficient duration. The Committee concluded that these limitations meant that the studies did not provide an adequate basis for a nutritional or toxicological evaluation.

Because of the high projected intake of salatrim products (90th percentile levels for "all ages" and for 3–5-year-olds are 37 and 26 g per day, respectively) and given that no systemic effects were seen in animal studies, the Committee paid particular attention to the results of the five studies in humans. Of these, one was a free-living trial, the other four were clinic-based with different experimental designs.

In the four clinic-based studies the experimental protocols provided intakes of up to 60 g of salatrim per person per day for periods of 1, 4 or 7 days. Although these studies provided some indication that the consumption of salatrim in the diet was associated with an increased incidence of mild gastrointestinal symptoms and significantly elevated serum enzymes, the treatment periods were short and the numbers of study participants were few.

The free-living study was a randomized, double-blind, multiple-dose, parallel comparison between diets in which the fat was replaced by salatrim oil (23SO, 4SO or 43SO) and control diets in which the fat was soy oil. At least 12 women and 12 men were recruited for each of the two control groups and each of the five treatment groups which received 30, 45 or 60 g per day of 23SO, 60 g per day of 4SO or 60 g per day of 43SO. The total duration of the study was 6 weeks. Subjects in the treatment groups received soy oil during weeks 1 and 6 and salatrim during weeks 2–5, while those in the control groups received soy oil throughout the study.

A total of 183 subjects started the study; 34 dropped out, four of whom were controls. Of those who dropped out, 20 had received salatrim and recorded adverse effects as the reason for leaving the study. The Committee noted inconsistencies between the published

and unpublished reports of the study in that there were differences in the recorded numbers of subjects dropping out.

The consumption of 60 g of salatrim per day was associated with more reports (compared to controls) of stomach cramps and nausea in a substantial number of subjects. Transient elevations of the levels of alanine aminotransferase and aspartate aminotransferase were recorded. Due to the short duration of the study, the high drop-out rate and the modest number of participants, the Committee concluded that it was not possible to determine whether these observations were clinically significant.

The Committee concluded that the available studies did not provide an adequate basis for evaluating the safety and nutritional effects of salatrim. The Committee recommended that additional appropriately designed studies be performed to assess fully both the safety and nutritional consequences of ingestion of salatrim.

A monograph addressing the safety and nutritional effects of salatrim, which included an analysis of the caloric content of the salatrim family, was prepared.

The Committee considered salatrim to be a replacement for conventional fats and oils and therefore regarded it as a food ingredient and not as a food additive. At the request of the Codex Committee on Food Additives and Contaminants the Committee nevertheless prepared new specifications to describe this food ingredient.

4. Substances evaluated using the Procedure for the Safety Evaluation of Flavouring Agents

Six groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as modified at the present meeting (section 2.2.1 and Fig. 1).

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.

The Committee first considered the metabolic pathways common to the groups of flavouring agents evaluated at the present meeting.

Hydrolysis of esters

Linear alkyl esters are hydrolysed rapidly to their component alcohols and carboxylic acids in the intestinal tract, blood and liver, and most tissues throughout the body. Hydrolysis is catalysed by classes of enzymes recognized as carboxylesterases or esterases. For simple linear esters, as considered at this meeting, the rate of hydrolysis increases with increased chain length of either the acid or alcohol component. The rate of hydrolysis of straight-chain esters is approximately 100 times that of branched-chain esters. The rates of hydrolysis of the alkenyl esters citronellyl acetate (3,7-dimethyl-6-octen-1-yl acetate) and citronellyl phenylacetate, by artificial pancreatic juice, were similar to the rates for simple branched-chain esters.

Oxidation of alkyl primary alcohols and aldehydes

Most linear and branched-chain, saturated and unsaturated primary alcohols are oxidized rapidly to their corresponding aldehydes by alcohol dehydrogenase. The rate of oxidation increases with increased chain length, and the presence of a double bond.

The subsequent oxidation of the aldehydes to their corresponding acids is catalysed by dehydrogenase and oxidase enzymes. The most active is a NAD^+/NADH-dependent aldehyde dehydrogenase present in the cytosol, the activity of which increases with increasing relative molecular mass of the aldehyde substrate. Aldehydes may also be reduced to alcohols or conjugated with sulfhydryl-containing substances, such as glutathione. Aldehyde dehydrogenase-catalysed oxidation of aldehydes of low relative molecular mass requires glutathione, which suggests that the free aldehyde may be conjugated rapidly with glutathione *in vivo* to form a thiohemiacetal that is subsequently oxidized to the corresponding acid. Branched-chain aldehydes are rapidly oxidized by aldehyde dehydrogenase, and the rate of oxidation of 2-methylpropanal is similar to that of acetaldehyde.

Oxidation of linear saturated carboxylic acids

Aliphatic linear saturated carboxylic acids are metabolized in the fatty acid β-oxidation pathway, the tricarboxylic acid cycle, or the C_1-tetrahydrofolate pathway. Oxidation of formic acid to carbon dioxide and water occurs primarily in the liver and is catalysed by tetrahydrofolate in humans and other primates.

Other carboxylic acids are condensed with coenzyme A (CoA) to yield thioesters that undergo β-cleavage to acetyl CoA. Carboxylic acids containing an even number of carbon atoms give acetyl CoA, whereas those containing an odd number yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly, whereas propionyl CoA is first converted to succinyl CoA.

Oxidation of branched-chain saturated carboxylic acids

Short-chain (containing six or fewer carbon atoms) branched-chain saturated aliphatic acids undergo β-oxidation preferentially in the longer chain, followed by cleavage to yield linear acid fragments that are metabolized via the fatty acid pathway or the tricarboxylic acid cycle. Isobutyric acid (2-methylpropanoic acid), isovaleric acid (3-methylbutanoic acid) and 2-methylbutyric acid (2-methylbutanoic acid) are formed during the oxidative deamination of endogenous branched-chain amino acids and are metabolized by normal pathways of intermediary metabolism. At high dose levels, longer branched-chain acids may undergo ω-oxidation to yield diacids that undergo further oxidation and cleavage.

Acids with a methyl substituent are extensively metabolized to carbon dioxide via β-oxidation, unless the methyl group is located at the β-position (e.g. 3-methylpentanoic acid), in which case α-oxidation

occurs, yielding short-chain acid fragments capable of being completely metabolized.

The presence of a 2-ethyl substituent prevents the β-oxidation of aliphatic carboxylic acids, and these compounds undergo ω-oxidation and ω-1 oxidation to yield polar metabolites that are excreted primarily in the urine. Saturation of this ω-oxidation pathway may lead to formation of the 2-substituted carboxylic acid that may be excreted as the glucuronic acid conjugate.

4.1 Allyl 2-furoate

Twenty-one allyl esters used as flavouring agents in food were evaluated by the Committee at its forty-sixth meeting using the Procedure for the Safety Evaluation of Flavouring Agents modified at that meeting (Annex 1, reference *122*). The Committee concluded that the use of 20 of the 21 allyl esters that were evaluated posed no safety concerns at their estimated levels of intake. The evaluation of one of the flavouring agents in the group, allyl 2-furoate (2-propenyl furan-2-carboxylate), was postponed pending consideration of the last step on the right-hand side of the Procedure in which a decision criterion of 1.5 μg per person per day is applied to a substance for which adequate data on metabolism and toxicity are lacking. At the present meeting, allyl 2-furoate was evaluated in accordance with the Procedure inclusive of this step (see Fig. 1).

Intake data

On the basis of a reported annual volume of production of 1 kg in Europe (International Organization of the Flavor Industry, personal communication, 1995) and <0.01 kg in the USA (*10*), the estimated daily per capita intake of allyl 2-furoate is 0.14 μg in Europe and <0.01 μg in the USA. These estimates were calculated assuming under-reporting of the poundage data and consumption by 10% of the population as described in Annex 5 of WHO Food Additives Series, No. 35 (Annex 1, reference *117*).

Information on absorption, metabolism and elimination

No data on the metabolism of allyl 2-furoate, an ester of allyl alcohol and 2-furoic acid, were available. The Committee recognized that allyl esters are generally hydrolysed to allyl alcohol and their corresponding carboxylic acids. At its forty-sixth meeting, however, the Committee determined that there was insufficient evidence to conclude that allyl 2-furoate would be rapidly and completely hydrolysed in humans.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to allyl 2-furoate, the Committee assigned the compound to structural class III (*11*).

Step 2. No data on the metabolism of allyl 2-furoate were available and the substance could not be predicted to be metabolized to innocuous products. Accordingly, the right-hand side of the decision tree was further considered.

Step B3. The intake estimates for allyl 2-furoate were below the threshold for class III (90 μg per day).

Step B4. There were no toxicity data on allyl 2-furoate or on a structurally related substance to provide a NOEL to indicate whether an adequate margin of safety exists under conditions of intended use.

Step B5. The conditions of use of allyl 2-furoate do not result in an intake greater than 1.5 μg per day. The estimated intake is approximately one-tenth of this value.

Conclusion

No multiple-dose toxicity studies on allyl 2-furoate were available. At its forty-sixth meeting, the Committee considered the available toxicity data on the other allyl esters as inapplicable to the evaluation of allyl 2-furoate, because they were all expected to be rapidly and completely hydrolysed in humans.

If hydrolysis of allyl 2-furoate were assumed, the ADI for allyl alcohol and knowledge of the metabolism of 2-furoic acid would support a conclusion of no safety concern for this substance. In accordance with the Procedure, the Committee concluded that allyl 2-furoate would not be expected to present a safety concern at the estimated level of current intake.

A toxicological monograph was not prepared. The tentative specifications prepared at the forty-sixth meeting of the Committee were maintained.

4.2 Saturated aliphatic acyclic linear primary alcohols, aldehydes and acids

The Committee evaluated a group of 38 flavouring agents that included selected saturated aliphatic acyclic linear primary alcohols, aldehydes and acids of chain length C_{1-18} (Table 1) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Table 1
Summary of the results of safety evaluations of 38 saturated aliphatic acyclic linear primary alcohols, aldehydes and acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on levels of current intake
Formic acid[c]	0079	No Europe: 800 USA: 160	NR	Formic acid is produced endogenously in humans and is a normal component of intermediate metabolism	No safety concern
Acetaldehyde	0080	Yes Europe: 11000 USA: 9700	Yes	Acetaldehyde is oxidized to acetic acid which is metabolized via the citric acid cycle; acetaldehyde can also be reduced to ethanol	
Acetic acid[c]	0081	Yes Europe: ND USA: 360000	Yes	Acetic acid is metabolized to carbon dioxide; it acetylates amines and can be incorporated into proteins	
Propyl alcohol	0082	Yes Europe: 420 USA: 2700	Yes	Propyl alcohol is oxidized to propionaldehyde which is oxidized to propionic acid; propionic acid is metabolized via the citric acid cycle	
Propionaldehyde	0083	No Europe: 33 USA: 140	NR	Propionaldehyde is oxidized to propionic acid which is metabolized via the citric acid cycle	

Propionic acid[c]	0084	Yes Europe: 1100 USA: 5200	Yes	Propionic acid is metabolized via the citric acid cycle	No safety concern
Butyl alcohol	0085	Yes Europe: 1900 USA: 8100	Yes	Butyl alcohol is oxidized to butyraldehyde which is oxidized to butyric acid; butyric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Butyraldehyde	0086	No Europe: 26 USA: 17	NR	Butyraldehyde is oxidized to butyric acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Butyric acid	0087	Yes Europe: 10000 USA: 5900	Yes	Butyric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Amyl alcohol	0088	No Europe: 97 USA: 44	NR	Amyl alcohol is oxidized to valeraldehyde which is rapidly oxidized to valeric acid; valeric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Valeraldehyde	0089	Yes Europe: 3000 USA: 8.8	Yes	Valeraldehyde is rapidly oxidized to valeric acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Valeric acid	0090	No Europe: 140 USA: 850	NR	Valeric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Hexyl alcohol	0091	Yes Europe: 1900 USA: 800	Yes	Hexyl alcohol is oxidized to hexanal which is rapidly oxidized to hexanoic acid; hexanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Hexanal	0092	No Europe: 780 USA: 260	NR	Hexanal is rapidly oxidized to hexanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Hexanoic acid	0093	Yes Europe: 3500 USA: 1300	Yes	Hexanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	

Table 1 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Heptyl alcohol	0094	No Europe: 12 USA: 7	NR	Heptyl alcohol is oxidized to heptanal which is rapidly oxidized to heptanoic acid; heptanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	No safety concern
Heptanal	0095	No Europe: 200 USA: 3.2	NR	Heptanal is rapidly oxidized to heptanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Heptanoic acid	0096	No Europe: 170 USA: 5.3	NR	Heptanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
1-Octanol	0097	No Europe: 230 USA: 32	NR	1-Octanol is oxidized to octanal which is rapidly oxidized to octanoic acid; octanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Octanal[c]	0098	No Europe: 170 USA: 90	NR	Octanal is rapidly oxidized to octanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Octanoic acid	0099	Yes Europe: 3800 USA: 650	Yes	Octanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Nonyl alcohol	0100	No Europe: 8.1 USA: 2.1	NR	Nonyl alcohol is oxidized to nonanal which is rapidly oxidized to nonanoic acid; nonanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Nonanal[c]	0101	No Europe: 130 USA: 17	NR	Nonanal is rapidly oxidized to nonanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	

Nonanoic acid	0102	No Europe: 64 USA: 63	NR	Nonanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	No safety concern
1-Decanol	0103	No Europe: 290 USA: 7	NR	1-Decanol is oxidized to decanal which is rapidly oxidized to decanoic acid; decanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Decanal	0104	No Europe: 288 USA: 61	NR	Decanal is rapidly oxidized to decanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Decanoic acid	0105	No Europe: 1400 USA: 980	NR	Decanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways; at high concentrations, it undergoes ω-oxidation	
Undecyl alcohol	0106	No Europe: 0.9 USA: 11	NR	Undecyl alcohol is oxidized to undecanal which is rapidly oxidized to undecanoic acid; undecanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Undecanal	0107	No Europe: 480 USA: 1.5	NR	Undecanal is rapidly oxidized to undecanoic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Undecanoic acid	0108	No Europe: 4.6 USA: 8.8	NR	Undecanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Lauryl alcohol	0109	No Europe: 170 USA: 80	NR	Lauryl alcohol is oxidized to lauric aldehyde which is rapidly oxidized to lauric acid; lauric acid is metabolized via the fatty acid and tricarboxylic acid pathways	

Table 1 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Comments	Conclusion based on current levels of intake
Lauric aldehyde	0110	No Europe: 52 USA: 21	NR	Lauric aldehyde is rapidly oxidized to lauric acid which is metabolized via the fatty acid and tricarboxylic acid pathways	No safety concern
Lauric acid	0111	No Europe: 590 USA: 1200	NR	Lauric acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Myristaldehyde	0112	No Europe: 9.4 USA: 25	NR	Myristaldehyde is rapidly oxidized to myristic acid which is metabolized via the fatty acid and tricarboxylic acid pathways	
Myristic acid	0113	No Europe: 160 USA: 72	NR	Myristic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
1-Hexadecanol	0114	No Europe: 3.6 USA: 0.2	NR	1-Hexadecanol is oxidized to hexadecanal which is rapidly oxidized to hexadecanoic acid; hexadecanoic acid is metabolized via the fatty acid and tricarboxylic acid pathways	
Palmitic acid	0115	No Europe: 89 USA: 234	NR	β-oxidation of palmitic acid yields 2-carbon units which enter the tricarboxylic acid cycle	
Stearic acid	0116	Yes Europe: 58 USA: 1900	Yes	β-oxidation of stearic acid yields 2-carbon units which enter the tricarboxylic acid cycle	

NR: Not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the procedure. ND: no intake data reported

[a] *Step 1*: All of the substances in this group are in structural class I, the human intake threshold of which is 1800 μg per day.
Step 2: All of the substances in this group are metabolized to innocuous products.

[b] All intake values are expressed in μg per day.

[c] The ADI for this substance was maintained.

Several substances in the group have been evaluated previously by the Committee. At the seventeenth meeting, group ADIs "not limited" were allocated to acetic acid and its potassium and sodium salts and to propionic acid (propanoic acid) and its calcium, potassium and sodium salts, and an ADI of 0–3 mg/kg of body weight was allocated to formic acid (Annex 1, reference *32*), which was made a group ADI with ethyl formate at the twenty-third meeting (Annex 1, reference *50*). A group ADI of 0–0.1 mg/kg of body weight was established for octanal and nonanal, singly or in combination, at the twenty-eighth meeting (Annex 1, reference *66*). An ADI "not limited" was allocated to the aluminium, ammonium, calcium, magnesium, potassium and sodium salts of myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) acids at the seventeenth meeting (Annex 1, reference *32*). At the twenty-ninth meeting, the Committee did not establish ADIs for these acids due to lack of information on the manufacture and use of the food-grade material, but noted that they are normal constituents of coconut oil, butter and other edible oils. ADIs have not been allocated to butyl alcohol (1-butanol), decanal or propyl alcohol (1-propanol) because the data were considered to be inadequate (Annex 1, references *38*, *14* and *56* respectively).

The intake of one substance, ethyl alcohol (ethanol), which is structurally related to the group was considered at the forty-sixth meeting (Annex 1, reference *122*), when the Committee evaluated ethyl esters used as flavouring agents. At that time, the Committee concluded that ethyl alcohol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.

Intake data

The total annual volume of production of the 38 substances from their use as flavouring agents is approximately 2100 tonnes in the USA. Approximately 90% of the total volume is accounted for by acetic acid, which includes the amount produced for uses (acidulant or solvent) in food other than as a flavouring agent. No specific data were available on the use of acetic acid as a flavouring agent in Europe. Disregarding acetic acid, the total reported annual volume of production of the remaining 37 aliphatic substances used as flavouring agents is approximately 300 tonnes in Europe and 200 tonnes in the USA.

According to production statistics and derived estimated intakes of flavouring agents in Europe and the USA, acetaldehyde (ethanal), butyl alcohol and butyric acid (butanoic acid) are the most important substances in this group. Acetaldehyde and butyric acid account for

about 50% of the daily per capita intake in Europe, and acetaldehyde and butyl alcohol account for about 46% of the daily per capita intake in the USA. Other flavouring agents in this group with high intake levels (i.e. >1800 μg per person per day) include octanoic acid, hexanoic acid, valeraldehyde (pentanal), butyl alcohol and hexyl alcohol (1-hexanol) in Europe and butyric acid, propionic acid, propyl alcohol and stearic acid in the USA (Table 1).

Saturated linear aliphatic alcohols, aldehydes and acids are ubiquitous in nature. Alcohols and acids of low relative molecular mass have been detected in almost every known fruit and vegetable. There are a limited number of reports of the natural occurrence of the corresponding aldehydes. In the USA, the available quantitative data indicate that the dietary consumption of saturated linear aliphatic alcohols, aldehydes and acids from naturally occurring sources exceeds their consumption from use as flavouring substances.

Information on absorption, metabolism and elimination

Linear aliphatic acyclic alcohols, aldehydes and carboxylic acids are absorbed through the gastrointestinal tract. Their half-lives in plasma are difficult to measure since many alcohols of low relative molecular mass (e.g. ethyl alcohol), aldehydes and carboxylic acids (e.g. acetic acid and propionic acid) are endogenous in humans. Acetaldehyde has been detected in whole blood (<0.2 mg/l) and acetate is a blood buffer.

The flavouring agents in this group of selected saturated aliphatic linear alcohols, aldehydes and acids are all metabolized via the fatty acid and tricarboxylic acid pathways (see pages 26–27).

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned saturated aliphatic linear alcohols, aldehydes and acids, the Committee assigned all 38 substances to structural class I.

Step 2. All of the flavouring agents in this group are known to be, or can be readily predicted to be, efficiently metabolized to innocuous substances. Accordingly, the left-hand side of the decision tree was further considered.

Step A3. The intake estimates for 27 substances in this group fall below the threshold for human intake for class I (1800 μg per day); therefore, these substances were considered to be of no safety concern.

Step A4. The intake estimates for 11 substances in this group exceeded the threshold for human intake for class I. In all cases, the substances could be predicted to undergo complete metabolism to endogenous products via the fatty acid and tricarboxylic acid pathways. In the opinion of the Committee, the endogenous levels of metabolites from these substances would not give rise to perturbations outside the physiological range. Therefore, these 11 substances were also considered to be of no safety concern.

Table 1 summarizes the evaluation of the 38 saturated aliphatic acyclic linear primary alcohols, aldehydes and acids using the Procedure.

Consideration of combined intakes

In the unlikely event that all foods containing all of the 38 substances in this group were consumed simultaneously on a daily basis, the estimated daily per capita intake in Europe and the USA (excluding intakes of acetic acid and propionic acid which have ADIs "not limited") would be approximately 40 mg and 30 mg respectively, i.e. above the threshold for human intake for substances in class I.

All of the substances in this group and their metabolites are innocuous and endogenous and their combined intake was judged by the Committee not to give rise to perturbations outside the physiological range.

Conclusion

The Committee concluded that the substances in this group would not present safety concerns at the estimated current levels of intake.

No toxicity data were required for the application of the Procedure. However, the Committee noted that the toxicity data that were available were consistent with the results of using the Procedure. In cases where ADIs had been established previously, they were maintained at the present meeting.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.3 Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids

The Committee evaluated a group of 25 flavouring agents that included selected saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids (see Table 2) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Table 2
Summary of the results of safety evaluations of 25 saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids[a]

Substance	No.	*Step A3* Does intake exceed the threshold for human intake?[b]	Conclusion based on current levels of intake
Structural class I: methyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
Isobutyl alcohol	0251	No Europe: 530 USA: 290	
Isobutyraldehyde	0252	No Europe: 130 USA:100	
Isobutyric acid	0253	No Europe: 820 USA: 140	
2-Methylbutyraldehyde	0254	No Europe: 4.9 USA: 370	
2-Methylbutyric acid	0255	No Europe: 1200 USA: 480	
3-Methylbutyraldehyde	0258	No Europe: 110 USA: 140	
Isovaleric acid	0259	No Europe: 480 USA: 96	
2-Methylpentanal	0260	No Europe: 12 USA: 8.5	No safety concern
2-Methylvaleric acid	0261	No Europe: 680 USA: 2.3	
3-Methylpentanoic acid	0262	No Europe: 2.9 USA: 8.8	
3-Methyl-1-pentanol	0263	No Europe: 5.9 USA: 4.2	
4-Methylpentanoic acid	0264	No Europe: 1.6 USA: 55	
2-Methylhexanoic acid	0265	No Europe: 15 USA: 2.3	
5-Methylhexanoic acid	0266	No Europe: 0.0 USA: 8.6	
3,5,5-Trimethyl-1-hexanol	0268	No Europe: 13 USA: 0.76	

Table 2 (*continued*)

Substance	No.	*Step A3* Does intake exceed the threshold for human intake?[b]	Conclusion based on current levels of intake
3,5,5-Trimethylhexanal	0269	No Europe: 0.29 USA: 150	
2-Methyloctanal	0270	No Europe: 0.14 USA: 0.95	
4-Methyloctanoic acid	0271	No Europe: 11 USA: 0.10	
3,7-Dimethyl-1-octanol	0272	No Europe: 94 USA: 2.9	No safety concern
2,6-Dimethyloctanal	0273	No Europe: 0.01 USA: 6.7	
4-Methylnonanoic acid	0274	No Europe: 1.00 USA: 1.5	
2-Methylundecanal	0275	No Europe: 0.61 USA: 0.10	
Structural class II: ethyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
2-Ethylbutyraldehyde[c]	0256	No Europe: 0.57 USA: 0.17	
2-Ethylbutyric acid[c]	0257	No Europe: 60 USA: 31	No safety concern
2-Ethyl-1-hexanol[c, d]	0267	No Europe: 86 USA: 40	

[a] *Step 2:* All of the substances in this group are metabolized to innocuous products.
[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 2-ethyl substituent inhibits the β-oxidation of aliphatic alcohols, aldehydes and carboxylic acids. These compounds undergo ω- and ω-1-oxidation to yield polar metabolites which are primarily excreted in urine.
[d] The ADI for this substance was maintained.

Twenty-two of these substances contain one or more methyl substituents and the remaining three have ethyl substituents in the α-position.

Two of the substances have been evaluated previously by the Committee. Isobutyl alcohol (2-methyl-1-propanol) was considered at the twenty-third meeting, when an ADI was not allocated because of a

lack of information (Annex 1, reference *50*). An ADI of 0–0.5 mg per kg of body weight was allocated to 2-ethyl-1-hexanol at the forty-first meeting (Annex 1, reference *107*).

Intake data

The total annual volume of production of the 22 methyl-substituted saturated aliphatic branched-chain primary alcohols, aldehydes and acids from their use as flavouring substances is approximately 29 tonnes in Europe and 9.8 tonnes in the USA. In Europe, more than 85% of the total annual volume is accounted for by five substances (isobutyl alcohol, isobutyric acid, 2-methylbutyric acid, isovaleric acid and 2-methylvaleric acid (2-methylpentanoic acid)). In the USA more than 80% of the total annual volume is accounted for by seven substances (isobutyl alcohol, isobutyraldehyde (2-methylpropanal), isobutyric acid, 2-methylbutyraldehyde (2-methylbutanal), 2-methylbutyric acid, 3-methylbutyraldehyde (3-methylbutanal) and isovaleric acid).

The total reported annual volume of production of the three 2-ethyl substituted substances for use as flavouring agents is 1000 kg in Europe and 370 kg in the USA.

Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids have been detected as natural components of a wide variety of foods such as cheese, fruits, vinegar and alcoholic beverages. Quantitative data on the natural occurrence of these flavouring agents has been reported for 11 of the 25 substances in the group and their total annual consumption in food is estimated at 1.5 million kg per year.

Information on absorption, metabolism and elimination

The metabolism of methyl- and ethyl-substituted saturated aliphatic acyclic branched-chain alcohols, aldehydes and carboxylic acids is described on pages 26–27.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids, the Committee assigned all 22 methyl-substituted substances to structural class I. The three ethyl-substituted substances (2-ethylbutyraldehyde (2-ethylbutanal), 2-ethylbutyric acid (2-ethylbutanoic

acid) and 2-ethyl-1-hexanol) contain sterically hindered functional groups and were therefore assigned to structural class II.

Step 2. At their current levels of intake from use as flavouring agents (see Table 2), the 22 methyl-substituted alcohols, aldehydes and carboxylic acids and the three ethyl-substituted alcohols, aldehydes and carboxylic acids would not be expected to saturate the metabolic pathways and all the compounds were predicted to be metabolized to innocuous products.

Step A3. The intake estimates for all the 22 methyl-substituted substances in this group in both Europe and the USA are below the threshold for human intake for class I (1800 μg per day). Therefore, these substances were considered to be of no safety concern when used as flavouring agents at current estimated levels of intake.

The intake estimates for the three ethyl-substituted substances (2-ethylbutyraldehyde, 2-ethylbutyric acid and 2-ethyl-1-hexanol) in this group in both Europe and the USA are below the threshold for human intake for class II (540 μg per day). Therefore, these substances were also considered to be of no safety concern when used as flavouring agents at current estimated levels of intake.

Table 2 summarizes the evaluation of the 25 saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and carboxylic acids using the Procedure.

Consideration of combined intake

In the unlikely event that all foods containing all 22 methyl-substituted alcohols, aldehydes and acids as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake of these substances would be 410 μg in Europe and 1900 μg in the USA.

In the unlikely event that all foods containing all three ethyl-substituted alcohols, aldehydes and acids were consumed simultaneously on a daily basis, the estimated total daily per capita intake of these three substances would be less than 145 μg in Europe and less than 71 μg in the USA.

The Committee judged that the combined intake of substances in this group is of no safety concern, since all the substances are expected to be efficiently metabolized and the combined level of intake is not expected to saturate metabolic pathways.

Conclusion

The Committee concluded that the use of the above-mentioned substances as flavouring agents would not present safety concerns at the estimated current levels of intake.

No toxicity data were required for the application of the Procedure. However, the Committee noted that where toxicity data were available they were consistent with the results of the Procedure. The ADI established previously for 2-ethyl-1-hexanol was maintained.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.4 Aliphatic lactones

The Committee evaluated a group of 35 aliphatic lactones used as flavouring agents in food (Table 3) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Two substances in the group, γ-nonalactone and γ-undecalactone, were previously evaluated by the Committee at its eleventh meeting, when ADIs of 0–1.25 mg/kg of body weight were established for each substance (Annex 1, reference *14*).

Intake data

The total annual volume of production of the 35 substances from their use as flavouring agents is approximately 160 tonnes in Europe and 27 tonnes in the USA. The estimated total daily per capita intakes of all aliphatic lactones resulting from their use as flavouring agents are 30.3 mg in Europe and less than 5.3 mg in the USA. In Europe, γ-decalactone and δ-dodecalactone account for two-thirds of the daily per capita intake of lactones used as flavouring agents. In the USA, four substances (γ-decalactone, δ-decalactone, γ-dodecalactone and δ-dodecalactone) account for most of the daily per capita intake of aliphatic lactones used as flavouring agents.

The four lactones that are α,β-unsaturated (5-hydroxy-2,4-decadienoic acid δ-lactone, 5-hydroxy-2-decenoic acid δ-lactone, 5-hydroxy-2-dodecenoic acid δ-lactone, and a mixture of 5-hydroxy-2-decenoic acid δ-lactone, 5-hydroxy-2-dodecenoic acid δ-lactone and 5-hydroxy-2-tetradecenoic acid δ-lactone) and the two hydroxyfuranones (5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone and 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one) are estimated to have very low total daily per capita intakes. The combined estimated per capita intakes of these six substances from their use in food is 27 μg in Europe and less than 9 μg in the USA.

Table 3
Summary of the results of safety evaluations of 35 aliphatic lactones

Substance	No.	*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/A5* Adequate NOEL for substance or related substance?	Conclusion based on current levels of intake
Structural class I						
4-Hydroxybutyric acid lactone (γ-butyrolactone)	0219	Yes	No Europe: 130 USA: 100	NA	NA	
γ-Valerolactone	0220	Yes	No Europe: 140 USA: 57	NA	NA	
γ-Hexalactone	0223	Yes	No Europe: 190 USA: 19	NA	NA	No safety concern
δ-Hexalactone	0224	Yes	No Europe: 380 USA: 2.5	NA	NA	
γ-Heptalactone	0225	Yes	No Europe: 190 USA: 41	NA	NA	

Table 3 (*continued*)

Substance	No.	*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/A5* Adequate NOEL for substance or related substance?	Conclusion based on current levels of intake
γ-Octalactone	0226	Yes	No Europe: 490 USA: 90	NA	NA	No safety concern
δ-Octalactone	0228	Yes	No Europe: 270 USA: 17	NA	NA	
γ-Nonalactone[b]	0229	Yes	No Europe: 1200 USA: 470	NA	NA	
Hydroxynonanoic acid δ-lactone	0230	Yes	No Europe: 150 USA: 11	NA	NA	
γ-Decalactone	0231	Yes	Yes Europe: 1800 USA: 370	No	Yes	
δ-Decalactone	0232	Yes	Yes Europe: 8400 USA: 1900	No	Yes	
ε-Decalactone	0241	Yes	No Europe: 0.01 USA: 0	NA	NA	
γ-Undecalactone[b]	0233	Yes	No Europe: 1400 USA: 550	NA	NA	

5-Hydroxyundecanoic acid δ-lactone	0234	Yes	No Europe: 350 USA: 180	NA	NA	No safety concern
γ-Dodecalactone	0235	Yes	No Europe: 220 USA: 110	NA	NA	
δ-Dodecalactone	0236	Yes	Yes Europe: 6800 USA: 1140	No	Yes	
ε-Dodecalactone	0242	Yes	No Europe: 0.01 USA: 0.17	NA	NA	
δ-Tetradecalactone	0238	Yes	No Europe: 120 USA: 2.5	NA	NA	
ω-Pentadecalactone	0239	Yes	No Europe: 84 USA: 51	NA	NA	
4-Hydroxy-3-pentenoic acid lactone	0221	Yes	No Europe: NR USA: 4.8	NA	NA	
5-Hydroxy-7-decenoic acid δ-lactone	0247	Yes	No Europe: 0.26 USA: 0.10	NA	NA	

Table 3 (*continued*)

Substance	No.	*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/A5* Adequate NOEL for substance or related substance?	Conclusion based on current levels of intake
5-Hydroxy-8-undecenoic acid δ-lactone	0248	Yes	No Europe: 0.01 USA: 8.6	NA	NA	No safety concern
1,4-Dodec-6-enolactone	0249	Yes	No Europe: 0.01 USA: 8.6	NA	NA	
ω-6-Hexadecenlactone	0240	Yes	No Europe: 6 USA: 0.10	NA	NA	
4,4-Dibutyl-γ-butyrolactone	0227	Yes	No Europe: 0.14 USA: 0.10	NA	NA	
3-Heptyldihydro-5-methyl-2(3*H*)-furanone	0244	Yes	No Europe: 0.04 USA: 0.1	NA	NA	
4-Hydroxy-3-methyloctanoic acid γ-lactone	0437	Yes	No Europe: 0 USA: 8.6	NA	NA	
6-Hydroxy-3,7-dimethyloctanoic acid lactone	0237	Yes	No Europe: 0.1 USA: 0	NA	NA	
γ-Methyldecalactone	0250	Yes	No Europe: 0 USA: 43	NA	NA	

Structural class III						
5-Hydroxy-2-decenoic acid δ-lactone	0246	No	No Europe: 12 USA: 0.10	NA	Insufficient information	Not evaluated[c]
5-Hydroxy-2,4-decadienoic acid δ-lactone	0245	No	No Europe: 0.33 USA: 0.10	NA	Insufficient information	
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	No	— Europe: 0 USA: 2	NA	Insufficient information	
5-Hydroxy-2-dodecenoic acid δ-lactone	0438	No	— Europe: 0 USA: 8.6	NA	Insufficient information	
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	0222	No	No Europe: 13 USA: 6.1	NA	Yes	No safety concern
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	0243	No	No Europe: 2.1 USA: 0.1	NA	Yes	

NA: not applicable.
[a] The thresholds for human intake for classes I and III are 1800 μg per day and 90 μg per day, respectively. All intake values are expressed in μg per day.
[b] The ADI for this substance was maintained.
[c] Evaluation deferred, pending consideration of other α,β-unsaturated compounds.

Most of the aliphatic lactones have been reported to occur naturally in traditional foods. The four aliphatic lactones that are used most as flavouring agents (γ-decalactone, δ-decalactone, γ-dodecalactone and δ-dodecalactone) are ubiquitous in food, occurring mainly in fruits, alcoholic beverages, meats and dairy products.

Information on absorption, metabolism and elimination

Lactones are generally formed by acid-catalysed intramolecular cyclization of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as urine, the lactone ring is favoured. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract.

The aliphatic lactones in this group can be divided into three subgroups on the basis of their predicted metabolism, namely, lactones derived from linear and branched-chain hydroxycarboxylic acids, lactones which are α,β-unsaturated, and the two hydroxyfuranones. The metabolism of the members of each of these subgroups is discussed below.

Lactones derived from linear saturated 5-hydroxycarboxylic acids. Linear saturated 5-hydroxycarboxylic acids (formed from δ-lactones) are converted, via acetyl CoA, to hydroxythioesters which then undergo β-oxidation and cleavage to yield an acetyl CoA fragment and a new β-hydroxythioester reduced by two carbons. Acids containing even numbers of carbon atoms continue to be oxidized and cleaved to yield acetyl CoA while those containing odd numbers of carbon atoms yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly while propionyl CoA is transformed into succinyl CoA which then enters the citric acid cycle.

Lactones derived from linear saturated 4- or 6-hydroxycarboxylic acids. Linear saturated 4- or 6-hydroxycarboxylic acids (formed from γ- or ε-lactones) participate in the same pathway as described above for linear saturated 5-hydroxycarboxylic acids; however, loss of an acetyl CoA fragment produces an α-hydroxythioester which undergoes α-oxidation and α-decarboxylation to yield a linear carboxylic acid and eventually carbon dioxide. γ-Butyrolactone, the only lactone in this group formed from a primary alcohol, may participate in an alternative oxidation pathway, namely, oxidation by alcohol dehydrogenase and succinate-semialdehyde dehydrogenase to succinate which then enters the citric acid cycle.

Lactones derived from linear unsaturated hydroxycarboxylic acids. If the lactone is formed from a linear hydroxycarboxylic acid which is unsaturated, cleavage of acetyl CoA units continues along the carbon chain until the position of unsaturation is reached. If the unsaturation begins at an odd-numbered carbon, acetyl CoA fragmentation will eventually yield a 3-enoyl CoA which is converted to the *trans*-Δ^2-enoyl CoA before entering the fatty acid pathway. If unsaturation begins at an even-numbered carbon, acetyl CoA fragmentation yields a Δ^2-enoyl CoA product which is a substrate for further fatty acid oxidation. If the stereochemistry of the double bond is *cis*, hydration yields (*R*)-3-hydroxyacyl CoA which is isomerized to (*S*)-3-hydroxyacyl CoA by 3-hydroxyacyl CoA epimerase prior to entering the fatty acid pathway.

Lactones derived from branched-chain hydroxycarboxylic acids. For branched-chain hydroxycarboxylic acids, the principal metabolic pathways utilized for detoxication are influenced by the chain length and the position and size of the alkyl substituents. Short-chain (< six carbon atoms) branched aliphatic hydroxycarboxylic acids may be excreted unchanged as the glucuronic acid conjugate, or undergo α- or β-oxidation followed by cleavage and complete metabolism to carbon dioxide via the fatty acid pathway and the tricarboxylic acid cycle. Alternatively, as chain length, substitution and lipophilicity increase, the hydroxycarboxylic acid may undergo a combination of ω-, ω-1 and β-oxidation to yield polar hydroxyacid, ketoacid and hydroxydiacid metabolites which are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces. These metabolites are considered to be innocuous.

α,β-Unsaturated lactones. For the four substances which are α,β-unsaturated there was no direct evidence of hydrolysis. While hydrolysis to the corresponding ring-opened α,β-unsaturated hydroxycarboxylic acids may occur, no information was available on the four substances considered to enable the Committee to predict that this is the major route of metabolism. If hydrolysis to the corresponding ring-opened form occurs, condensation of the α,β-unsaturated hydroxycarboxylic acid with acetyl CoA would yield a Δ^2-enoyl CoA product, which is a substrate in the fatty acid pathway. Since the stereochemistry of the double bond in a lactone is *cis*, hydration would yield (*R*)-3-hydroxyacyl CoA, which is then isomerized to (*S*)-3-hydroxyacyl CoA by 3-hydroxyacyl CoA epimerase prior to entering the fatty acid pathway.

Alternatively, the lactones which are α,β-unsaturated may conjugate with glutathione and be excreted as cysteine or mercapturic acid

derivatives. Evidence for this alternative pathway comes from two structurally related lactones which are α,β-unsaturated. The Committee considered that further information was required in order to clarify the metabolic route(s) of these substances.

Hydroxyfuranones. There was no direct evidence available of hydrolysis of the two hydroxyfuranones (nos 0222 and 0243, see Table 3) to the corresponding ring-opened compound. The Committee considered that alternative metabolic pathways are likely and that no prediction of a metabolic route is possible for these substances.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the aliphatic lactones, the Committee assigned 29 substances to structural class I; the four α,β-unsaturated substances and the two hydroxyfuranones were assigned to structural class III.

Step 2. The available data indicate that for the 29 lactones in class I derived from saturated linear and branched-chain hydroxycarboxylic acids, the corresponding aliphatic hydroxycarboxylic acids are metabolized via the fatty acid pathway. For these substances, the evaluation should proceed via the left-hand side of the decision-tree. For the four lactones in class III which are α,β-unsaturated, metabolism may occur either via hydrolysis followed by β-oxidation or via conjugation with glutathione. There was insufficient information available to predict the route of metabolism of these four substances with confidence. The Committee considered that further information on their metabolism was required and that they should be evaluated together with other α,β-unsaturated substances and that their evaluation should therefore be deferred. No information was available to indicate the route of metabolism for the two lactones in class III which are hydroxyfuranones and therefore the evaluation of these two substances should proceed via the right-hand side of the decision-tree.

Step A3/B3. For the 29 lactones derived from saturated linear and branched-chain hydroxycarboxylic acids in class I, three lactones (γ-decalactone, δ-decalactone and δ-dodecalactone) had intake estimates equal to or greater than the threshold for class I (1800 μg per day). The evaluation of these three substances therefore proceeded to step A4. For the other 26 lactones of similar structure the intake estimates are below the threshold for class I and they would therefore not be expected to be of safety concern. For the two hydroxyfuranones in class III, the intake estimates are well below the

threshold for class III (90 μg per day). The evaluation of these substances therefore proceeded to step B4.

Step A4. None of the three lactones derived from saturated linear hydroxycarboxylic acids (γ-decalactone, δ-decalactone and δ-dodecalactone) are known to be endogenous or to be metabolized to endogenous substances. The safety evaluation of these substances therefore proceeded to step A5.

Step A5. Although adequate studies on which to base a NOEL for the three lactones derived from saturated linear hydroxycarboxylic acids were not available, the following NOELs have been reported for structurally related lactones in 2-year studies in rats: 250 mg/kg of body weight per day for γ-nonalactone and γ-undecalactone and 110 mg/kg of body weight per day for γ-butyrolactone. In a 2-year study in mice with γ-butyrolactone a NOEL of 260 mg/kg of body weight per day was found. The studies on γ-nonalactone and γ-undecalactone were considered previously by the Committee and ADIs were established at the eleventh meeting (Annex 1, reference *14*). Although these studies were not conducted according to modern standards, the results are considered to be valid. These NOELs provide an adequate margin of safety (>1000) for γ-decalactone, δ-decalactone and δ-dodecalactone and therefore these substances would not be expected to be of safety concern.

Step B4. The Committee considered the results of studies on the two hydroxyfuranones in class III. In a 90-day study in rats administered 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone in the diet, the NOEL was 1.3 mg/kg of body weight per day, and in a 1-year study in rats administered 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one in the diet, the NOEL was 46 mg/kg of body weight per day. These NOELs provide an adequate margin of safety (>1000) for these substances and therefore they would not be expected to be of safety concern.

Table 3 summarizes the evaluation of the 35 aliphatic lactones using the Procedure.

Consideration of combined intakes

From the available data, the 29 lactones derived from linear and branched-chain hydroxycarboxylic acids would be expected to be efficiently metabolized via commonly known biochemical pathways to innocuous substances. In the unlikely event that all foods containing all 29 substances as flavouring agents were consumed simultaneously on a daily basis, the estimated daily per capita consumption in Europe and the USA would exceed the threshold for human intake for substances in class I but, in the opinion of the Committee, this

would not give rise to perturbations outside the physiological range.

For the two hydroxyfuranones whose route of metabolism is unknown, their combined estimated intake was very low (15 μg per day) compared to the known NOELs for each of these substances and was not considered to present a safety concern.

Conclusions

The Committee concluded that the evaluation of the four substances which are α,β-unsaturated should be deferred, pending consideration of other α,β-unsaturated substances. The safety evaluation of the two hydroxyfuranones proceeded because of the existence of supporting data from toxicity studies.

On the basis of the results of the evaluation of the 29 lactones derived from linear and branched-chain hydroxycarboxylic acids and the substances 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone and 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one, the Committee concluded that the use of these substances as flavouring agents would not present safety concerns at the estimated current levels of intake.

In using the Procedure, the Committee noted that where toxicity data were available, they were consistent with the results of the safety evaluation.

The ADIs for γ-nonalactone and γ-undecalactone were maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.5 Esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids

The Committee evaluated a group of 32 flavouring agents that included selected esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids (Table 4) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

The Committee had previously evaluated one member of the group, ethyl isovalerate, at its eleventh meeting (Annex 1, reference *14*), but because of a lack of data, was unable to allocate an ADI.

Intake data

The total annual volume of production of the 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids from their use as flavouring substances is approximately 32 tonnes in Europe and 16 tonnes in the USA. In Europe, more than 90% of the

Table 4

Summary of the results of safety evaluations of 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Methyl isobutyrate	0185	No Europe: 23 USA: 270	NR	No safety concern
Ethyl isobutyrate	0186	No Europe: 750 USA: 470	NR	
Propyl isobutyrate	0187	No Europe: 15 USA: 0.08	NR	
Butyl isobutyrate	0188	No Europe: 2.7 USA: 1.9	NR	
Hexyl isobutyrate	0189	No Europe: 3.00 USA: 0.57	NR	
Heptyl isobutyrate	0190	No Europe: 0.00 USA: 3.0	NR	
trans-3-Heptenyl 2-methylpropanoate	0191	No Europe: 0.01 USA: 2.3	NR	
Octyl isobutyrate	0192	No Europe: 11 USA: 5.0	NR	
Dodecyl isobutyrate	0193	No Europe: 50 USA: 0.76	NR	
Isobutyl isobutyrate	0194	No Europe: 65 USA: 2.3	NR	
Methyl isovalerate	0195	No Europe: 7.8 USA: 110	NR	
Ethyl isovalerate	0196	No Europe: 760 USA: 540	NR	
Propyl isovalerate	0197	No Europe: 2.00 USA: 0.10	NR	
Butyl isovalerate	0198	No Europe: 94 USA: 500	NR	
Hexyl 3-methyl-butanoate	0199	No Europe: 2.3 USA: 3.1	NR	
Octyl isovalerate	0200	No Europe: 7.3 USA: 0.57	NR	
Nonyl isovalerate	0201	No Europe: 0.01 USA: 0.08	NR	

Table 4 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
3-Hexenyl 3-methylbutanoate	0202	No Europe: 9.4 USA: 30	NR	No safety concern
2-Methylpropyl 3-methylbutyrate	0203	No Europe: 78 USA: 130	NR	
2-Methylbutyl 3-methylbutanoate	0204	No Europe: 0.86 USA: 0.95	NR	
Methyl 2-methylbutyrate	0205	No Europe: 390 USA: 69	NR	
Ethyl 2-methylbutyrate[c]	0206	Yes Europe: 2200 USA: 560	Yes	
n-Butyl 2-methylbutyrate	0207	No Europe: 26 USA: 0.02	NR	
Hexyl 2-methylbutanoate	0208	No Europe: 4.9 USA: 8.6	NR	
Octyl 2-methylbutyrate	0209	No Europe: 0.01 USA: 0.10	NR	
Isopropyl 2-methylbutyrate	0210	No Europe: 4.9 USA: 0.10	NR	
3-Hexenyl 2-methylbutanoate	0211	No Europe: 5 USA: 8.8	NR	
2-Methylbutyl 2-methylbutyrate	0212	No Europe: 3.6 USA: 0.04	NR	
Methyl 2-methylpentanoate	0213	No Europe: 0.17 USA: 0.02	NR	
Ethyl 2-methylpentanoate	0214	No Europe: 7.6 USA: 320	NR	
Ethyl 3-methylpentanoate	0215	No Europe: 0.31 USA: 5.90	NR	
Methyl 4-methylvalerate	0216	No Europe: 0.03 USA: 0.10	NR	

NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the procedure.

[a] *Step 1*: All of the esters in this group are in structural class I.
Step 2: All of the esters in this group are metabolized to innocuous products.

[b] The threshold for human intake for class I is 1800 µg per day. All intake values are expressed in µg per day.

[c] The components ethanol and 2-methylbutyric acid are endogenous. The acid is an intermediate in the metabolism of the amino acid.

total annual volume is accounted for by ethyl isobutyrate (ethyl 2-methylpropanoate), ethyl isovalerate (ethyl 3-methylbutanoate), ethyl 2-methylbutyrate (ethyl 2-methylbutanoate) and methyl 2-methylbutyrate (methyl 2-methylbutanoate). In the USA, approximately 67% of the total annual volume is accounted for by ethyl isobutyrate, ethyl isovalerate, butyl isovalerate (butyl 3-methylbutanoate) and ethyl 2-methylbutyrate.

Esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids have been detected as natural components of a wide variety of foods. The available quantitative data on the natural occurrence of these esters indicate that the total intake from natural food sources is approximately 14 tonnes per year. This estimated intake is approximately equal to the estimated intake from their use as flavouring substances. In the USA, the consumption of isobutyrate esters from natural food sources is equivalent to their consumption from use as flavouring substances. The consumption of isovalerate esters and 2-methylbutyrate esters from natural food sources is several orders of magnitude higher than that from their use as flavouring agents.

Information on absorption, metabolism and elimination

It is expected that the esters in this group will be readily hydrolysed to their component alcohols and carboxylic acids in the intestinal tract, blood and liver. The metabolism of the hydrolysis products is discussed on pages 26–27.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids, the Committee assigned all 32 substances to structural class I.

Step 2. At the estimated current levels of intake (see Table 4), these esters would not be expected to saturate the metabolic pathways, and they were all predicted to be metabolized to innocuous products. The left-hand side of the decision-tree was therefore considered.

Step A3. The estimated daily per capita intakes of all but one of the 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids in Europe and the USA were below the threshold for class I (1800 μg per day), so that they were considered to be of no safety concern when used at estimated current levels of

intake as flavouring agents. Only ethyl 2-methylbutyrate has an estimated intake greater than the threshold for class I.

Step A4. Ethyl 2-methylbutyrate is expected to be hydrolysed to ethyl alcohol and 2-methylbutyric acid, which are endogenous. Therefore, this substance was determined to be of no safety concern on the basis of its structural class and known metabolism.

Table 4 summarizes the evaluation of the 32 esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids used as flavouring substances.

Consideration of combined intakes

In the unlikely event that all foods containing all of the substances in this group as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake would be 4.6 mg in Europe and 3 mg in the USA. The estimated daily per capita intake of the branched-chain acids (i.e. isobutyric acid, isovaleric acid and 2-methylbutyric acid) formed via hydrolysis of these esters is 3.1 mg in Europe and 2 mg in the USA.

These estimated combined intakes would exceed the threshold for class I. Since all the 32 substances in this group are expected to be efficiently metabolized, they would not be expected to saturate the metabolic 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 substances in this group would not present safety concerns at the estimated current levels of intake.

No toxicity data were required for application of the Procedure. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.6 Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids

The Committee evaluated a group of 67 esters of aliphatic linear and branched-chain saturated and monounsaturated primary alcohols

with aliphatic linear saturated carboxylic acids (see Table 5) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

One member of the group, butyl acetate, had been previously evaluated at the eleventh meeting of the Committee, but no ADI was established due to a lack of data (Annex 1, reference *14*).

Intake data

The total annual volume of production of the 67 esters in this group for use as flavouring agents is approximately 65 tonnes in Europe and 19 tonnes in the USA. In Europe, more than 75% of the total annual volume of production is accounted for by butyl butyrate (*n*-butyl *n*-butanoate), *n*-amyl butyrate (pentyl butanoate) and the acetate esters of methyl alcohol (methanol), butyl alcohol, hexyl alcohol, *cis*-3-hexenol and isobutyl alcohol. In the USA, more than 70% of the total annual volume of production is accounted for by *n*-amyl butyrate, *cis*-3- and *trans*-2-hexenyl propionate (*cis*-3- and *trans*-2-hexenyl propanoate) and the acetate esters of propyl alcohol, isobutyl alcohol and 2-methylbutyl alcohol (2-methylbutanol). On the basis of the reported annual volume of production in Europe and the USA, the total estimated daily per capita intake of the 67 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids from their use as flavouring agents is 9.2 mg in Europe and 3.8 mg in the USA. The use of seven of the esters (heptyl formate, octyl propionate (octyl propanoate), decyl propionate (decyl propanoate), decyl butyrate (decyl butanoate), butyl heptanoate, butyl laurate (butyl dodecanoate), and *cis*-3- and *trans*-2-hexenyl propionate) has been reported in the USA but not in Europe.

Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids are principal components of alcoholic beverages and of a wide variety of fruits. Quantitative data on the natural occurrence in food for 37 substances in the group have been reported from the USA, which indicate that the intake of these substances from natural sources exceeds the intake from their use as flavouring agents.

Information on absorption, metabolism and elimination

In general, it is expected that esters of aliphatic linear and branched-chain primary alcohols with aliphatic linear saturated carboxylic acids would be hydrolysed to their component alcohols and carboxylic acids. The metabolism of the saturated acids and alcohols is described on pages 26–27.

Esters of the three monounsaturated alcohols in this group are expected to be oxidized via their corresponding aldehydes to carboxylic

Table 5
Summary of the results of safety evaluations of 67 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Propyl formate	0117	No Europe: 5.0 USA: 0.38	NA	
Butyl formate	0118	No Europe: 21 USA: 0.17	NA	
n-Amyl formate	0119	No Europe: 29 USA: 110	NA	
Hexyl formate	0120	No Europe: 8.7 USA: 8.0	NA	
Heptyl formate	0121	No Europe: 0.00 USA: 0.10	NA	
Octyl formate	0122	No Europe: 0.14 USA: 0.95	NA	
cis-3-Hexenyl formate	0123	No Europe: 43 USA: 1.7	NA	
Isobutyl formate	0124	No Europe: 4.7 USA: 1.5	NA	
Methyl acetate	0125	No Europe: 460 USA: 110	NA	No safety concern
Propyl acetate	0126	No Europe: 180 USA: 440	NA	
Butyl acetate	0127	No Europe: 1200 USA: 170	NA	
Hexyl acetate	0128	Yes Europe: 3200 USA: 160	Yes[c]	
Heptyl acetate	0129	No Europe: 56 USA: 2.3	NA	
Octyl acetate	0130	No Europe: 83 USA: 9.5	NA	
Nonyl acetate	0131	No Europe: 6.6 USA: 2.5	NA	
Decyl acetate	0132	No Europe: 7.3 USA: 21	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[a] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Lauryl acetate	0133	No Europe: 9.3 USA: 0.57	NA	No safety concern
cis-3-Hexenyl acetate	0134	No Europe: 640 USA: 57	NA	
trans-3-Heptenyl acetate	0135	No Europe: 0.24 USA: 0.76	NA	
10-Undecen-1-yl acetate	0136	No Europe: 0.83 USA: 0.10	NA	
Isobutyl acetate	0137	No Europe: 1200 USA: 1300	NA	
2-Methylbutyl acetate	0138	No Europe: 130 USA: 360	NA	
2-Ethylbutyl acetate	0140	No Europe: 4.0 USA: 0.17	NA	
Methyl propionate	0141	No Europe: 9.3 USA: 30	NA	
Propyl propionate	0142	No Europe: 9.6 USA: 44	NA	
Butyl propionate	0143	No Europe: 10 USA: 1.1	NA	
Hexyl propionate	0144	No Europe: 5.7 USA: 3.0	NA	
Octyl propionate	0145	No Europe: 0.00 USA: 0.02	NA	
Decyl propionate	0146	No Europe: 0.00 USA: 0.95	NA	
cis-3-Hexenyl propionate and *trans*-2-hexenyl propionate	0147	No Europe: 0.00 USA: 430	NA	Not evaluated[c]
Isobutyl propionate	0148	No Europe: 12 USA: 6.5	NA	No safety concern
Methyl butyrate	0149	No Europe: 220 USA: 44	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Propyl butyrate	0150	No Europe: 75 USA: 38	NA	
Butyl butyrate	0151	No Europe: 390 USA: 63	NA	
n-Amyl butyrate	0152	No Europe: 450 USA: 200	NA	
Hexyl butyrate	0153	No Europe: 110 USA: 27	NA	
Heptyl butyrate	0154	No Europe: 6.0 USA: 3.8	NA	
Octyl butyrate	0155	No Europe: 16 USA: 0.38	NA	
Decyl butyrate	0156	No Europe: 0.00 USA: 0.08	NA	
cis-3-Hexenyl butyrate	0157	No Europe: 160 USA: 4.8	NA	
Isobutyl butyrate	0158	No Europe: 47 USA: 7.4	NA	No safety concern
Methyl valerate	0159	No Europe: 30 USA: 11	NA	
Butyl valerate	0160	No Europe: 3.7 USA: 0.10	NA	
Propyl hexanoate	0161	No Europe: 14 USA: 0.17	NA	
Butyl hexanoate	0162	No Europe: 15 USA: 1.9	NA	
n-Amyl hexanoate	0163	No Europe: 8.7 USA: 8.8	NA	
Hexyl hexanoate	0164	No Europe: 150 USA: 13	NA	
cis-3-Hexenyl hexanoate	0165	No Europe: 42 USA: 1.3	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Isobutyl hexanoate	0166	No Europe: 6.1 USA: 1.7	NA	
Methyl heptanoate	0167	No Europe: 5.7 USA: 0.10	NA	
Propyl heptanoate	0168	No Europe: 0.14 USA: 0.38	NA	
Butyl heptanoate	0169	No Europe: 0.00 USA: 4.4	NA	
n-Amyl heptanoate	0170	No Europe: 0.61 USA: 0.02	NA	
Octyl heptanoate	0171	No Europe: 0.21 USA: 0.38	NA	
Isobutyl heptanoate	0172	No Europe: 0.01 USA: 1.9	NA	
Methyl octanoate	0173	No Europe: 9.7 USA: 0.17	NA	
n-Amyl octanoate	0174	No Europe: 3.4 USA: 1.9	NA	No safety concern
Hexyl octanoate	0175	No Europe: 1.3 USA: 0.95	NA	
Heptyl octanoate	0176	No Europe: 0.71 USA: 0.95	NA	
Octyl octanoate	0177	No Europe: 0.03 USA: 2.3	NA	
Nonyl octanoate	0178	No Europe: 0.14 USA: 0.95	NA	
Methyl nonanoate	0179	No Europe: 0.86 USA: 2.3	NA	
Methyl laurate	0180	No Europe: 5.1 USA: 0.76	NA	
Butyl laurate	0181	No Europe: 0.00 USA: 0.10	NA	

Table 5 (*continued*)

Substance	No.	*Step A3*[c] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	Conclusion based on current levels of intake
Isoamyl laurate	0182	No Europe: 0.14 USA: 0.57	NA	No safety concern
Methyl myristate	0183	No Europe: 62 USA: 46	NA	
Butyl stearate	0184	No Europe: 5.1 USA: 5.5	NA	

[a] *Step 1*: All of the esters in this group are in structural class I except 2-ethylbutyl acetate, which is in structural class II.
Step 2: Evaluation of *cis*-3- and *trans*-2-hexenyl propionate was postponed. All of the other substances in this group are metabolized to innocuous products.
[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] Hexanoic acid, the metabolite of the component hexyl alcohol, and acetic acid are endogenous in humans.
[d] Evaluation postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

acids, which then undergo β-oxidation in the fatty acid and other well-known metabolic pathways.

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids, the Committee assigned all but one of the 67 esters to structural class I. 2-Ethylbutyl acetate contains a sterically hindered functional group and was therefore assigned to structural class II.

Step 2. At this step the evaluation of *cis*-3- and *trans*-2-hexenyl propionate was postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

The available data indicate that the remaining esters in this group would be hydrolysed in humans to their component alcohols and carboxylic acids. The aliphatic acyclic primary alcohols are oxidized to their corresponding carboxylic acids, which are either conjugated and excreted in the urine, or undergo β-oxidation and cleavage. The aliphatic linear saturated carboxylic acids are endogenous in humans. At the current levels of per capita intake these esters would not be expected to saturate the metabolic pathways. Therefore, the remaining 66 esters of aliphatic acyclic primary alcohols with aliphatic linear

saturated carboxylic acids were predicted to be metabolized to innocuous products. Accordingly, the Committee considered the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of all but one of the remaining 65 class I esters in this group in Europe and the USA were below the threshold for human intake (1800 μg). Only hexyl acetate had an estimated daily per capita intake greater than the threshold for class I. The estimated daily per capita intake of 2-ethylbutyl acetate in Europe and the USA was below the threshold for human intake for class II (540 μg).

Step A4. This step was considered only for hexyl acetate which was the only substance in this group for which the estimated level of intake exceeded the threshold for class I. The component hexyl alcohol is oxidized to hexanoic acid which is endogenous as it is an intermediary metabolite in the fatty acid pathway, and acetate is a component of the tricarboxylic acid cycle. In the opinion of the Committee the endogenous levels of these two metabolites would not give rise to perturbations outside the physiological range. Therefore, hexyl acetate was also determined to be of no safety concern on the basis of its structural class and known metabolism.

Table 5 summarizes the evaluation of the 67 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids used as flavouring substances.

Consideration of combined intakes

In the unlikely event that all foods containing all of the 66 esters evaluated as flavouring substances were consumed simultaneously on a daily basis, the estimated daily per capita intake in Europe and the USA would exceed the threshold for human intake for substances in class I. All the flavouring agents in this group of esters are expected to be metabolized via well-known biochemical pathways to innocuous metabolites and/or endogenous substances and in the opinion of the Committee the endogenous levels of these metabolites would not give rise to perturbations outside the physiological range. Accordingly, the combined intake of these substances was considered to be of no safety concern.

Conclusions

On the basis of the results of the Procedure, the Committee concluded that the 66 esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids evaluated pose no safety concern when used at the estimated levels of current intake as

flavouring agents. The evaluation of *cis*-3- and *trans*-2-hexenyl propionate was postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

No toxicity data were required for the application of the Procedure for this group of esters. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.7 Esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids

A safety evaluation of a group of 26 terpenoid esters used as flavouring agents (see Table 6) was conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

One member of the group, geranyl acetate (*trans*-3,7-dimethyl-2,6-octadien-1-yl acetate), was previously evaluated at the twenty-third meeting of the Committee (Annex 1, reference *50*). It was evaluated as part of a group of other terpenoid flavouring substances, citral, citronellol and linalool, which have close chemical, biochemical and toxicological relationships. The Committee allocated a group ADI of 0–0.5 mg/kg of body weight, expressed as citral, to these substances on the basis of their clearly defined metabolism and their low toxicity in short-term toxicity studies.

Intake data

The total annual volume of production of the 26 terpenoid esters used as flavouring agents is approximately 13 tonnes in Europe and 2 tonnes in the USA. In both Europe and the USA approximately 60% of the total annual volume is accounted for by the acetate and butyrate esters of citronellol, geraniol and nerol. On the basis of these annual volumes of production, the total estimated daily per capita intake of the 26 terpenoid esters used as flavouring substances is 1800 μg in Europe and 410 μg in the USA. The total estimated daily per capita intake of the terpenoid alcohols (i.e. citronellol, geraniol, nerol and rhodinol) formed via hydrolysis of these esters is 1400 μg in Europe and 320 μg in the USA.

Terpenoid esters are principal flavour components of citrus fruit and citrus peel oils, and have also been detected in a wide variety of other fruits, spices and vegetables. The terpenoid esters are usually found at concentrations of up to 1 mg/kg in citrus fruit juices, 20 g/kg in citrus

Table 6
Summary of the results of safety evaluations of 26 esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids[a]

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	Conclusion based on current levels of intake
Citronellyl formate	0053	No Europe: 103 USA: 2.5	
Geranyl formate	0054	No Europe: 330 USA: 48	
Neryl formate	0055	No Europe: 0.01 USA: 0.04	
Rhodinyl formate	0056	No Europe: ND USA: 0.10	
Citronellyl acetate	0057	No Europe: 217 USA: 36	
Geranyl acetate[c]	0058	No Europe: 580 USA: 205	
Neryl acetate	0059	No Europe: 180 USA: 63	
Rhodinyl acetate	0060	No Europe: 1.1 USA: 0.8	
Citronellyl propionate	0061	No Europe: 41 USA: 1.5	No safety concern
Geranyl propionate	0062	No Europe: 81 USA: 11	
Neryl propionate	0063	No Europe: 4.3 USA: 1.1	
Rhodinyl propionate	0064	No Europe: ND USA: 0.02	
Citronellyl butyrate	0065	No Europe: 32 USA: 5	
Geranyl butyrate	0066	No Europe: 60 USA: 25	
Neryl butyrate	0067	No Europe: 0.4 USA: 0.02	
Rhodinyl butyrate	0068	No Europe: ND USA: 1.0	

Table 6 (*continued*)

Substance	No.	*Step A3*[b] Does intake exceed the threshold for human intake?	Conclusion based on current levels of intake
Citronellyl valerate	0069	No Europe: 0.7 USA: 4.0	
Geranyl hexanoate	0070	No Europe: 0.07 USA: 0.5	
Citronellyl isobutyrate	0071	No Europe: 13 USA: 1.3	
Geranyl isobutyrate	0072	No Europe: 124 USA: 3.0	
Neryl isobutyrate	0073	No Europe: 2.0 USA: 0.4	
Rhodinyl isobutyrate	0074	No Europe: 0.03 USA: 0.04	No safety concern
Geranyl isovalerate	0075	No Europe: 43 USA: 1.7	
Neryl isovalerate	0076	No Europe: 0.03 USA: 0.04	
Rhodinyl isovalerate	0077	No Europe: 0.01 USA: 0.02	
Geranyl 2-ethylbutanoate	0078	No Europe: 0.6 USA: 0	

ND, no data reported.
[a] *Step 1*: All of the esters in this group are in structural class I.
Step 2: All of the esters in this group are metabolized to innocuous products.
[b] The threshold for human intake for class I is 1800 µg per day. All intake values are expressed in µg per day.
[c] The ADI for this substance was maintained.

peel oils and 50 g/kg in spices. In the USA terpenoid esters are consumed predominantly as components of traditional foods and the total annual volume of consumption of the most common terpenoid esters as natural components of food is estimated to be approximately 300 tonnes (Stofberg & Grundschober, personal communication, 1987).

Information on absorption, metabolism and elimination

The terpenoid esters are hydrolysed to their corresponding terpenoid alcohols (geraniol, citronellol, nerol and rhodinol) and aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, hexanoic,

isobutyric and isovaleric acids). Both the hydrolysis and the metabolism of aliphatic carboxylic acids are discussed on pages 26–27.

Following hydrolysis, the terpenoid alcohols undergo a complex pattern of alcohol oxidation, ω-oxidation, hydration, selective hydrogenation and subsequent conjugation to form oxygenated polar metabolites which are excreted primarily in the urine. Geraniol, related terpenoid alcohols (citronellol and nerol) and the aldehydes (geranial and neral) follow similar metabolic pathways in animals (Fig. 2).

Application of the Procedure

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned terpenoid esters, the Committee assigned all 26 esters to structural class I.

Step 2. The esters in this group are expected to be readily hydrolysed to their component alcohols and carboxylic acids, which are considered to be innocuous. The terpenoid alcohols are expected to undergo ω-oxidation and functional group oxidation to yield polar metabolites which are excreted as the glucuronic acid conjugate in the urine. Eight of the nine component carboxylic acids are endogenous in humans and are metabolized in the fatty acid β-oxidation pathway, amino acid pathways, the citric acid cycle or the C_1-tetrahydrofolate pathway to eventually yield carbon dioxide and water. The remaining carboxylic acid, 2-ethylbutyric acid, undergoes oxidation to polar metabolites which are conjugated with glucuronic acid and excreted. At current levels of intake these esters and their component terpenoid alcohols and aliphatic carboxylic acids would not be expected to saturate these metabolic pathways.

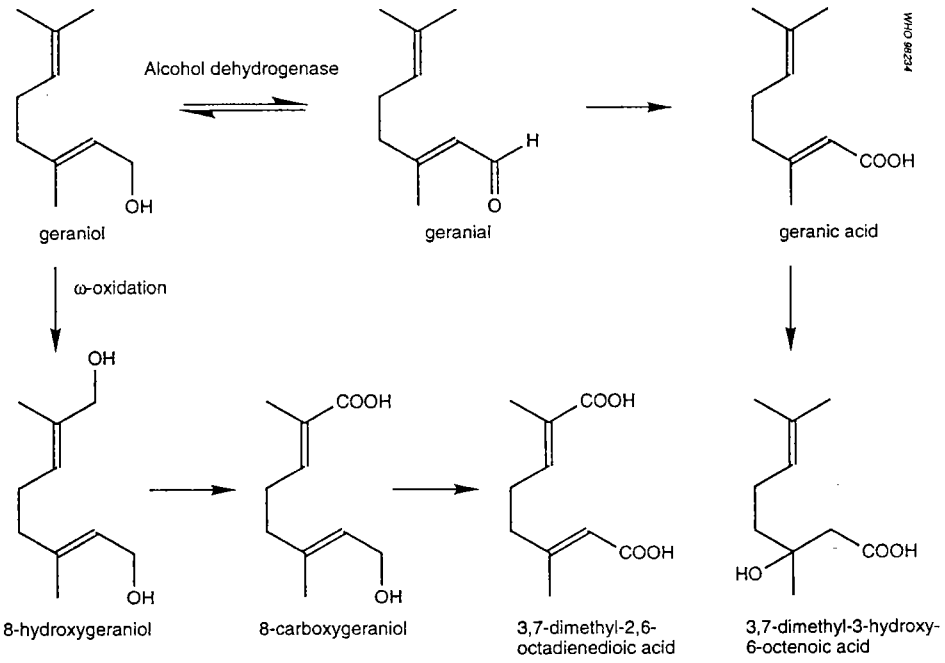
Step A3. The daily per capita intakes of each of the 26 terpenoid esters in both Europe and the USA are below the threshold for class I (1800 μg). Therefore none of the 26 terpenoid esters evaluated were considered to pose a safety concern when used at current levels of intake as flavouring substances.

Table 6 summarizes the evaluation of the 26 terpenoid esters used as flavouring substances.

Consideration of combined intake

In the unlikely event that all foods containing these 26 terpenoid esters used as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake would still be below the threshold for human intake for class I (1800 μg). The

Figure 2
Metabolism of geraniol in rats



Committee noted that the terpene alcohols, geraniol, citronellol and linalool, are used as flavouring agents and that the combined intakes of these alcohols and esters would be less than the group ADI.

Conclusions

The Committee concluded that the esters derived from branched-chain terpenoid alcohols and aliphatic acyclic linear and branched-chain carboxylic acids present no safety concern at the estimated levels of current intake.

No toxicity data were required for the application of the Procedure. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure.

The Committee noted that some of the esters are metabolized to α,β-unsaturated carbonyl compounds, but concluded that these had been adequately evaluated previously (Annex 1, reference *50*). The Committee maintained the group ADI of 0–0.5 mg/kg of body weight for geranyl acetate, citral, citronellol and linalool.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.8 Specifications for flavouring agents

The specifications for 173 flavouring agents on the modified agenda were reviewed. New specifications were prepared for 153 flavouring agents, 28 of which were designated as "tentative". The existing specifications for 11 flavouring agents reviewed toxicologically and for two flavouring agents reviewed for specifications only (see section 6) were revised. In addition, the existing specifications for the 52 flavouring agents in FAO Food and Nutrition Paper, No. 52, Add. 4 (Annex 1, reference *124*) were given identification numbers.

5. Contaminants: aflatoxins

Aflatoxins B_1, B_2, G_1 and G_2 are mycotoxins that may be produced by three species of *Aspergillus*: *A. flavus*, *A. parasiticus* and *A. nomius*, which contaminate plants and plant products. Aflatoxins M_1 and M_2, the hydroxylated metabolites of aflatoxins B_1 and B_2, may be found in milk or milk products obtained from livestock that have ingested contaminated feed. Of these six aflatoxins, aflatoxin B_1 is the most frequently present in contaminated samples and aflatoxins B_2, G_1 and G_2 are generally not reported in the absence of aflatoxin B_1. Most of the toxicological data relate to aflatoxin B_1. Intake of aflatoxins in the diet arises mainly from eating contaminated maize and groundnuts and their products.

The aflatoxins were evaluated at the thirty-first meeting of the Committee (Annex 1, reference *77*). At that time, the Committee considered aflatoxins to be potential human carcinogens. Sufficient information was not available to establish a figure for a tolerable level of intake. The Committee urged that the intake of dietary aflatoxins be reduced to the lowest practicable levels so as to reduce the potential risk as far as possible. A working group convened by the International Agency for Research on Cancer also concluded that naturally occurring aflatoxins are carcinogenic to humans (*12*).

At its forty-sixth meeting (Annex 1, reference *122*), the Committee considered estimates of the carcinogenic potency of aflatoxins and the potential risks associated with their intake. In view of the value of such estimates, the Committee recommended that this task be continued at its next meeting and that a monograph be published summarizing the data and analyses.

At its present meeting, the Committee reviewed a wide range of studies in both animals and humans that provided qualitative and quantitative information on the hepatocarcinogenicity of aflatoxins. The Committee evaluated the potency of these contaminants, linked these potencies to intake estimates and discussed the impact of hypothetical standards on sample populations and their overall risks.

Carcinogenicity

The aflatoxins are among the most potent mutagenic and carcinogenic substances known. Extensive experimental evidence from test species shows that aflatoxins are capable of inducing liver cancer in most animal species studied. In addition, most epidemiological studies show a correlation between exposure to aflatoxin B_1 and an increased incidence of liver cancer. Aflatoxins are metabolized in humans and test species to the corresponding epoxide which is usually considered to be the ultimate reactive intermediate. There is some evidence suggesting that humans are at substantially lower risk from exposure to aflatoxins than test species. The Committee was aware of epidemiological studies which suggest that intake of aflatoxins poses no detectable independent risk and of studies which suggest that they pose risks only in the presence of other risk factors such as hepatitis B infection. Several current studies are likely to allow more accurate estimates of the risks to humans from the intake of aflatoxins, most notably cohort studies in China (Qidong and Shanghai) and Thailand and hepatitis B vaccination trials in China (Qidong and Taiwan) and the Gambia. When these studies are complete, the Committee may wish to re-evaluate the risks of aflatoxins in humans.

A number of factors influence the risk of primary liver cancer, most notably carriage of hepatitis B virus as determined by the presence in serum of the hepatitis B surface antigen (presence denoted $HBsAg^+$ and absence denoted $HbsAg^-$). The potency of aflatoxin B_1 appears to be significantly enhanced in individuals with simultaneous hepatitis B infection. This interaction makes it difficult to interpret the epidemiological studies to determine the extent to which aflatoxins act as independent risk factors. The conclusions of the Committee regarding the carcinogenic potency of aflatoxins are therefore contingent upon the dynamics of hepatitis B infection in a human population.

The identification of hepatitis C virus is an important recent advance in understanding the etiology of liver cancer. Two studies have inves-

tigated interactions between hepatitis C infection, aflatoxins and liver cancer, but the results so far are inconclusive. It is estimated that between 50 and 100% of cases of liver cancer are associated with persistent infection with hepatitis B and/or hepatitis C.

The Committee considered that the weight of scientific evidence, which includes epidemiological data, studies in laboratory animals and *in vivo* and *in vitro* studies of metabolism, supports a conclusion that aflatoxins should be treated as carcinogenic food contaminants, the intake of which should be reduced to levels as low as reasonably achievable.

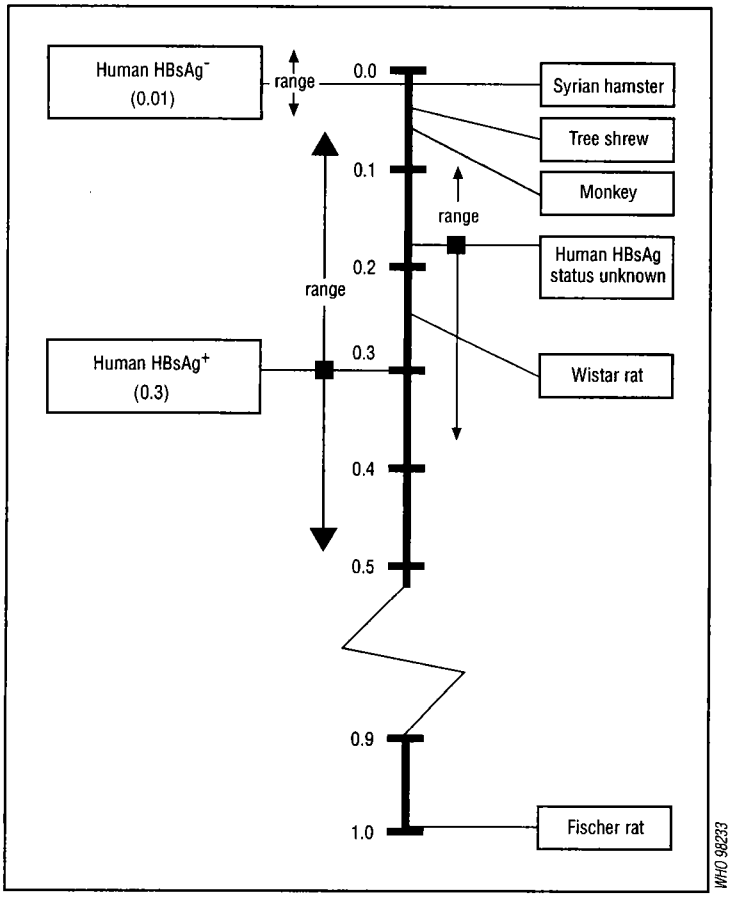
Carcinogenic potency

The Committee reviewed dose–response analyses that have been performed on aflatoxins. All of these analyses have limitations, three of which predominate. First, all of the epidemiological data from which a dose–response relationship can be determined are confounded by concurrent infection with hepatitis B. The epidemiological data are from geographical areas where both the prevalence of $HBsAg^+$ individuals and of contamination with aflatoxins are high; the relationship between these risk factors in areas in which aflatoxin contamination and prevalence of hepatitis B are low is unknown. Second, the reliability and precision of the estimates of exposure to aflatoxins in the relevant study populations are unknown. For example, aflatoxin biomarkers in humans do not reflect long-term intake of aflatoxins; analyses of crops for aflatoxins do not reflect the levels of aflatoxins consumed in foods after selection and processing. Finally, the shape of the dose–response curve is unknown and this introduces an additional element of uncertainty when choosing mathematical models for interpolation.

Observations concerning the interaction between hepatitis B infection and aflatoxins suggest two separate aflatoxin potencies; one is apparent in populations in which chronic hepatitis infections are common, the other in populations in which chronic hepatitis infections are rare. In analyses based on toxicological and epidemiological data, potency estimates for aflatoxins were divided into two basic groups, potencies applicable to individuals without hepatitis B infection and those applicable to individuals with chronic hepatitis B infection. The Committee found these estimates useful even though, through the use of differing mathematical models, they covered a broad range of possible values (Fig. 3). Potencies calculated from epidemiological studies in which hepatitis B infection status was unknown were in the range of potencies found in individuals with and without hepatitis B infection. The review also considered the extrapolation of animal data

Figure 3

Potency estimates for human liver cancer resulting from exposure to aflatoxin B_1 (cases per year/100 000 people per ng of aflatoxin B_1/kg of body weight per day), based on epidemiological and toxicological studies



HBsAg⁺: Hepatitis B surface antigen detected in serum; HBsAg⁻: hepatitis B surface antigen not detected in serum.

to estimate potency in humans; these estimates also generally fell within the range of the potency estimates derived from the epidemiological data.

There are several potential biases in the potency estimates depicted in Fig. 3:

- Only studies showing a positive association between aflatoxins and liver cancer were used as opposed to considering all studies (negative as well as positive); this leads to overestimation of the aflatoxin potency.

- When current levels of intake (i.e. using biomarkers or dietary surveys) are related to current levels of liver cancer (which presumably has a long induction period), historical levels of intake are ignored, and since intakes are likely to have been higher in the past, aflatoxin potency will be overestimated.
- The earliest studies systematically underestimated the prevalence of hepatitis B infection in patients with liver cancer by as much as 20–30% due to limitations in the methodology used to detect hepatitis B virus, which also leads to an overestimate of the relative potency of any other factor, including aflatoxins.
- Histological confirmation of the cases of liver cancer is limited in most epidemiological studies, allowing the possibility that cases of non-primary liver cancer have been included, which could lead to an underestimation or overestimation of the aflatoxin potency.

When these biases are taken into account, the values in Fig. 3 should be viewed as overestimates of the potency of the aflatoxins, leading to the hypothesis that it is possible that humans are in fact less sensitive to aflatoxins than the animal species tested in laboratory experiments.

The Committee reviewed the extensive data available on the metabolism of aflatoxins in various species. It was agreed that differences in the carcinogenic potency of aflatoxins between species can be partially attributed to differences in metabolism. However, there was insufficient quantitative information available about competing aspects of metabolic activation and detoxification of aflatoxin B_1 in various species to identify an adequate animal model for humans and to explain the apparent differences in potency between species.

The intake assessments used in many of the epidemiological studies ignored the contributions to total aflatoxin intake made by milk and milk products. Thus, the potencies shown in Fig. 3 do not generally apply to aflatoxin M_1. The results of a comparative toxicity study in rats suggest that aflatoxin M_1 has a potency approximately one order of magnitude less than that of aflatoxin B_1 in this species.

The Committee reviewed the potency estimates from the epidemiological studies which showed a positive association between aflatoxins and liver cancer and chose separate potency estimates and ranges for $HBsAg^+$ and $HBsAg^-$ individuals. Potency values of 0.3 cancers per year/100 000 population per ng of aflatoxins/kg of body weight per day (range 0.05–0.5) for $HBsAg^+$ individuals and of 0.01 cancers per year/100 000 population per ng of aflatoxins/kg of body weight per day (range 0.002–0.03) in $HBsAg^-$ individuals were chosen.

Population risks

The fraction of the incidence of liver cancer in a population attributable to intake of aflatoxins was derived by combining estimates of aflatoxin potency (risk per unit dose) and estimates of aflatoxin intake (dose per person). The Committee reviewed the frequency and amount of aflatoxin contamination in a variety of products (e.g. groundnuts, cereals and maize) in numerous countries. Many of the data on levels of aflatoxin contamination were derived from non-random samples which appeared to be biased upwards because monitoring studies focus on products that are thought to be contaminated. Some of the data on levels of contamination are not likely to be based on current Codex Alimentarius Commission sampling recommendations for aflatoxins. Accordingly, data on levels of contamination should be interpreted with caution and used only to infer patterns of importance in setting standards and not to provide exact contamination estimates.

The Committee considered the possible impact of applying hypothetical standards to aflatoxin contamination. It noted that the magnitude of the difference between two hypothetical standards is substantially larger than the magnitude of the difference in the mean contamination levels resulting from application of the separate standards. For example, in the case of maize from the USA, which has a mean level of aflatoxin contamination of 4.7 μg/kg, application of a hypothetical standard of 20 μg/kg would result in rejection of 3.9% of the crop and a mean level of aflatoxin contamination of 0.9 μg/kg. Imposition of a stricter hypothetical standard of 10 μg/kg would result in rejection of 6.2% of the crop and reduce the mean level of aflatoxin contamination by 0.3 μg/kg to 0.6 μg/kg (Table 7). Similar results would be obtained if aflatoxin B_1 levels in maize and total aflatoxins or aflatoxin B levels in groundnuts were examined.

Using the Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme regional diets combined with data on levels of aflatoxin contamination, the Committee was able to provide relative estimates of the mean dietary intake of aflatoxins for various regions. If these intakes are linked to the potency estimates shown in Fig. 3, it is possible to calculate the overall population risks on the basis of the prevalence of hepatitis B infection in various regions.

From its analysis the Committee noted that the application of a standard would prevent human consumption of the most highly contaminated samples, thus greatly reducing average estimated intakes of aflatoxins. The use of standards by all countries should be encour-

Table 7
Distribution of total aflatoxin contamination in maize from the USA and comparison of the impact of two different hypothetical standards on the percentage of samples rejected and the mean contamination level

Concentration of total aflatoxins in maize (µg/kg)	Percentile	Impact of hypothetical standard on percentage of samples rejected and mean contamination level[a]
0.1	10.0	
0.2	30.0	
0.3	50.0	
0.4	70.0	
0.5	80.0	
1.0	88.0	
5.0	90.6	
10	93.8	6.2% of samples rejected at a standard of 10 µg/kg (mean contamination level = 0.6 µg/kg)
15	95.0	
20	96.1	3.9% of samples rejected at a standard of 20 µg/kg (mean contamination level = 0.9 µg/kg)
30	96.8	
40	97.6	
50	98.0	

[a] The mean level of aflatoxin contamination with no standard was 4.7 µg/kg.

aged. The Committee considered the effect of modifying a given standard through the use of several hypothetical calculations. Two illustrations are given below.

The first example pertains to areas in which the level of contamination of food by aflatoxins is low and the proportion of the population who are $HBsAg^+$ is small. For this purpose, monitoring data from Europe on aflatoxin B_1 levels in groundnuts, maize[1] and their products were used. In this example, 1% of the population were assumed to be $HBsAg^+$. From the potency estimates given earlier (see Fig. 3), this yields an estimated potency for this population of $0.01 \times 99\% + 0.3 \times 1\% = 0.013$ cancers per year/100 000 people per ng of aflatoxins/kg of body weight per day (range 0.002–0.035). If it is assumed that all samples with levels of contamination above 20 µg/kg are removed and that these foods are ingested according to the "European diet", the mean estimated intake of aflatoxins is 19 ng per person per day. Assuming that an adult human weighs 60 kg, the estimated population

[1] The Committee noted that the aflatoxin data for Europe were for "all cereals". However, in these calculations, it was assumed that the aflatoxin level for "all cereals" applied to maize only.

risk (potency × intake) is 0.0041 cancers per year per 100000 people (range 0.0006–0.01). In contrast, if the same assumptions are used but a hypothetical standard of 10 μg/kg is applied, the average aflatoxin intake is 18 ng per person per day, resulting in an estimated population risk of 0.0039 cancers per year per 100000 people (range 0.0006–0.01). Thus, reducing the hypothetical standard from 20 μg/kg to 10 μg/kg yields a reduction in the estimated population risk of approximately two cancers per year per 1000 million people.

The second example pertains to areas with higher levels of aflatoxin contamination (for these purposes monitoring data from China on aflatoxin B_1 levels in groundnuts, maize and their products were used) and where a larger percentage of the population are carriers (in this case, 25% were assumed to be $HBsAg^+$). The estimated potency for this population is $0.01 \times 75\% + 0.3 \times 25\% = 0.083$ cancers per year per ng of aflatoxins ingested/kg of body weight per day (range 0.014–0.15). If a hypothetical standard of 20 μg/kg and the "Far Eastern diet" are used, the average estimated intake is 125 ng per person per day, yielding an average population risk of 0.17 cancers per year per 100000 people (range 0.03–0.3). If a hypothetical standard of 10 μg/kg is used, the average estimated intake falls to 103 ng per person per day, yielding an estimated population risk of 0.14 cancers per year per 100000 people (range 0.02–0.3). Thus, reducing the hypothetical standard for this population from 20 μg/kg to 10 μg/kg yields a reduction in the estimated population risk of 300 cancers per year per 1000 million people.

Conclusions

1. Aflatoxins are considered to be human liver carcinogens. Aflatoxin B_1 is the most potent carcinogen of the aflatoxins and most of the toxicological data available are related to aflatoxin B_1. Aflatoxin M_1, the hydroxylated metabolite of B_1, has a potency approximately one order of magnitude less than that of B_1.
2. The potency of aflatoxins in $HBsAg^+$ individuals is substantially higher than in $HBsAg^-$ individuals. Thus, reduction of the intake of aflatoxins in populations with a high prevalence of $HBsAg^+$ individuals will result in a greater reduction in liver cancer rates than reduction of the intake of aflatoxins in populations with a low prevalence of $HBsAg^+$ individuals.
3. Vaccination against hepatitis B will reduce the number of carriers of the virus. The present analysis suggests that reducing the number of carriers would reduce the potency of the aflatoxins in vaccinated populations and consequently reduce the risk of liver cancer.

4. Analyses of the application of hypothetical standards for aflatoxin contamination in food (10 μg/kg or 20 μg/kg) to model populations indicates that:
   (a) populations in which the prevalence of $HBsAg^+$ individuals is low and/or in which the mean intake of aflatoxins is low (less than 1 ng/kg of body weight per day) are unlikely to exhibit detectable[1] differences in population risks;
   (b) populations in which both the prevalence of $HBsAg^+$ and the intake of aflatoxins are high would benefit from reductions in aflatoxin intake.
5. The Committee has previously noted that reductions in the intake of aflatoxins can be achieved through avoidance measures such as improved farming and proper storage practices and/or through enforcing standards for levels of contamination in food or feed within countries and across borders (Annex 1, reference 77).
6. When two alternative standards for aflatoxin contamination in food are being considered, if the fraction of the samples excluded under the two standards is similar, the higher standard will yield essentially the same risk of liver cancer as the lower standard. When a substantial fraction of the current food supply is heavily contaminated with aflatoxins, reducing the levels of contamination may result in a detectable[1] reduction in rates of liver cancer. Conversely, when only a small fraction of the current food supply is heavily contaminated, reducing the standard by an apparently substantial amount may have little appreciable effect on health.

6. Revision of certain specifications

A total of 40 substances were examined for specifications only (see Annex 2), and the specifications for 30 were revised.

The specifications for agar, carthamus yellow, microcrystalline wax, propylene glycol, propylene glycol alginate and gellan gum were revised, with minor changes.

The specifications for alginic acid, ammonium alginate, calcium alginate, potassium alginate and sodium alginate were revised and the requirements for relative molecular mass range were changed in

[1] In the context of this statement "detectable" refers to an aflatoxin-induced change in liver cancer rates which exceeds the year-to-year variability around the current incidence and mortality rates. Hence "detectable" refers to the ability to observe a significant effect on the occurrence of liver cancer following intervention and will depend on the quality of the data available on historical trends in incidence and mortality.

order to reflect more precisely the products on the market. Some minor changes were also made.

The specifications for aluminium powder, mixed carotenoids, ethyl hydroxyethyl cellulose, propylene glycol esters of fatty acids and talc were revised and the "tentative" qualifications were deleted.

The specifications for citric acid, calcium propionate (calcium propanoate), potassium propionate (potassium propanoate) and sodium propionate (sodium propanoate) were revised, with minor changes. Citric acid was also added to the section on flavouring agents in the specifications monograph.

The specifications for propionic acid used as a preservative and an antimould and antirope agent were revised and designated as "tentative". The Committee requested information on the method of analysis and levels of readily oxidizable substances.

The Committee agreed to revise the specifications for modified starches to include enzyme treatment as an alternative method for modifying food starches. In addition, the Committee agreed to delete the minimum and maximum levels of reagents used in the processing of the starches since the end-product specifications were deemed sufficient to assure the quality and safety of the various modified starches. As a result of these recommendations, the existing tentative monograph for enzyme-treated starches was withdrawn.

The specifications for allyl cyclohexane propionate, ethyl octanoate, ethyl nonanoate, isoamyl acetate, isoamyl butyrate, isoamyl isobutyrate and isoamyl isovalerate used as flavouring agents were reviewed and given identification numbers. The specifications for isoamyl acetate used as a carrier solvent were revised in order to reflect that the substance is a mixture.

The existing tentative specifications for carbon dioxide and sulfur dioxide were revised in order to define purer products and the "tentative" designation was deleted.

The specifications for gum arabic obtained from certain species of acacia trees were last revised at the forty-fourth meeting of the Committee (Annex 1, reference *116*). In revising the specifications for gum arabic at its present meeting, the Committee considered two new extensive project reports from FAO (*13*, *14*) on the production, marketing and physicochemical characterization of this substance. The reports demonstrated that chemometric analysis of analytical compositional data is a suitable procedure for evaluating the chemical rela-

tionship of gums obtained from various acacia species. In the light of these reports, the Committee reiterated that gum from other acacia species closely related to *Acacia senegal* and meeting the newly revised specifications would adequately reflect the materials that had been toxicologically evaluated. The specifications were revised to make a clear distinction between gum arabic obtained from *A. seyal* and from *A. senegal.*

New specifications for enzyme-hydrolysed sodium carboxymethyl cellulose were prepared and designated as "tentative". The Committee requested information on the physical state of the substance as manufactured, the nature and proportion of material of low relative molecular mass present in the substance, and tests that distinguish the substance from sodium carboxymethyl cellulose.

The existing tentative specifications for carthamus red were revised and the "tentative" designation was maintained. The Committee requested information on the content of carthamin and method of assay.

The existing tentative specifications for petroleum jelly were revised, with minor corrections, and the "tentative" designation was maintained. The Committee requested information on the method of analysis and levels of viscosity at 100 °C, carbon number at 5% distillation point, average relative molecular mass and oil content. Unless this information was received by 31 March 1998, the specifications would be withdrawn.

The Committee compared the specifications for "diacetyltartaric and fatty acid esters of glycerol" (DATEM) with the existing tentative specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and noted that the substances appear very similar. Both substances were toxicologically evaluated at the seventeenth meeting of the Committee in 1973 (Annex 1, reference *32*). At that meeting, DATEM was assigned an ADI of 0–50 mg/kg of body weight, whereas the mixed tartaric, acetic and fatty acids of glycerol were assigned an ADI "not limited". The Committee has not received information which would allow these substances to be distinguished analytically. The Committee decided to maintain the tentative status of the existing specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and requested data that would distinguish this substance from DATEM. Unless such data were provided by 31 March 1998, the Committee would consider combining the two specifications. The existing specifications of DATEM were revised.

The Committee withdrew the tentative specifications for anoxomer since none of the information requested at its twenty-sixth meeting (Annex 1, reference *59*) has been submitted. The Committee was informed that anoxomer has been neither produced nor used for many years.

The specifications for turmeric as a food colour were on the agenda for revision. However, as the use of this substance was considered to be as a spice and not as a food colour, the Committee decided to withdraw the specifications.

7. Recommendations

1. In view of the large number of food additives, food ingredients 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. One of the substances referred to the present meeting for evaluation is an example of materials that have been defined as "novel foods" by some countries and organizations. The Committee recommended that FAO and WHO arrange at future meetings of the Committee for the review of procedures outlined in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*) for the safety evaluation of these types of products and for the development of guidelines for assessing their safety and wholesomeness, taking into account guidelines developed by other organizations.

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Annex 1

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101. *Evaluation of certain food additives and naturally occurring toxicants* (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 828, 1992.
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103. *Compendium of food additive specifications, addendum 1.* FAO Food and Nutrition Paper, No. 52, Add. 1, 1992.
104. *Evaluation of certain veterinary drug residues in food* (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 31, 1993.
106. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/5, 1993.
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108. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 32, 1993.
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111. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 33, 1994.
112. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/6, 1994.
113. *Evaluation of certain veterinary drug residues in food* (Forty-third report of the

Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 34, 1995.
115. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/7, 1995.
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117. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 35, 1996.
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120. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 36, 1996.
121. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/8, 1996.
122. *Evaluation of certain food additives and contaminants* (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 37, 1996.
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126. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 38, 1996.
127. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/9, 1997.
128. *Evaluation of certain veterinary drug residues in food* (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 39, 1997.
130. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/10, 1998.

Annex 2

Acceptable Daily Intakes, other toxicological information, and information on specifications

Substance	Specifications[a]	Acceptable Daily Intake (ADI) in mg/kg of body weight and other toxicological recommendations
Antioxidant		
tert-Butylhydroquinone (TBHQ)	R	0–0.7
Emulsifiers		
Microcrystalline cellulose	R	Not specified[b, c]
Sucrose esters of fatty acids and sucroglycerides	R	0–30 (group ADI)
Enzyme preparations		
α-Acetolactate decarboxylase	N, T	Not specified (temporary)[b, d]
Maltogenic amylase	N, T	Not specified (temporary)[b, d]
Flavouring agent		
trans-Anethole	R	0–0.6 (temporary)[e]
Glazing agent		
Hydrogenated poly-1-decene	N	No ADI allocated because insufficient data were available
Sweetening agent		
Maltitol syrup	R	Not specified[b, c]
Miscellaneous substance		
Salatrim (short- and long-chain acyltriglyceride molecules)	N	Adequate information was not available to evaluate its safety and nutritional effects

[a] N, new specifications prepared; R, existing specifications revised; 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] This ADI applies to the substance conforming to the revised specifications.

[d] Temporary ADI, pending consideration of the "tentative" qualification of the specifications. The "tentative" qualification of 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* (Rome, Food and Agriculture Organization of the United Nations, 1992) will be reviewed in 1998.

[e] Temporary ADI extended to 1998, pending the submission of the results of studies currently underway that were requested at earlier meetings of the Committee.

Flavouring agents

The substances listed here were evaluated using the Procedure for the 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 levels of intake
Allyl ester			
Allyl 2-furoate (2-propenyl furan-2-carboxylate)	0021	S, T	No safety concern
Saturated aliphatic acyclic linear primary alcohols, aldehydes and acids			
Formic acid[c]	0079	R	
Acetaldehyde (ethanal)	0080	N	
Acetic acid[c]	0081	R	
Propyl alcohol (1-propanol)	0082	R	
Propionaldehyde (propanal)	0083	N	
Propionic acid[c] (propanoic acid)	0084	N	
Butyl alcohol (1-butanol)	0085	R	
Butyraldehyde (butanal)	0086	N	
Butyric acid (butanoic acid)	0087	N	
Amyl alcohol (1-pentanol)	0088	N	
Valeraldehyde (pentanal)	0089	N	
Valeric acid (pentanoic acid)	0090	N	
Hexyl alcohol (1-hexanol)	0091	N	
Hexanal	0092	N	
Hexanoic acid	0093	N	
Heptyl alcohol (1-heptanol)	0094	N	
Heptanal	0095	N	
Heptanoic acid	0096	N	
1-Octanol	0097	N	No safety concern
Octanal[c]	0098	R	
Octanoic acid	0099	N	
Nonyl alcohol (1-nonanol)	0100	N	
Nonanal[c]	0101	R	
Nonanoic acid	0102	N	
1-Decanol	0103	N	
Decanal	0104	R	
Decanoic acid	0105	N	
Undecyl alcohol (1-undecanol)	0106	N	
Undecanal	0107	N	
Undecanoic acid	0108	N	
Lauryl alcohol (1-dodecanol)	0109	N	
Lauric aldehyde (dodecanal)	0110	N	
Lauric acid (dodecanoic acid)	0111	N	
Myristaldehyde (tetradecanal)	0112	N	
Myristic acid (tetradecanoic acid)	0113	N	
1-Hexadecanol	0114	N	
Palmitic acid (hexadecanoic acid)	0115	N	
Stearic acid (octadecanoic acid)	0116	N	
Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
Structural class I: methyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
Isobutyl alcohol (2-methyl-1-propanol)	0251	NR	No safety concern
Isobutyraldehyde (2-methylpropanal)	0252	NR	
Isobutyric acid (2-methylpropanoic acid)	0253	NR	
2-Methylbutyraldehyde (2-methylbutanal)	0254	NR	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
2-Methylbutyric acid (2-methylbutanoic acid)	0255	NR	No safety concern
3-Methylbutyraldehyde (3-methylbutanal)	0258	NR	
Isovaleric acid (3-methylbutanoic acid)	0259	NR	
2-Methylpentanal	0260	NR	
2-Methylvaleric acid (2-methylpentanoic acid)	0261	NR	
3-Methylpentanoic acid	0262	NR	
3-Methyl-1-pentanol	0263	NR	
4-Methylpentanoic acid	0264	NR	
2-Methylhexanoic acid	0265	NR	
5-Methylhexanoic acid	0266	NR	
3,5,5-Trimethyl-1-hexanol	0268	NR	
3,5,5-Trimethylhexanal	0269	NR	
2-Methyloctanal	0270	NR	
4-Methyloctanoic acid	0271	NR	
3,7-Dimethyl-1-octanol	0272	NR	
2,6-Dimethyloctanal	0273	NR	
4-Methylnonanoic acid	0274	NR	
2-Methylundecanal	0275	NR	
Structural class II: ethyl-substituted saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids			
2-Ethylbutyraldehyde (2-ethylbutanal)	0256	NR	No safety concern
2-Ethylbutyric acid (2-ethylbutanoic acid)	0257	NR	
2-Ethyl-1-hexanol[c]	0267	NR	
Aliphatic lactones			
Structural class I			
4-Hydroxybutyric acid lactone (γ-butyrolactone)	0219	NR	No safety concern
γ-Valerolactone	0220	NR	
γ-Hexalactone	0223	NR	
δ-Hexalactone	0224	NR	
γ-Heptalactone	0225	NR	
γ-Octalactone	0226	NR	
δ-Octalactone	0228	NR	
γ-Nonalactone[c]	0229	NR	
Hydroxynonanoic acid δ-lactone	0230	NR	
γ-Decalactone	0231	NR	
δ-Decalactone	0232	NR	
ε-Decalactone	0241	NR	
γ-Undecalactone[c]	0233	NR	
5-Hydroxyundecanoic acid δ-lactone	0234	NR	
γ-Dodecalactone	0235	NR	
δ-Dodecalactone	0236	NR	
ε-Dodecalactone	0242	NR	
δ-Tetradecalactone	0238	NR	
ω-Pentadecalactone	0239	NR	
4-Hydroxy-3-pentenoic acid lactone	0221	NR	
5-Hydroxy-7-decenoic acid δ-lactone	0247	NR	
5-Hydroxy-8-undecenoic acid δ-lactone	0248	NR	
1,4-Dodec-6-enolactone	0249	NR	
ω-6-Hexadecenlactone	0240	NR	
4,4-Dibutyl-γ-butyrolactone	0227	NR	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
3-Heptyldihydro-5-methyl-2(3*H*)-furanone	0244	NR	No safety concern
4-Hydroxy-3-methyloctanoic acid γ-lactone	0437	NR	
6-Hydroxy-3,7-dimethyloctanoic acid lactone	0237	NR	
γ-Methyldecalactone	0250	NR	
Structural class III			
5-Hydroxy-2-decenoic acid δ-lactone	0246	NR	
5-Hydroxy-2,4-decadienoic acid δ-lactone	0245	NR	
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	NR	Not evaluated[d]
5-Hydroxy-2-dodecenoic acid δ-lactone	0438	NR	
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	0222	NR	No safety concern
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	0243	NR	
Esters of aliphatic acyclic primary alcohols with branched-chain aliphatic acyclic acids			
Methyl isobutyrate (methyl 2-methylpropanoate)	0185	N	No safety concern
Ethyl isobutyrate (ethyl 2-methylpropanoate)	0186	N	
Propyl isobutyrate (*n*-propyl 2-methylpropanoate)	0187	N	
Butyl isobutyrate (butyl 2-methylpropanoate)	0188	N	
Hexyl isobutyrate (hexyl 2-methylpropanoate)	0189	N	
Heptyl isobutyrate (heptyl 2-methylpropanoate)	0190	N	
trans-3-Heptenyl 2-methylpropanoate	0191	N	
Octyl isobutyrate (octyl 2-methylpropanoate)	0192	N	
Dodecyl isobutyrate (dodecyl 2-methylpropanoate)	0193	N	
Isobutyl isobutyrate (2-methylpropyl 2-methylpropanoate)	0194	N	
Methyl isovalerate (methyl 3-methylbutanoate)	0195	N	
Ethyl isovalerate (ethyl 3-methylbutanoate)	0196	R	
Propyl isovalerate (propyl 3-methylbutanoate)	0197	N	
Butyl isovalerate (butyl 3-methylbutanoate)	0198	N	
Hexyl 3-methylbutanoate	0199	N	
Octyl isovalerate (octyl 3-methylbutanoate)	0200	N	
Nonyl isovalerate (nonyl 3-methylbutanoate)	0201	N	
3-Hexenyl 3-methylbutanoate	0202	N	
2-Methylpropyl 3-methylbutyrate (2-methylpropyl 3-methylbutanoate)	0203	N	
2-Methylbutyl 3-methylbutanoate	0204	N, T	
Methyl 2-methylbutyrate (methyl 2-methylbutanoate)	0205	N	
Ethyl 2-methylbutyrate (ethyl 2-methylbutanoate)	0206	N	
n-Butyl 2-methylbutyrate (butyl 2-methylbutanoate)	0207	N	
Hexyl 2-methylbutanoate	0208	N	
Octyl 2-methylbutyrate (octyl 2-methylbutanoate)	0209	N	
Isopropyl 2-methylbutyrate (1-methylethyl 2-methylbutanoate)	0210	N, T	
3-Hexenyl 2-methylbutanoate	0211	N, T	
2-Methylbutyl 2-methylbutyrate (2-methylbutyl 2-methylbutanoate)	0212	N	
Methyl 2-methylpentanoate	0213	N, T	
Ethyl 2-methylpentanoate	0214	N	
Ethyl 3-methylpentanoate	0215	N, T	
Methyl 4-methylvalerate	0216	N	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids			
Propyl formate	0117	N	No safety concern
Butyl formate	0118	N	
n-Amyl formate (pentyl formate)	0119	N	
Hexyl formate	0120	N	
Heptyl formate	0121	N, T	
Octyl formate	0122	N	
cis-3-Hexenyl formate (*cis*-3-hexenyl-1-yl formate)	0123	N	
Isobutyl formate (2-methylpropyl formate)	0124	N	
Methyl acetate	0125	N	
Propyl acetate	0126	N	
Butyl acetate	0127	R	
Hexyl acetate	0128	N	
Heptyl acetate	0129	N	
Octyl acetate	0130	N	
Nonyl acetate	0131	N	
Decyl acetate	0132	N	
Lauryl acetate (dodecyl acetate)	0133	N	
cis-3-Hexenyl acetate (*cis*-3-hexenyl-1-yl acetate)	0134	N	
trans-3-Heptenyl acetate (*trans*-3-heptenyl-1-yl acetate)	0135	N	
10-Undecen-1-yl acetate	0136	N	
Isobutyl acetate (2-methylpropyl acetate)	0137	N	
2-Methylbutyl acetate (2-methyl-1-butyl acetate)	0138	N	
2-Ethylbutyl acetate	0140	N, T	
Methyl propionate (methyl propanoate)	0141	N	
Propyl propionate (*n*-propyl propanoate)	0142	N	
Butyl propionate (butyl propanoate)	0143	N	
Hexyl propionate (*n*-hexyl propanoate)	0144	N	
Octyl propionate (octyl propanoate)	0145	N	
Decyl propionate (decyl propanoate)	0146	N	
cis-3- and *trans*-2-Hexenyl propionate (*cis*-3- and *trans*-2-hexenyl propanoate)	0147	N	Not evaluated[e]
Isobutyl propionate (2-methylpropyl propanoate)	0148	N	No safety concern
Methyl butyrate (methyl butanoate)	0149	N	
Propyl butyrate (*n*-propyl butanoate)	0150	N	
Butyl butyrate (*n*-butyl *n*-butanoate)	0151	N	
n-Amyl butyrate (pentyl butanoate)	0152	N	
Hexyl butyrate (hexyl butanoate)	0153	N	
Heptyl butyrate (heptyl butanoate)	0154	N, T	
Octyl butyrate (octyl butanoate)	0155	N, T	
Decyl butyrate (decyl butanoate)	0156	N, T	
cis-3-Hexenyl butyrate (*cis*-3-hexen-1-yl butanoate)	0157	N	
Isobutyl butyrate (2-methylpropyl butanoate)	0158	N	
Methyl valerate (methyl pentanoate)	0159	N	
Butyl valerate (butyl pentanoate)	0160	N	
Propyl hexanoate	0161	N	
Butyl hexanoate	0162	N	
n-Amyl hexanoate (pentyl hexanoate)	0163	N	
Hexyl hexanoate	0164	N	
cis-3-Hexenyl hexanoate (*cis*-3-hexen-1-yl hexanoate)	0165	N, T	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
Isobutyl hexanoate (2-methylpropyl hexanoate)	0166	N	No safety concern
Methyl heptanoate	0167	N	
Propyl heptanoate	0168	N, T	
Butyl heptanoate	0169	N, T	
n-Amyl heptanoate (pentyl heptanoate)	0170	N	
Octyl heptanoate	0171	N, T	
Isobutyl heptanoate (2-methylpropyl heptanoate)	0172	N, T	
Methyl octanoate	0173	N	
n-Amyl octanoate (pentyl octanoate)	0174	N	
Hexyl octanoate	0175	N	
Heptyl octanoate	0176	N, T	
Octyl octanoate	0177	N, T	
Nonyl octanoate	0178	N, T	
Methyl nonanoate	0179	N	
Methyl laurate (methyl dodecanoate)	0180	N	
Butyl laurate (butyl dodecanoate)	0181	N	
Isoamyl laurate (3-methylbutyl dodecanoate)	0182	N, T	
Methyl myristate (methyl *n*-tetradecanoate)	0183	N	
Butyl stearate (butyl octadecanoate)	0184	N, T	
Esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids			
Citronellyl formate (3,7-dimethyl-6-octen 1-yl formate)	0053	N	No safety concern
Geranyl formate (*trans*-3,7-dimethyl-2,6-octadien-1-yl formate)	0054	N	
Neryl formate (*cis*-3,7-dimethyl-2,6-octadien-1-yl formate)	0055	N	
Rhodinyl formate (3,7-dimethyl-7-octen-1-yl formate)	0056	N	
Citronellyl acetate (3,7-dimethyl-6-octen-1-yl acetate)	0057	N	
Geranyl acetate[c] (*trans*-3,7-dimethyl-2,6-octadien-1-yl acetate)	0058	R	
Neryl acetate (*cis*-3,7-dimethyl-2,6-octadien-1-yl acetate)	0059	N	
Rhodinyl acetate (3,7-dimethyl-7-octen-1-yl acetate)	0060	N	
Citronellyl propionate (3,7-dimethyl-6-octen-1-yl propanoate)	0061	N	
Geranyl propionate (*trans*-3,7-dimethyl-2,6-octadien-1-yl propanoate)	0062	N	
Neryl propionate (*cis*-3,7-dimethyl-2,6-octadien-1-yl propanoate)	0063	N	
Rhodinyl propionate (3,7-dimethyl-7-octen-1-yl propanoate)	0064	N, T	
Citronellyl butyrate (3,7-dimethyl-6-octen-1-yl butanoate)	0065	N	
Geranyl butyrate (*trans*-3,7-dimethyl-2,6-octadien-1-yl butanoate)	0066	N	
Neryl butyrate (*cis*-3,7-dimethyl-2,6-octadien-1-yl butanoate)	0067	N	

Substance[a]	No.	Specifications[b]	Conclusion based on current levels of intake
Rhodinyl butyrate (3,7-dimethyl-7-octen-1-yl butanoate)	0068	N	No safety concern
Citronellyl valerate (3,7-dimethyl-6-octen-1-yl pentanoate)	0069	N, T	
Geranyl hexanoate (*trans*-3,7-dimethyl-2,6-octadien-1-yl hexanoate)	0070	N, T	
Citronellyl isobutyrate (3,7-dimethyl-6-octen-1-yl 2-methyl propanoate)	0071	N	
Geranyl isobutyrate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 2-methyl propanoate)	0072	N, T	
Neryl isobutyrate (*cis*-3,7-dimethyl-2,6-octadien-1-yl 2-methyl propanoate)	0073	N	
Rhodinyl isobutyrate (3,7-dimethyl-7-octen-1-yl 2-methyl propanoate)	0074	N, T	
Geranyl isovalerate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 3-methyl butanoate)	0075	N, T	
Neryl isovalerate (*cis*-3,7-dimethyl-2,6-octadien-1-yl 2-methyl butanoate)	0076	N	
Rhodinyl isovalerate (3,7-dimethyl-7-octen-1-yl 3-methylbutanoate)	0077	N, T	
Geranyl 2-ethylbutanoate (*trans*-3,7-dimethyl-2,6-octadien-1-yl 2-ethylbutanoate)	0078	N, T	

[a] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 5, 1997). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.
[b] N, new specifications prepared; NR, specifications not reviewed; 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.
[c] The ADI for this substance was maintained.
[d] Evaluation postponed, pending consideration of other α,β-unsaturated compounds.
[e] Evaluation postponed, pending consideration of other α,β-unsaturated carbonyl compounds.

Contaminant	Toxicological recommendations
Aflatoxins	Potencies were estimated

Substance[a] (considered for specifications only)	No.	Specifications[b]
Flavouring agents		
Allyl cyclohexane propionate (2-propenyl cyclohexane propanoate)	0013	S
Ethyl octanoate	0033	S
Ethyl nonanoate	0034	S
Isoamyl acetate (3-methylbutyl acetate)	0043	R
Isoamyl butyrate (3-methylbutyl butanoate)	0045	S
Isoamyl isobutyrate (3-methylbutyl 2-methylpropanoate)	0049	S
Isoamyl isovalerate (3-methylbutyl 3-methylbutanoate)	0050	S
Food additives		
Agar	—	R
Alginic acid	—	R
Aluminium powder	—	R
Ammonium alginate	—	R

Substance[a] (considered for specifications only)	No.	Specifications[b]
Anoxomer	—	W
Calcium alginate	—	R
Calcium propionate (calcium propanoate)	—	R
Carbon dioxide	—	R
Carthamus red	—	R, T[c]
Carthamus yellow	—	R
Citric acid	—	R
Diacetyltartaric and fatty acid esters of glycerol (DATEM)	—	R
Enzyme-hydrolysed sodium carboxymethyl cellulose	—	N, T[d]
Enzyme-treated starches	—	W
Ethyl hydroxyethyl cellulose	—	R
Gellan gum	—	R
Gum arabic	—	R
Microcrystalline wax	—	R
Mixed carotenoids	—	R
Modified starches	—	R
Petroleum jelly	—	R, T[e]
Potassium alginate	—	R
Potassium propionate (potassium propanoate)	—	R
Propionic acid	—	R, T[f]
Propylene glycol	—	R
Propylene glycol alginate	—	R
Propylene glycol esters of fatty acids	—	R
Sodium alginate	—	R
Sodium propionate (sodium propanoate)	—	R
Sulfur dioxide	—	R
Talc	—	R
Tartaric, acetic and fatty acid esters of glycerol, mixed	—	R, T[g]
Turmeric	—	W

[a] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 5, 1997). 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; 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] The existing tentative specifications for carthamus red were revised and the "tentative" designation was maintained. The Committee requested information on the content of carthamin and method of assay.

[d] New specifications for enzyme-hydrolysed sodium carboxymethyl cellulose were prepared and designated as "tentative". The Committee requested information on the physical state of the substance as manufactured, the nature and proportion of material of low relative molecular mass present, and tests that distinguish it from sodium carboxymethyl cellulose.

[e] The existing tentative specifications for petroleum jelly were revised and the "tentative" designation was maintained The Committee requested information on the method of analysis and levels of viscosity at 100°C, carbon number at 5% distillation point, average relative molecular mass and oil content. Unless this information was received by 31 March 1998, the specifications would be withdrawn.

[f] The specifications for propionic acid used as a preservative and an antimould and antirope agent were revised and designated as "tentative". The Committee requested information on the method of analysis and levels of readily oxidizable substances.

[g] The Committee compared the specifications for "diacetyltartaric and fatty acid esters of glycerol" (DATEM) with the existing tentative specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and noted that the two substances appear very similar. The Committee has not received information which would allow these substances to be distinguished analytically. It therefore decided to maintain the tentative status of the existing specifications for "tartaric, acetic and fatty acid esters of glycerol, mixed" and requested data that would distinguish this substance from DATEM. Unless such data were provided by 31 March 1998, the Committee would consider combining the specifications for the two substances.

European Food Safety Authority

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Flavouring Group Evaluation 1, Revision 1 (FGE.01Rev 1): Branched-chain aliphatic saturated aldehydes, carboxylic acids and related esters of primary alcohols and branched-chain carboxylic acids from chemical groups 1 and 2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000)[1]

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

(Question No EFSA-Q-2008-033)

Adopted on 4 July 2007

PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Scientific Panel is asked to evaluate the 21 flavouring substances in this Flavouring Group 1 Evaluation, Revision 1 (FGE.01Rev1), using the procedure as referred to in the Commission

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission Flavouring Group Evaluation 1, Revision 1 (FGE.01Rev 1): Branched-chain aliphatic saturated aldehydes, carboxylic acids and related esters of primary alcohols and branched-chain carboxylic acids from chemical groups 1 and 2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal 617, 1-44.

* 1 member of the Panel did not participate in the discussion due to a declared interest. For details see the minutes of the 24thAFC Panel meeting : http://www.efsa.europa.eu/EFSA/ScientificPanels/AFC/efsa_locale-1178620753812_Meetings424.htm

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Regulation (EC) No 1565/2000. These 21 flavouring substances belong to chemical groups 1 and 2, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation deals with 21 branched-chain aliphatic acyclic saturated aldehydes, carboxylic acids and esters derived from aliphatic acyclic primary saturated alcohols and branched-chain aliphatic acyclic saturated carboxylic acids.

Fifteen of the flavouring substances possess a chiral centre. In three of these cases, the substance has been presented without indication whether the commercial flavouring substance was dominated by one of the chiral isomers.

All 21 flavouring substances are classified into structural class I.

Thirteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intakes(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, the 21 flavouring substances in this group have intakes in Europe from 0.0012 to 9.0 microgram/*capita*/day, which are below the threshold of concern value for structural class I substances of 1800 microgram/person/day.

Genotoxicity data are only available for one structurally related substance and the genotoxicity could therefore not be assessed adequately for the substances in the present group. However, the data available do not preclude evaluation of the flavouring substances in the present group using the Procedure.

All 21 flavouring substances in the present flavouring group are expected to be metabolised to innocuous products.

It was noted that where toxicity data were available, they were consistent with the conclusions in the present FGE using the Procedure.

On the basis of the default MSDI approach the Panel concluded that these 21 flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 1000 to 3500 microgram/person/day for 20 flavouring substances from structural class I. For one of the flavouring substances use levels were lacking and the mTAMDI could not be calculated. Except for six flavouring substances the intakes were above the threshold of concern for a structural class I

substance of 1800 microgram/person/day. The six substances which have mTAMDI intake estimates below the threshold of concern for structural class I, are also expected to be metabolised to innocuous products.

Thus, for 14 of the 21 flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these 14 substances, as well as for the one substance [FL-no: 05.219], for which use levels are missing, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for 19 of the 21 flavouring candidate substances, except that information on geometrical isomerism/chirality is missing for three of the substances [FL-no: 05.160, 05.211 and 05.219]. For two substances [FL-no: 09.418 and 09.585] the purity criteria are deficient in one of the parameters.

Thus, the final evaluation of the materials of commerce cannot be performed for five substances [FL-no: 05.160, 05.211, 05.219, 09.418 and 09.585], pending further information. The remaining 16 substances [FL-no: 05.164, 05.166, 05.167, 05.169, 08.094, 08.095, 08.096, 08.097, 08.115, 09.387, 09.392, 09.499, 09.663, 09.679, 09.698 and 09.839] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

KEYWORDS

Flavourings, safety, branched-chain, aliphatic, saturated aldehydes, carboxylic acids, esters, primary alcohols.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all others in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2006/252/EC (EC, 2006). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000), which is broadly based on the opinion of the Scientific Committee on Food (SCF, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The Flavouring Group Evaluation (FGE) is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the positive list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996).

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.01	Adopted by SCF, 3 December 2003	http://europa.eu.int/comm/food/food/chemicalsafety/flavouring/scientificadvice_en.htm	17
FGE.01Rev1	4 July 2007	http://www.efsa.europa.eu/EFSA/ScientificPanels/AFC/efsa_locale-1178620753812_Opinions425.htm	21

The present revision of FGE.01, FGE.01Rev1, includes the assessment of four additional flavouring substances [FL-no: 05.160, 05.211, 05.219 and 09.418]. Information on acute toxicity for one of the substances [FL-no: 05.160] is included. Additional information on 12 substances [FL-no: 05.164, 05.167, 08.094, 08.095, 08.096, 08.097, 08.115, 09.387, 09.585, 09.663, 09.679 and 09.698] has been made available since FGE.01 was published.

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

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ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 1 Revision 1

1.1. Description

The present Flavouring Group Evaluation 01, Revision 1 (FGE.01Rev1), using the procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure - shown in schematic form in Annex I), deals with 21 branched-chain aliphatic acyclic saturated aldehydes, carboxylic acids and esters derived from aliphatic acyclic primary saturated alcohols and branched-chain aliphatic acyclic saturated carboxylic acids. These 21 flavouring substances belong to chemical groups 1 and 2, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000).

The 21 flavouring substances (candidate substances) under consideration, with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1. Seven of the 21 candidate substances are aliphatic branched-chain aldehydes [FL-no: 05.160, 05.164, 05.166, 05.167, 05.169, 05.211 and 05.219]; five are branched-chain aliphatic acyclic carboxylic acids [FL-no: 08.094 - 08.097 and 08.115] and nine are esters of aliphatic acyclic primary alcohols and branched-chain aliphatic acyclic carboxylic acids [FL-no: 09.387, 09.392, 09.418, 09.499, 09.585, 09.663, 09.679, 09.698 and 09.839].

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

The 21 candidate substances are closely related structurally to 31 flavouring substances (supporting substances) evaluated at the 49th meeting of the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) in the group "Esters of Aliphatic Acyclic Primary Alcohols with Branched-chain Aliphatic Acyclic Acids" (JECFA, 1998a; JECFA, 1999b).

The names and structures for the 31 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; JECFA, 1999b; SCF, 1995).

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variation of 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.).

Fifteen of the 21 flavouring substances in the group posses a chiral centre [FL-no: 05.160, 05.164, 05.167, 05.211, 05.219, 08.094, 08.095, 08.096, 08.097, 08.115, 09.387, 09.585, 09.663, 09.679 and 09.698]. For 12 of these 15 flavouring substances [FL-no: 05.164, 05.167, 08.094, 08.095, 08.096, 08.097, 08.115, 09.387, 09.585, 09.663, 09.679 and 09.698] Industry has informed that they are used as “racemate”. The substances [FL-no: 05.160, 05.211 and 05.219] have been presented

without indication whether the commercial flavouring substance was dominated by one of the optical isomers (see Table 1).

1.3. Natural Occurrence in Food

Thirteen of the 21 candidate substances in the present group have been reported to occur naturally in a wide variety of fruits, as well as in beer, cheese, meat, black tea and essiential oil. Quantitative data on natural occurrence have been reported for six of the substances.

These reports are:

- 6-Methyloctanal [FL-no: 05.211]: 6 mg/kg in citrus
- 4-Methylhexanoic acid [FL-no: 08.097]: 3.2 mg/kg in cheese, 0.08 mg/kg in lamb and 0.75 mg/kg in mutton
- Pentyl isobutyrate [FL-no: 09.418]: 25 mg/kg in monkey orange *Strychnos spinosa*
- Isobutyl 2-methylbutyrate [FL-no: 09.585]: 700 mg/kg in myrtle berry
- 2-Methylbutyl isobutyrate [09.663]: up to 44000 mg/kg in hop oil
- Propyl 2-methylbutyrate [FL-no: 09.698]: trace amounts in apples

Eight of the substances [FL-no: 05.160, 05.166, 05.167, 05.169, 05.219, 08.094, 08.115 and 09.839] have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

2. Specifications

Purity criteria for the 21 candidate substances have been provided by the flavour industry (EFFA, 2000a; EFFA, 2000b), (see Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), this information is adequate for 19 of the 21 substances. The purity criteria for the remaining two substances [FL-no: 09.418 and 09.585] are deficient in one or more of the parameters (Table 1). Otherwise the specifications are adequate for all 21 candidate substances, except that, information on optical isomerism is needed for three substances [FL-no: 05.160, 05.211 and 05.219] (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 in 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, 2004a).

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

The Maximised Survey-derived Daily Intake (MSDI (SCF, 1999)) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 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 population[2] (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 FGE.01Rev1, the total annual volume of production of the 21 candidate substances for use as flavouring substances in Europe has been reported to be 155 kg (EFFA, 2000c; EFFA, 2003s; EFFA, 2004ac; Flavour Industry, 2006b) and for the 31 supporting substances 32000 kg cited by JECFA (JECFA, 1999b).

On the basis of the annual volumes of production reported for the 21 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a). More than 75 % of the total annual volume of production for the candidate substances (EFFA, 2000c; EFFA, 2003s) is accounted for by four of these flavourings: pentyl isobutyrate [FL-no: 09.418], isobutyl 2-methylbutyrate [FL-no: 09.585], pentyl isovalerate [FL-no: 09.499] and pentyl 2-methylbutyrate [FL-no: 09.679]. The estimated daily *per capita* intake from use as a flavouring substance is 1.3 microgram/day of pentyl isobutyrate, 1.7 microgram/day of isobutyl 2-methylbutyrate, 9.0 microgram/day of pentyl isovalerate and 1.9 microgram/day of pentyl 2-methylbutyrate (Table 2a). The daily *per capita* intakes for each of the remaining substances are all less than 0.75 microgram (Table 2a).

[2] *EU figure 375 millions (Eurostat, 1998). 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*

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 21 candidate substances, information on food categories and normal and maximum use levels[3,4,5] were submitted for 20 of the substances by the Flavour Industry (EFFA, 2000c; EFFA, 2003s; EFFA, 2004ac; Flavour Industry, 2006b). The 20 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 1	Dairy products, excluding products of category 2	All
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All
Category 3	Edible ices, including sherbet and sorbet	All
Category 4.1	Processed fruits	All
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 5	Confectionery	All
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All
Category 7	Bakery wares	All
Category 8	Meat and meat products, including poultry and game	All
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All
Category 13	Foodstuffs intended for particular nutritional uses.	All except [FL-no: 09.387, 09.585]
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	Only [FL-no: 05.211, 09.418]
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All
Category 15	Ready-to-eat savouries	All except [FL-no: 09.418, 09.585]
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All

According to the Flavour Industry the normal use levels for 20 of the 21 candidate substances are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg

[3] *"Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i)*

[4] *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).*

[4] *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).*

(EFFA, 2002i; EFFA, 2000c; EFFA, 2003s; EFFA, 2004ac; Flavour Industry, 2006b). Information on use levels is lacking for [FL-no: 05.219].

The mTAMDI values for 20 of the 21 candidate substances from structural class I, for which use levels are submitted, range from 1000 to 3500 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

Data for short and medium length branched-chain aldehydes, carboxylic acids and esters (and their corresponding alcohol and carboxylic acid moieties) included in the present Flavouring Group Evaluation and general information for this class of chemicals indicate that they are rapidly absorbed from the gastrointestinal tract, metabolised and excreted. Also, *in vitro* hydrolysis data from studies with the supporting substances, as well as other closely related chemicals, indicate that the esters included in the present evaluation can be hydrolysed to yield corresponding alcohols and carboxylic acids (Gangolli & Shilling, 1968; Grundschober, 1977; Leegwater & Straten, 1974a; Longland et al., 1977).

General discussions of the biotransformations of branched-chain aliphatic carboxylic acids and aliphatic linear alcohols, as well as specific discussions of the metabolic pathways for isobutyric acid, isovaleric acid, 2-methylbutyric acid, 2-methylpentanoic acid, 4-methylpentanoic acid and 3-methylpentanoic acid as well as hydrolysis of their esters, are provided in Annex III.

In summary it is anticipated that the nine esters [FL-no: 09.387, 09.392, 09.418 09.499, 09.585, 09.663, 09.679, 09.698 and 09.839] will undergo hydrolysis to yield their corresponding aliphatic alcohols and branched-chain carboxylic acids. The resulting aliphatic alcohols and branched-chain carboxylic acids, as well as the seven branched-chain aldehydes [FL-no: 05.160, 05.164, 05.166, 05.167, 05.169, 05.211 and 05.219] and five branched-chain carboxylic acids [FL-no: 08.094 - 08.097 and 08.115], are expected to be completely oxidised to carbon dioxide via the fatty acid pathway followed by the tricarboxylic acid cycle. The alcohols can also be conjugated, especially the branched ones, and excreted via the urine.

It is anticipated that all the 21 candidate substances are metabolised to innocuous products. See Annex III.

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

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

For the safety evaluation of the 21 candidate substances from chemical groups 1 and 2 the Procedure (Annex I) was applied. The stepwise evaluations of the 21 substances are summarised in Table 2a.

Step 1

All the 21 candidate substances are classified in structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2.

All 21 candidate substances can be predicted to be metabolised to innocuous products and accordingly the evaluation of these substances proceed via the A-side of the Procedure scheme (Annex I).

Step A3

The 21 candidate substances which have all been assigned to class I have estimated European daily *per capita* intakes (MSDI) from 0.0012 to 9.0 microgram (EFFA, 2000c; EFFA, 2003s). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I substances.

Based on the results of the safety evaluation sequence of the Procedure, all 21 candidate substances do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

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

The estimated intakes for 20 of the candidate substances, all from structural class I, based on the mTAMDI range from 1000 to 3500 microgram/person/day. For 14 of these 20 substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day for structural class I. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1. For one substance [FL-no: 05.219] the mTAMDI could not be estimated due to lack of data on use levels.

For 14 of the 20 candidate substances, for which use levels have been provided, further information is required. This would include more reliable intake data and, if required, additional toxicological data.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
05.160	2-Methyldecanal	0.011	1000	Class I	1800
05.164	2-Methylhexanal	0.61	1000	Class I	1800
05.166	4-Methylpentanal	0.0012	1000	Class I	1800
05.167	12-Methyltetradecanal	0.12	1000	Class I	1800
05.169	12-Methyltridecanal	0.24	1000	Class I	1800
05.211	6-Methyloctanal	0.012	1600	Class I	1800
05.219	3-Methylhexanal	0.12	Lacking	Class I	1800
08.094	4-Methyldecanoic acid	0.24	2200	Class I	1800
08.095	8-Methyldecanoic acid	0.71	2200	Class I	1800
08.096	3-Methylhexanoic acid	0.24	2200	Class I	1800
08.097	4-Methylhexanoic acid	0.73	1900	Class I	1800
08.115	4-Methylheptanoic acid	0.24	2200	Class I	1800
09.387	Heptyl 2-methylbutyrate	0.0012	2300	Class I	1800

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
09.392	Heptyl isovalerate	0.0012	2300	Class I	1800
09.418	Pentyl isobutyrate	1.3	3500	Class I	1800
09.499	Pentyl isovalerate	9.0	2300	Class I	1800
09.585	Isobutyl 2-methylbutyrate	1.7	1900	Class I	1800
09.663	2-Methylbutyl isobutyrate	0.73	2300	Class I	1800
09.679	Pentyl 2-methylbutyrate	1.9	2300	Class I	1800
09.698	Propyl 2-methylbutyrate	0.12	2300	Class I	1800
09.839	Decyl 3-methylbutyrate	0.012	2300	Class I	1800

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 Flavouring Group Evaluation 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 volumes of production in Europe (EFFA, 2002i; EFFA, 2000c; EFFA, 2003s; EFFA, 2004ac; Flavour Industry, 2006b) the combined estimated daily *per capita* intake of the 21 flavouring substances from structural class I as flavouring substances is 19 microgram/day, which does not exceed the threshold of concern for a compound belonging to class I of 1800 microgram per person per day.

The 21 candidate substances are structurally related to 31 flavouring substances, also from structural class I, evaluated by JECFA at its 49th session (JECFA, 1998a; JECFA, 1999b). The total combined estimated intake of the candidate and supporting substances is 3900 microgram/*capita*/day which exceeds the threshold of concern for a compound belonging to structural class I. However, at the level of exposure resulting from the use as flavourings, all the candidate and supporting substances are expected to be efficiently metabolised and would not be expected to saturate the metabolic pathways. For these reasons and in the light of toxicological data on supporting substances (Annex IV), the combined intake based on the MSDI approach of these substances would not be expected to be of safety concern.

8. Toxicity

8.1. Acute Toxicity

Data are available for two candidate substances and for several supporting substances. The LD_{50} values range from 4000 to 16000 mg/kg body weight (bw) in rats.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

There are no studies available on the candidate substances in the present flavouring group, but several subacute/subchronic studies on supporting substances.

The toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data are available for two candidate substances.

The developmental and reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

There are no data for any of the candidate substances of this group and only *in vitro* data on a single supporting substance, ethyl isovalerate [FL-no: 09.447]. The tests for mutagenicity in bacteria and mammalian cells (chromosomal aberrations) were negative. However, the data available do not preclude evaluation of the candidate substances using the Procedure.

The genotoxicity data are summarised in Annex IV, Table IV.4 and IV.5.

9. Conclusions

The present Flavouring Group Evaluation deals with 21 branched-chain aliphatic acyclic saturated aldehydes, carboxylic acids and esters derived from aliphatic acyclic primary saturated alcohols and branched-chain aliphatic acyclic saturated carboxylic acids from chemical groups 1 and 2.

Fifteen of the 21 flavouring substances in the group posses a chiral centre. Three of these substances [FL-no: 05.160, 05.211 and 05.219] have been presented without indication whether the commercial flavouring substance was dominated by one of the optical isomers.

All 21 flavouring substances are classified into structural class I according to the decision tree approach presented by Cramer et al., 1978.

Thirteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the 21 flavouring substances in this group have intakes in Europe from 0.0012 to 9.0 microgram/*capita*/day, which are below the threshold of concern value for a structural class I substance of 1800 microgram/person/day.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 21 of the candidate substances belonging to structural class I would result in a total intake of approximately 19 microgram/*capita*/day. This value is below the threshold of concern for structural class I substances of 1800 microgram/person/day. The total combined estimated intake of the 31 supporting substances from structural class I and of the 21 candidate substances is approximately 3900 microgram/*capita*/day, which exceeds the threshold of concern for structural class I (1800 microgram/person/day). However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

Genotoxicity data are available only for one supporting substance and the genotoxicity could not be assessed adequately for the present flavouring group. However, the data available do not preclude evaluation of the candidate substances using the Procedure.

All 21 candidate substances in the present flavouring group are expected to be metabolised to innocuous products.

It was noted that where toxicity data were available, they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that on the basis of the default MSDI approach these 21 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1000 to 3500 microgram/person/day for 20 of the 21 flavouring substances from structural class I for which there were intake data. Thus, the intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for six flavouring substances [FL-no: 05.160, 05.164, 05.166, 05.167, 05.169 and 05.211]. The estimated intake of one flavouring substance also assigned to structural class I could not be calculated [FL-no: 05.219] due to lack of data. The six substances, which have mTAMDI intake estimates below the threshold of concern for structural class I, are also expected to be metabolised to innocuous products.

Thus for 14 of the 21 flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class. Therefore, for these 14 substances, as well as for the one substance for which use levels are lacking, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 21 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 tests for the materials of commerce have been provided for 19 of the 21 flavouring candidate substances, except that information on geometrical isomerism/chirality is missing for three of the substances [FL-no: 05.160, 05.211 and 05.219]. For two substances [FL-no: 09.418 and 09.585] the purity criteria are deficient in one of the parameters.

Thus, the final evaluation of the materials of commerce cannot be performed for five substances [FL-no: 05.160, 05.211, 05.219, 09.418 and 09.585], pending further information. The remaining 16 substances [FL-no: 05.164, 05.166, 05.167, 05.169, 08.094, 08.095, 08.096, 08.097, 08.115, 09.387, 09.392, 09.499, 09.663, 09.679, 09.698 and 09.839] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

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

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

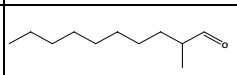
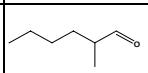
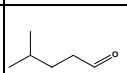
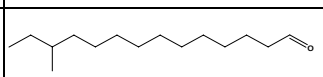
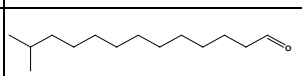
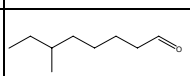
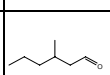
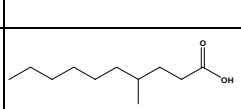
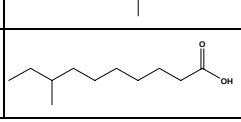
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
05.160	2-Methyldecanal 6)		19009-56-4	Liquid $C_{11}H_{22}O$ 170.29	Practically insoluble or insoluble 1 ml in 1 ml	102 (8 hPa) MS 95 %	1.428-4.434 0.826-0.832	CASrn in Register refers to the racemate.
05.164	2-Methylhexanal		925-54-2	Liquid $C_7H_{14}O$ 114.19	Insoluble 1 ml in 1 ml	141 MS 95 %	1.411-1.417 0.801-0.807	Racemate.
05.166	4-Methylpentanal		10369 1119-16-0	Liquid $C_6H_{12}O$ 100.16	Insoluble 1 ml in 1 ml	116 MS 95 %	1.399-1.415 0.799-0.805	
05.167	12-Methyltetradecanal		75853-50-8	Liquid $C_{15}H_{30}O$ 226.40	Insoluble 1 ml in 1 ml	291 NMR 95 %	1.425-1.448 0.827-0.833	Racemate.
05.169 1229	12-Methyltridecanal		4005 75853-49-5	Liquid $C_{14}H_{28}O$ 212.38	Insoluble Soluble	141-143 (5 hPa) IR NMR MS 97 %	1.445-1.455 0.930-0.941	
05.211	6-Methyloctanal 6)		4433 30689-75-9	Liquid $C_9H_{18}O$ 142.24	Slightly soluble Soluble	184 IR NMR MS 98.5 %	1.424-1.430 0.824-0.830	CASrn in Register refers to the racemate.
05.219	3-Methylhexanal 6)		19269-28-4	Liquid $C_7H_{14}O$ 114.19	Insoluble 1 ml in 1 ml	143 IR NMR MS 98.7 %	1.410 0.805	CASrn in Register refers to the racemate.
08.094	4-Methyldecanoic acid		24323-24-8	Liquid $C_{11}H_{22}O_2$ 186.29	Insoluble 1 ml in 1 ml	152 (13 hPa) MS 95 %	1.436-1.442 n.a.	Racemate.
08.095	8-Methyldecanoic acid		5601-60-5	Liquid $C_{11}H_{22}O_2$ 186.29	Insoluble 1 ml in 1 ml	162 (15 hPa) NMR 95 %	1.443-1.449 n.a.	Racemate.

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

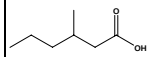
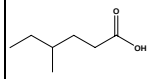
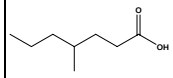
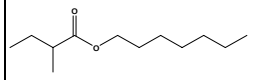
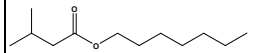
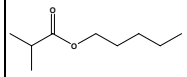
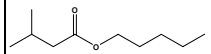
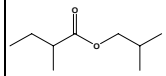
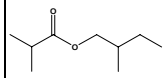
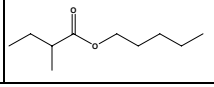
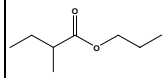
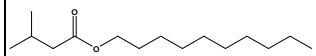
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
08.096	3-Methylhexanoic acid		3780-58-3	Liquid $C_7H_{14}O_2$ 130.19	Slightly soluble 1 ml in 1 ml	212 MS 95 %	1.419-1.425 0.916-0.922	Racemate.
08.097	4-Methylhexanoic acid		1561-11-1	Liquid $C_7H_{14}O_2$ 130.19	Slightly soluble 1 ml in 1 ml	218 MS 95 %	1.418-1.424 0.917-0.923	Racemate.
08.115	4-Methylheptanoic acid		3302-03-2	Liquid $C_8H_{16}O_2$ 144.21	Slightly soluble 1 ml in 1 ml	131 (20 hPa) MS 95 %	1.427-1.433 0.879-0.885	Racemate.
09.387	Heptyl 2-methylbutyrate		10668 50862-12-9	Liquid $C_{12}H_{24}O_2$ 200.32	Insoluble 1 ml in 1 ml	109 (13 hPa) MS 95 %	1.418-1.424 0.854-0.860	Racemate.
09.392	Heptyl isovalerate		10667 56423-43-9	Liquid $C_{12}H_{24}O_2$ 200.32	Insoluble 1 ml in 1 ml	108 (13 hPa) MS 95 %	1.422-1.428 0.862-0.868	
09.418	Pentyl isobutyrate		293 2445-72-9	Liquid $C_9H_{18}O_2$ 158.24	Practically insoluble or insoluble 1 ml in 1 ml	169 98 %	1.391-1.421 0.853-0.862	ID 7).
09.499	Pentyl isovalerate		2224 25415-62-7	Liquid $C_{10}H_{20}O_2$ 172.27	Insoluble 1 ml in 1 ml	108 (7 hPa) MS 98 %	1.413-1.415 0.856-0.862	
09.585	Isobutyl 2-methylbutyrate		10710 2445-67-2	Liquid $C_9H_{18}O_2$ 158.24	Insoluble 1 ml in 1 ml	166 (96 hPa) MS 95 %	1.403-1.409 0.845-0.870	Racemate. Specific gravity range.
09.663	2-Methylbutyl isobutyrate		10770 2445-69-4	Liquid $C_9H_{18}O_2$ 158.24	Insoluble 1 ml in 1 ml	170 MS 95 %	1.408-1.410 0.856 - 0.862	Racemate.
09.679	Pentyl 2-methylbutyrate		10875 68039-26-9	Liquid $C_{10}H_{20}O_2$ 172.27	Insoluble 1 ml in 1 ml	102 (5 hPa) MS 99 %	1.412-1.416 0.858-0.864	Racemate.

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

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.698	Propyl 2-methylbutyrate		10891 37064-20-3	Liquid $C_8H_{16}O_2$ 144.21	Insoluble 1 ml in 1 ml	156 (97 hPa) MS 99 %	1.399-1.406 0.862-0.868	Racemate.
09.839	Decyl 3-methylbutyrate		72928-48-4	Liquid $C_{15}H_{30}O_2$ 242.40	Insoluble 1 ml in 1 ml	287 MS 95 %	1.422-1.434 0.859-0.865	

1) Solubility in water, if not otherwise stated.

2) Solubility in 95% ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

7) ID: Missing identification test.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

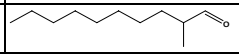
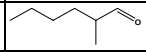
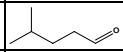
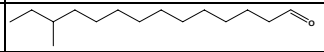
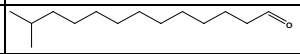
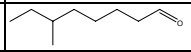
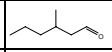
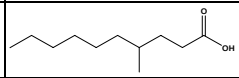
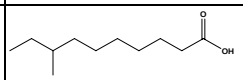
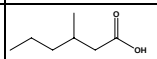
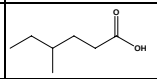
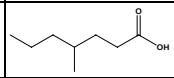
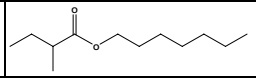
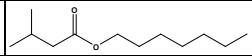
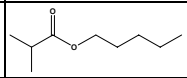
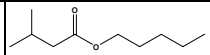
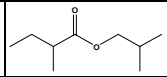
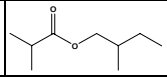
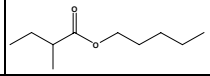
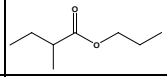
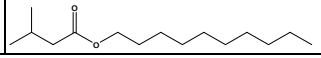
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
05.160	2-Methyldecanal		0.011	Class I A3: Intake below threshold	4)	7)	
05.164	2-Methylhexanal		0.61	Class I A3: Intake below threshold	4)	6)	
05.166	4-Methylpentanal		0.0012	Class I A3: Intake below threshold	4)	6)	
05.167	12-Methyltetradecanal		0.12	Class I A3: Intake below threshold	4)	6)	
05.169 1229	12-Methyltridecanal		0.24 0.5	Class I A3: Intake below threshold	4)	6)	
05.211	6-Methyloctanal		0.012	Class I A3: Intake below threshold	4)	7)	
05.219	3-Methylhexanal		0.12	Class I A3: Intake below threshold	4)	7)	
08.094	4-Methyldecanoic acid		0.24	Class I A3: Intake below threshold	4)	6)	
08.095	8-Methyldecanoic acid		0.71	Class I A3: Intake below threshold	4)	6)	
08.096	3-Methylhexanoic acid		0.24	Class I A3: Intake below threshold	4)	6)	
08.097	4-Methylhexanoic acid		0.73	Class I A3: Intake below threshold	4)	6)	
08.115	4-Methylheptanoic acid		0.24	Class I A3: Intake below threshold	4)	6)	
09.387	Heptyl 2-methylbutyrate		0.0012	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.392	Heptyl isovalerate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.418	Pentyl isobutyrate		1.3	Class I A3: Intake below threshold	4)	7)	
09.499	Pentyl isovalerate		9.0	Class I A3: Intake below threshold	4)	6)	
09.585	Isobutyl 2-methylbutyrate		1.7	Class I A3: Intake below threshold	4)	7)	
09.663	2-Methylbutyl isobutyrate		0.73	Class I A3: Intake below threshold	4)	6)	
09.679	Pentyl 2-methylbutyrate		1.9	Class I A3: Intake below threshold	4)	6)	
09.698	Propyl 2-methylbutyrate		0.12	Class I A3: Intake below threshold	4)	6)	
09.839	Decyl 3-methylbutyrate		0.012	Class I A3: Intake below threshold	4)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

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

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

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

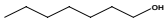
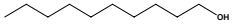
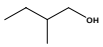
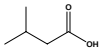
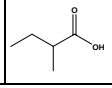
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.001	2-Methylpropan-1-ol 251		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.002	Propan-1-ol 82		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.021	Heptan-1-ol 94		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.024	Decan-1-ol 103		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern d) Category B c)	Class I A3: Intake below threshold	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.046	2-Methylbutyric acid 255		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	

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

2) No safety concern at estimated levels of intake.

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

4) Threshold of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (SCF, 1995).

b) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 2004a).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

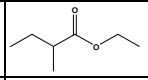
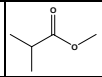
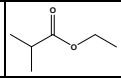
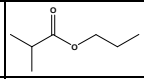
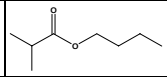
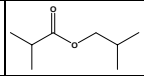
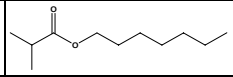
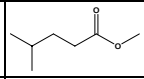
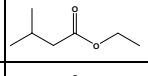
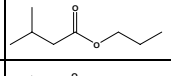
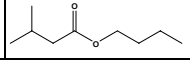
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.409	Ethyl 2-methylbutyrate		2443 265 7452-79-1	206 JECFA specification (JECFA, 2000d)	1900	No safety concern a) Category B b)	(R) or (S) enantiomer not specified by CASrn in Register.
09.412	Methyl isobutyrate		2694 287 547-63-7	185 JECFA specification (JECFA, 1997b)	20	No safety concern a) Category A b)	
09.413	Ethyl isobutyrate		2428 288 97-62-1	186 JECFA specification (JECFA, 1997b)	640	No safety concern a) Category A b)	
09.414	Propyl isobutyrate		2936 289 644-49-5	187 JECFA specification (JECFA, 1997b)	13	No safety concern a) Category A b)	
09.416	Butyl isobutyrate		2188 291 97-87-0	188 JECFA specification (JECFA, 1997b)	2.3	No safety concern a) Category A b)	
09.417	Isobutyl isobutyrate		2189 292 97-85-8	194 JECFA specification (JECFA, 1997b)	56	No safety concern a) Category A b)	
09.420	Heptyl isobutyrate		2550 295 2349-13-5	190 JECFA specification (JECFA, 1997b)	0.0012	No safety concern a) Category A b)	
09.432	Methyl 4-methylvalerate		2721 322 2412-80-8	216 JECFA specification (JECFA, 2000d)	0.024	No safety concern a) Category B b)	JECFA CASrn 2412-24-1 not valid.
09.447	Ethyl isovalerate		2463 442 108-64-5	196 JECFA specification (JECFA, 1997b)	650	No safety concern a) Category B b)	
09.448	Propyl isovalerate		2960 443 557-00-6	197 JECFA specification (JECFA, 1997b)	1.7	No safety concern a) Category B b)	
09.449	Butyl isovalerate		2218 444 109-19-3	198 JECFA specification (JECFA, 1997b)	80	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

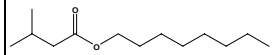
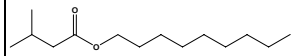
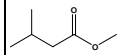
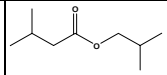
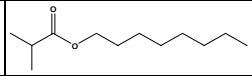
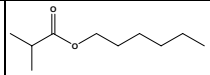
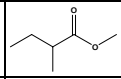
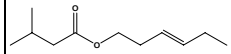
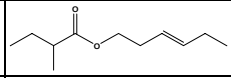
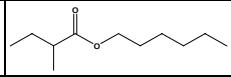
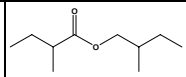
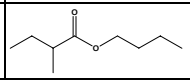
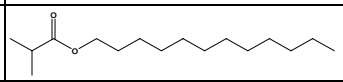
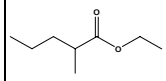
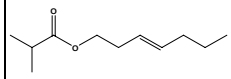
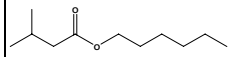
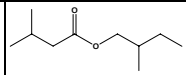
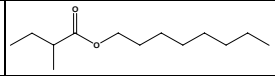
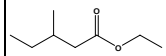
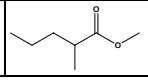
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.451	Octyl isovalerate		2814 446 7786-58-5	200 JECFA specification (JECFA, 1997b)	6.2	No safety concern a) Category B b)	
09.452	Nonyl isovalerate		2791 447 7786-47-2	201 JECFA specification (JECFA, 1997b)	0.012	No safety concern a) Category B b)	
09.462	Methyl isovalerate		2753 457 556-24-1	195 JECFA specification (JECFA, 1997b)	6.6	No safety concern a) Category B b)	
09.472	Isobutyl isovalerate		3369 568 589-59-3	203 JECFA specification (JECFA, 1997b)	66	No safety concern a) Category B b)	
09.473	Octyl isobutyrate		2808 593 109-15-9	192 JECFA specification (JECFA, 1997b)	9.4	No safety concern a) Category B b)	
09.478	Hexyl isobutyrate		3172 646 2349-07-7	189 JECFA specification (JECFA, 1997b)	2.6	No safety concern a) Category B b)	
09.483	Methyl 2-methylbutyrate		2719 2085 868-57-5	205 JECFA specification (JECFA, 2003b)	330	No safety concern a) Category B b)	
09.505	Hex-3-enyl isovalerate		3498 2344 10032-11-8	202 JECFA specification (JECFA, 1997b)	8.0	No safety concern a) Category B b)	(Z) or (E) isomer not specified by CASrn in Register.
09.506	Hex-3-enyl 2-methylbutyrate		3497 2345 10094-41-4	211 JECFA specification (JECFA, 2000d)	4.3	No safety concern a) Category B b)	(Z) or (E) isomer not specified by CASrn in Register.
09.507	Hexyl 2-methylbutyrate		3499 4132 10032-15-2	208 JECFA specification (JECFA, 2000d)	4.1	No safety concern a) Category B b)	(R) or (S) enantiomer not specified by CASrn in Register.
09.516	2-Methylbutyl 2-methylbutyrate		3359 10773 2445-78-5	212 JECFA specification (JECFA, 2003b)	3.0	No safety concern a)	(R) or (S) enantiomer not specified by CASrn in Register.
09.519	Butyl 2-methylbutyrate		3393 10534 15706-73-7	207 JECFA specification (JECFA, 2000d)	22	No safety concern a)	(R) or (S) enantiomer not specified by CASrn in Register.

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.523	Dodecyl isobutyrate		3452 10563 6624-71-1	193 JECFA specification (JECFA, 2000d)	0.17	No safety concern a)	
09.526	Ethyl 2-methylvalerate		3488 10616 39255-32-8	214 JECFA specification (JECFA, 2000d)	6.5	No safety concern a)	JECFA evaluated ethyl 2-methylpentanoate (CASrn 28959-02-6 which corresponds to the (S)-enantiomer). (R) or (S) enantiomer not specified by CASrn in Register.
09.528	trans-3-Heptenyl isobutyrate		3494 10663	191 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	CASrn to be introduced in the Register: 207801-32-9. JECFA evaluated trans-3-heptenyl 2-methyl propanoate (CASrn 67801-45-0 which corresponds to the (S)-enantiomer).
09.529	Hexyl isovalerate		3500 10692 10032-13-0	199 JECFA specification (JECFA, 1997b)	1.9	No safety concern a)	
09.531	2-Methylbutyl isovalerate		3506 10772 2445-77-4	204 JECFA specification (JECFA, 2000d)	0.73	No safety concern a)	(R) or (S) enantiomer not specified by CASrn in Register.
09.537	Octyl 2-methylbutyrate		3604 10866 29811-50-5	209 JECFA specification (JECFA, 2000d)	0.012	No safety concern a)	(R) or (S) enantiomer not specified by CASrn in Register.
09.541	Ethyl 3-methylvalerate		3679 5870-68-8	215 JECFA specification (JECFA, 2000d)	0.27	No safety concern a)	(R) or (S) enantiomer not specified by CASrn in Register.
09.549	Methyl 2-methylvalerate		3707 2177-77-7	213 JECFA specification (JECFA, 2000d)	0.15	No safety concern a)	(R) or (S) enantiomer not specified by CASrn in Register.

ND) No intake data reported.

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

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

3) No safety concern at estimated levels of intake.

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

a) (JECFA, 1999b).

b) (CoE, 1992).

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 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, 1996a; JECFA, 1997a; JECFA, 1999b).

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

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

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

- can the flavourings be predicted to be metabolised to innocuous products[6] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[7] (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.

[6] *"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).*

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

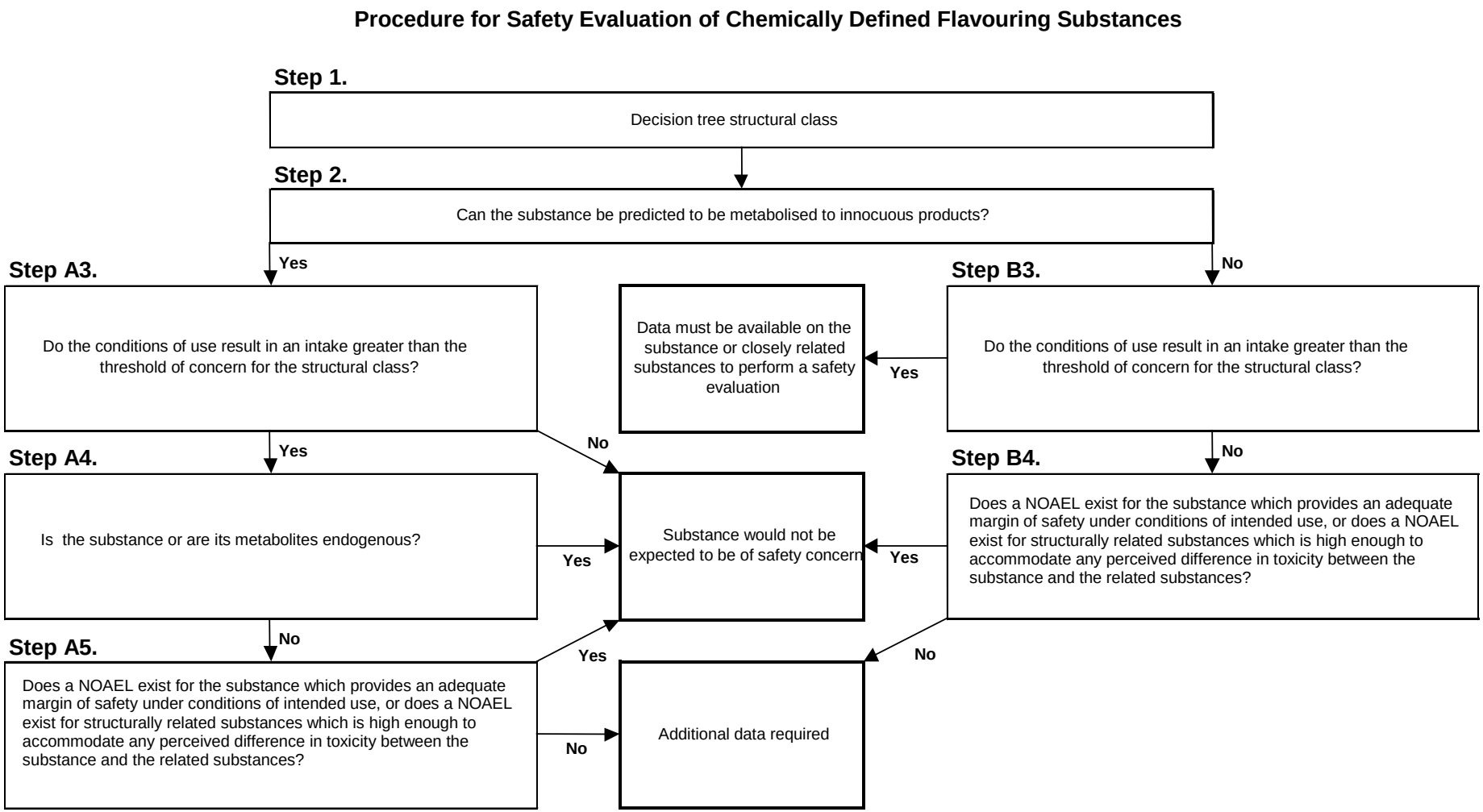


Figure I.1 *Procedure for Safety evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1. Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 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, 2002i). 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).

Table II.1.1 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 20 of the 21 candidate substances in the present flavouring group (Table II.1.2). For one flavouring substance [FL-no: 05.219] use levels are missing.

Table II.1.2. Normal and Maximum use levels (mg/kg) for candidate substances in FGE.01Rev1 (EFFA, 2000c; EFFA, 2003s; EFFA, 2004ac; Flavour Industry, 2006b)

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
05.160	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	- -	4 20	5 25	2 10
05.164	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	- -	4 20	5 25	2 10
05.166	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	2 10	2 10	- -	- -	2 10	3 15	- -	4 20	5 10	2 10
05.167	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	- -	4 20	5 25	2 10
05.169	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	- -	4 20	5 25	2 10
05.211	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5			2 10	3 15	2 10	4 20	5 25	2 10
08.094	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	- -	10 50	15 75	5 25
08.095	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	- -	10 50	15 75	5 25
08.096	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	- -	10 50	15 75	5 25
08.097	3	2	3	2	-	4	5	5	2	2	-	-	5	10	-	4	15	5

Table II.1.2. Normal and Maximum use levels (mg/kg) for candidate substances in FGE.01Rev1 (EFFA, 2000c; EFFA, 2003s; EFFA, 2004ac; Flavour Industry, 2006b)

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
	15	10	15	10	-	20	25	25	10	10	-	-	25	50	-	20	75	25
08.115	3	2	3	2	-	10	5	10	2	2	-	-	5	10	-	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	-	50	75	25
09.387	7	5	10	7	-	10	5	10	2	2	-	-	5	-	-	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	-	-	50	100	25
09.392	7	5	10	7	-	10	5	10	2	2	-	-	5	10	-	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	-	50	100	25
09.418	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	-	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	-	25
09.499	7	5	10	7	-	10	5	10	2	2	-	-	5	10	-	10	20	5
	35	25	50	35	-	200	25	50	10	10	-	-	25	50	-	200	100	25
09.585	7	5	10	7	-	10	5	10	2	2	-	-	5	-	-	10	-	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	-	-	50	-	25
09.663	7	5	10	7	-	10	5	10	2	2	-	-	5	10	-	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	-	50	100	25
09.679	3	2	3	7	-	10	2	10	2	2	-	-	5	10	-	10	20	5
	15	10	15	35	-	50	25	50	10	10	-	-	25	50	-	50	100	25
09.698	7	5	10	7	-	10	5	10	2	2	-	-	5	10	-	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	-	50	100	25
09.839	7	5	10	7	-	10	5	10	2	2	-	-	5	10	-	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	-	50	100	25

II.2. mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person consumes the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

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

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 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 II.2.2 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	Dairy products, excluding products of category 02.0	Food		
02	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03	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	Confectionery			Exception a
06	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07	Bakery wares	Food		
08	Meat and meat products, including poultry and game	Food		
09	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10	Eggs and egg products	Food		
11	Sweeteners, including honey			Exception a
12	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13	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	Ready-to-eat savouries			Exception b
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for the flavouring substances in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (EFFA, 2000c; EFFA, 2003s; EFFA, 2004ac; Flavour Industry, 2006b). The mTAMDI values are only given for highest reported normal use. Use levels for [FL-no: 05.211 and 05.219] are not submitted in the correct format.

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	**EU Register name**	**mTAMDI (µg/person/day)**	**Structural class**	**Threshold of concern (µg/person/day)**
05.160	2-Methyldecanal	1000	Class I	1800
05.164	2-Methylhexanal	1000	Class I	1800
05.166	4-Methylpentanal	1000	Class I	1800
05.167	12-Methyltetradecanal	1000	Class I	1800
05.169	12-Methyltridecanal	1000	Class I	1800
05.211	6-Methyloctanal	1600	Class I	1800
05.219	3-Methylhexanal	Lacking	Class I	1800
08.094	4-Methyldecanoic acid	2200	Class I	1800
08.095	8-Methyldecanoic acid	2200	Class I	1800
08.096	3-Methylhexanoic acid	2200	Class I	1800
08.097	4-Methylhexanoic acid	1900	Class I	1800
08.115	4-Methylheptanoic acid	2200	Class I	1800
09.387	Heptyl 2-methylbutyrate	2300	Class I	1800
09.392	Heptyl isovalerate	2300	Class I	1800
09.418	Pentyl isobutyrate	3500	Class I	1800
09.499	Pentyl isovalerate	2300	Class I	1800
09.585	Isobutyl 2-methylbutyrate	1900	Class I	1800
09.663	2-Methylbutyl isobutyrate	2300	Class I	1800
09.679	Pentyl 2-methylbutyrate	2300	Class I	1800
09.698	Propyl 2-methylbutyrate	2300	Class I	1800
09.839	Decyl 3-methylbutyrate	2300	Class I	1800

ANNEX III: METABOLISM

III.1. Absorption, distribution and excretion

Data for short- and medium-length branched-chain aldehydes, carboxylic acids and esters (and their component alcohols and carboxylic acids) included in this evaluation and more general information for this class of chemicals indicate that they are rapidly absorbed from the gastrointestinal tract (JECFA, 1998a; JECFA, 1999b). Information on distribution and excretion of these compounds has not been submitted, but as these flavouring substances can be expected to be extensively metabolised (see below), accumulation of metabolic intermediates and excretion of parent substances is not likely to be of relevance.

III.2. Biotransformation

A general discussion of the biotransformation of branched-chain aliphatic acids and aliphatic linear alcohols, as well as specific discussions of the metabolic pathways for isobutyric acid, isovaleric acid, 2-methylbutyric acid, 2-methylpentanoic acid, 4-methylpentanoic acid and 3-methylpentanoic acid are provided by JECFA (JECFA, 1998a; JECFA, 1999b). These discussions and conclusions apply equally well to the candidate substances as they do to the supporting substances.

The following additional discussion on the metabolism of the short- and medium-length branched-chain aldehydes, carboxylic acids and esters is structured according to the general metabolic reactions that have been demonstrated for these or similar chemicals. The likelihood that the candidate substances undergo these metabolic reactions depends on their chain length, degree of branching and functional groups. It is likely that multiple metabolic reactions will occur for some chemicals. The probable metabolic reactions are the following:

III.2.1 Oxidation of alcohols and aldehydes to acids
III.2.2 Reduction of aldehydes to alcohols
III.2.3 Metabolism to glucuronides and sulphates
III.2.4 Ester hydrolysis
III.2.5 Beta-oxidation of carboxylic acids
III.2.6 Omega-oxidation of carboxylic acids
III.2.7 Oxidation of branched-chain carboxylic acids

III.2.1. Oxidation of alcohols and aldehydes to acids

Linear and branched chain alcohols and aldehydes are oxidised to corresponding carboxylic acids by high capacity NAD+/NADH-dependent enzymes (Feron et al., 1991; Parkinson, 1996a; Voet & Voet, 1990).

Alcohol dehydrogenase (ADH) enzymes are cytosolic enzymes that are primarily responsible for the oxidation of alcohols to their corresponding aldehydes. Alcohols also can be oxidised to aldehydes by non-ADH enzymes present in the microsomes and peroxisomes, but these are generally quantitatively less important than ADH. Aldehyde dehydrogenases (ALDH) oxidise aldehydes to their corresponding carboxylic acids. Of the several ALDH enzymes involved in the oxidation of aldehydes, Class I ALDH enzymes are responsible for the oxidation of the widest

variety of aldehydes and are expected to be responsible for the oxidation of the candidate substances (Parkinson, 1996a). Branched-chain aliphatic alcohols and aldehydes also are excellent substrates for ADH and ALDH (Albro, 1975; Blair & Bodley, 1969; Hedlund & Kiessling, 1969a).

III.2.2. Reduction of aldehydes to alcohols

Aldehydes may also be reduced to alcohols, but reduction has a minor overall impact on aldehyde metabolism. Reduction is a reversible reaction, while oxidation is irreversible and the Km of ALD is substantially lower (higher affinity) than the Km of the reductases for the aldehydes (Sladek et al., 1989).

III.2.3. Metabolism to glucuronides and sulphates

Hydroxyl and carboxyl functional groups may undergo conjugation reactions forming glucuronides and sulphates (Parkinson, 1996a). Conjugation of hydroxyl and carboxyl groups with glucuronide and sulphate and subsequent urinary excretion is expected to compete with the other metabolic reactions described in this annex. Based on the metabolic profiles available, these conjugation reactions apparently comprise a small fraction of the overall metabolic disposition of short- and medium- length branched-chain alcohols, aldehydes, acids and esters. Conjugation with glucuronide may account for the elimination of up to 10 % of the dose for linear aliphatic alcohols with a chain length of about 6 to 8 carbon atoms. For linear alcohols with shorter chain length, this conjugation with glucuronic acid is even less important (Kamil et al., 1953a).

III.2.4. Ester hydrolysis

The esters included in this monograph are expected to be hydrolysed enzymatically to carboxylic acids and alcohols via carboxylesterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980).

Results of *in vitro* studies indicate that the affinity of the esterases for their substrates increases as the chain length of the ester increases and that the rate of hydrolyses of the straight-chain esters is approximately 100 times faster than the rate of hydrolysis of the branched-chain esters (Arndt & Krisch, 1973; Junge & Heymann, 1979).

While no hydrolysis data have been provided for the esters of the present group of flavourings, there are *in vitro* hydrolysis data for some structurally related esters.

Structurally related esters were shown to be hydrolysed rather slowly in artificial gastric juice (half life $T_{1/2}$ 146 - 1390 min) (Gangolli & Shilling, 1968; Longland et al., 1977). Hydrolysis in artificial pancreatic juice / pancreatin was found to be faster than in artificial gastric juice (Gangolli & Shilling, 1968; Leegwater & Straten, 1974a; Longland et al., 1977). However, there is a variation in the rate of hydrolysis between different structurally related esters. Hydrolysis by artificial pancreatic juice was rather fast for some esters ($T_{1/2}$ of isoamyl butyrate, benzyl isobutyrate and isoamyl isovalerate 11, 18 and 10 min, respectively) and relatively slow for other esters ($T_{1/2}$ of ethyl isovalerate and isoamyl caproate 198 and 38 min, respectively). Rat liver homogenate and small intestinal mucosa preparation were found to be much more efficient in hydrolysing esters. While half lives of ethyl isovalerate were 24 and 133 seconds for hydrolysis by liver homogenate and intestinal mucosa preparation, respectively, half lives of isoamyl butyrate and benzyl isobutyrate were less than one second in liver homogenate or intestinal mucosa preparation (Longland et al., 1977).

Based on these data on substances structurally related to the esters of the present group of flavourings it can be expected that the nine esters of the present group will be hydrolysed to their corresponding acids and alcohols in humans within a relatively short time.

III.2.5. Beta-oxidation of carboxylic acids

Beta-oxidation is a major route of metabolism for the candidate chemicals included in this monograph. Beta-oxidation of fatty acids results in acetate and the sequential removal of two-carbon units from carboxylic acids. The process is repeated until the end products are acetate, propionate or butyrate. The products of fatty acid oxidation depend on the chain length of the chemical. Acetate and butyrate are utilized for energy via the citric acid cycle or converted to acetoacetate and subsequently to other ketone bodies. Ketone bodies may be oxidised or excreted in the urine. Propionate originates from odd numbered-chain acids and is converted to carbohydrate and into lipids (Voet & Voet, 1990). The candidate chemicals included in this monograph are prime candidates for fatty acid beta-oxidation since the esters are expected to be hydrolysed to acids and alcohols, and the alcohols and aldehydes will be oxidised to carboxylic acids.

III.2.6. Omega-oxidation of carboxylic acids

Medium-chain length acids may also be partly oxidised via omega-oxidation, producing dicarboxylic acids, which may be attacked from either end by beta-oxidation. Omega-oxidation may occur when capacity for beta-oxidation is either exceeded because of a large dose of the chemical or blocked because of substitution in the alpha- or beta-position. Short-chain acids, such as butyric, caproic and caprylic acids may be converted to longer-chain fatty acids for incorporation into normal intermediary metabolism (Voet & Voet, 1990; Williams, 1959).

III.2.7. Oxidation of branched-chain carboxylic acids

Short-chain branched carboxylic acids (<6 C atoms) can be oxidised via beta-oxidation mainly in the longer chain, followed by cleavage to yield short-chain linear fragments that are metabolised via the normal pathways. Longer branched-chain fatty acids may undergo omega-oxidation to diacids, which may be further oxidised. Cleaved acids with a methyl substituent can be metabolised to carbon dioxide via alfa- and/or beta-oxidation (JECFA, 1998a; JECFA, 1999b).

III.3. Conclusion

The information in JECFA (JECFA, 1998a; JECFA, 1999b) and that discussed above allow the following assessment to be made:

It is anticipated that the nine candidate esters [FL-no: 09.387, 09.392, 09.418, 09.499, 09.585, 09.663, 09.679, 09.698, and 09.839] will undergo hydrolysis, either before or after absorption from the gastrointestinal tract, to yield their corresponding aliphatic alcohols and branched-chain carboxylic acids.

The hydrolysis products, any remaining non-hydrolysed candidate esters, the seven candidate branched-chain aldehydes [FL-no: 05.160, 05.164, 05.166, 05.167, 05.169, 05.211 and 05.219] and the five candidate branched-chain carboxylic acids [FL-no: 08.094-08.097 and 08.115], are all expected to be absorbed rapidly from the gastrointestinal tract.

Linear alcohols, resulting from ester hydrolysis, would be oxidised to their corresponding aldehydes and further to their corresponding linear carboxylic acids, which can be assumed to be metabolised to carbon dioxide via the fatty acid pathways and the tricarboxylic acid cycle.

Branched-chain alcohols, resulting from ester hydrolysis, would be oxidised to their corresponding aldehydes and these and the seven candidate branched-chain aldehydes would be oxidised to their corresponding branched carboxylic acids, which can be assumed to be metabolised to carbon dioxide via the fatty acid pathways and the tricarboxylic acid cycle.

Similarly, branched-chain carboxylic acids, resulting from ester hydrolysis, and the five candidate branched-chain aliphatic carboxylic acids can be assumed to be metabolised to carbon dioxide via the fatty acid pathways and the tricarboxylic acid cycle.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for two candidate substances of the present flavouring group evaluation and for 16 supporting substances evaluated by JECFA at the 49th meeting (JECFA, 1998a). The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Methyl isobutyrate [09.412])	Rat	NR	Oral	16000	(Patty, 1982a)	1
(Ethyl isobutyrate [09.413])	Rat	NR	Oral	>5000	(Moreno, 1975b)	1
(Propyl isobutyrate [09.414])	Rat	NR	Oral	15000	(Jenner et al., 1964)	1
(Butyl isobutyrate [09.416])	Rat	NR	Oral	>5000	(Levenstein, 1974a)	1
(Hexyl isobutyrate [09.478])	Rat	NR	Oral	>5000	(Moreno, 1977b)	1
(Octyl isobutyrate [09.473])	Rat	NR	Oral	>5000	(Levenstein, 1974a)	1
(Isobutyl isobutyrate [09.417])	Rat, Mouse	NR	Oral	12800	(Patty, 1982a)	1
(Methyl isovalerate [09.462])	Rabbit	NR	Oral	5690	(Munch, 1972)	1
(Ethyl isovalerate [09.447])	Rat	NR	Oral	>5000	(Levenstein, 1976a)	1
	Rabbit	NR	Oral	7030	(Munch, 1972)	1
(Propyl isovalerate [09.448])	Rabbit	NR	Oral	8220	(Munch, 1972)	1
(Butyl isovalerate [09.449])	Rat	NR	Oral	>5000	(Moreno, 1978b)	1
	Rabbit	NR	Oral	8230	(Munch, 1972)	1
(2-Methylpropyl isovalerate [09.472])	Rabbit	NR	Oral	>5000	(Munch, 1972)	1
	Rat	NR	Oral	6970	(Moreno, 1978b)	1
(2-Methylbutyl isovalerate [09.531])	Rat	NR	Oral	>5000	(Moreno, 1978b)	1
(Hexyl 2-methylbutyrate [09.507])	Rat	NR	Oral	>5000	(Moreno, 1977b)	1
(3-Hexenyl 2-methylbutyrate [09.506])	Rat	NR	Oral	>5000	(Moreno, 1977b)	1
4-Methylpentanal [05.166]	Rat	NR	Oral	5660	(Smyth et al., 1962)	
(Ethyl 3-methylvalerate [09.541])	Rat	NR	Oral	>4000	(Biosphere Research Center Inc., 1981)	1
2-Methyldecanal [05.160]	Rat	NR	Oral	>5000	(Moreno, 1975p)	

NR: not reported

1. Summarised by JECFA, 49th meeting (JECFA, 1998a).

Subacute/subchronic/chronic toxicity or carcinogenicity data are not available for any of the candidate substances from the present group evaluation but for 12 supporting substances evaluated at the 46th, 49th and 51st JECFA meetings. No carcinogenicity data are available (JECFA, 1997a; JECFA, 1998a; JECFA, 1999a).

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species / Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Ethyl isobutyrate [09.413])	Rat/ M, F 12	Oral	29.2 mg/kg bw/day	90 days	29.2	(Mecler & Craig, 1980)	1, 2
(Isobutyl isobutyrate [09.417])	Rat/ M, F 15	Gavage	0, 10, 100, 1000 mg/kg bw/day	18 weeks	1000	(Drake et al., 1978)	1, 2
(Ethyl isovalerate [09.447])	Rat/ M, F 12	Oral	M: 12.1 mg/kg bw/day F: 13.6 mg/kg bw/day	13 weeks	12.1	(Mecler & Craig, 1980)	1, 2
(Isovaleric acid [08.008])	Rat/ NR 6	Oral	2500 mg/kg bw/day	90 days	2500	(Amoore et al., 1978)	1, 2
(3-Methylbutyl 3-methylbutyrate [09.463])	Rat/ M, F	Diet	21.9, 69.2, or 219 mg/kg bw/day	90 days	219	(Damske et al., 1980)	1, 4
(Propyl alcohol [02.002])	Rat/ M 6	Drinking water	1 M equal to 60 mg/kg bw/day	4 months	60	(Hillbom et al., 1974b)	1, 2
(Butyl alcohol [02.004])	Rat/ M 30	Drinking water	5.6 mg/kg bw/day	13 weeks	5.6	(Wakabayashi et al., 1984b)	1, 2
	Rat/ M, F 6	Oral	0.69, 1.38, 2.75, 5.5 %, equal to 760, 1630, 3280, 6260 mg/kg bw/day for males, and 770, 1700, 3200, 6400 for females	14 days	1630	(PPG Industries Inc., 1991)	2
	Rat/ NR 10	Oral	0, 1000, 3500, 10000 mg/kg equal to 0, 90, 300, 940 mg/kg bw/day	28 days	940	(Bio-Fax, 1969)	1, 2
(Hexyl alcohol [02.005])	Beagle/ M, F 2	Oral	0.5 %, 1.0 % or 1000 mg/kg bw/day; 1.0 % equal to 230-695 mg/kg bw/day	13 weeks	230	(Eibert, 1992)	2
	Rat/ M, F 10	Oral	a) 0.25, 0.5 % b) 1 % for weeks 1-10; 2, 4, 4 % for weeks 11, 12, 13, respectively. 1 % equivalent to 577 mg/kg bw/day	13 weeks	577	(Eibert, 1992)	2
(Octanol [02.006])	Mouse/ NR	Oral	179 mg/kg bw/day	1 month	179	(Voskoboinikova, 1966)	1, 2

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species / Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	10						
(cis-3-Hexen-1-ol [02.056])	Rat/ M, F 15	Drinking water	0, 310, 1250, 5000 ppm equal to M: 0, 21, 85, 283 mg/kg bw/day, F: 0, 32, 131, 569 mg/kg bw/day	98 days	85	(Gaunt et al., 1969)	3
(Isobutanol [02.001])	Rat/ M, F 10	Oral	0, 1000, 4000, 16000 mg/l equal to 0, 60, 340, 1450 mg/kg bw/day	90 days	1450	(BASF, 1992a)	1, 2
	Rat/ M, F 20	Drinking water	0.2 % equivalent to 200 mg/kg bw/day	53-56 weeks	200	(Johannsen & Purchase, 1969)	1, 2
(Isoamyl alcohol [02.003])	Rat/ M, F 15	Gavage	0, 150, 500, 1000 mg/kg bw/day	17 weeks	1000	(Carpanini et al., 1973b)	1, 4
	Rat/ M, F 20	Drinking water	2 % equivalent to 2000 mg/kg bw/day	53-56 weeks	2000	(Johannsen & Purchase, 1969)	1, 4

NR = not reported.

M = Male.

F = Female.

1. The study was performed at a single dose level or multiple dose levels that produced no effects and, therefore, a NOAEL was not determined.

2. Summarised by JECFA, 49th meeting (JECFA, 1998a).

3. Summarised by JECFA, 51st meeting (JECFA, 1999a).

4. Summarised by JECFA 46th meeting (JECFA, 1997a).

Developmental and reproductive toxicity data are available for two candidate substances of the present flavouring group evaluation.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw /day), Including information of possible maternel toxicity	Reference	Comments
4-Methylhexanoic acid [08.097]	Dev. tox./ day 12 of gestation	Rat/ F 10	Gavage	14.1 mmol/kg corresponding to 1833 mg/kg bw/day	Offspring 1833 Maternal <1833	(Scott et al., 1994)	Isomer administered was not specified. No embryotoxicity observed despite severe maternal toxicity.
3-Methylhexanoic acid [08.096]	Dev. tox./ day 12 of gestation	Rat/ F 10	Gavage	14.1 mmol/kg corresponding to 1833 mg/kg bw/day	Offspring1833 Maternal<1833	(Scott et al., 1994)	Isomer administered was not specified. No embryotoxicity observed despite severe maternal toxicity.
	Dev. tox./ dosed during organogenesis	Rat/ F 15	Gavage	0, 375, 500 mg/kg/day	Offspring 500 Maternal <375	(Narotsky et al., 1994)	Chernoff/Kavlock assay. No signs of developmental toxicity were observed despite maternal mortality, respiratory toxicity and decreased maternal body weights.

F = Female

In vitro mutagenicity/genotoxicity data are not available for any of the candidate substances of the present flavouring group evaluation but for one of the supporting substances evaluated by JECFA at the 46th, 49th and 51st JECFA meetings. (JECFA, 1997a; JECFA, 1998a; JECFA, 1999a).

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Ethyl isovalerate [09.447])	Ames Test	S.typh. TA 92, TA 1535, TA 100, TA 1537, TA 94, TA 98, TA 2637	0.01-1 mg/plate	Negative	(Fujita & Sasaki, 1987)	With and without metabolic activation.
	Ames Test	S.typh. TA 92, TA 1535, TA 100, TA 1537, TA 94, TA 98, TA 2637	10 mg/plate	Negative	(Ishidate et al., 1984)	With and without metabolic activation.
	Rec. Assay	B. subtilis. H17 & M45	100-200 microlitre/ml	Positive	(Kuroda et al., 1984a)	This bacterial DNA-repair test system is of low predictive value for genotoxicity.
	Rec. Assay	B. subtilis. H17 (rec+) & M45 (rec-)	up to 20 microlitre/disc	Negative	(Yoo, 1986)	
	Chrom. Abs.	CHO fibroblasts	up to 2 mg/ml	Negative	(Ishidate et al., 1984)	
	Rec. Assay	B. subtilis. H17 & M45	17 microgram/plate	Negative	(Oda et al., 1979)	

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

No i*n vivo* mutagenicity/genotoxicity data are available for any of the candidate substances of the present flavouring group evaluation or for any of the supporting substances.

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Scientific Committee on Consumer Safety

SCCS

OPINION
on
Fragrance allergens in cosmetic products

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

About the Scientific Committees

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

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

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

SCCS

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

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Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1. Background

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

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

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

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

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

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

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

Furthermore, two fragrances (natural mixtures) were added

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

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

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

2. Terms of reference

1. *Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?*

2. *Can the SCCS establish any threshold for their safe use based on the available scientific data?*

3. *Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?*

3. Introduction

Fragrance ingredients

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

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

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

4. Clinical aspects of contact allergy to fragrance ingredients

4.1. Spectrum of reactions

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

4.1.1. Allergic contact dermatitis

Mechanism

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

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

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

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

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

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

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

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

Overview of clinical features

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

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

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

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

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

Hands

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

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

Axillae

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

Face

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

4.1.2. Irritant reactions (including contact urticaria)

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

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

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

4.1.3. Pigmentary anomalies

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

4.1.4. Photo-reactions

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

4.1.5. General/respiratory

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

4.2. Patch testing

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

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

4.3. Epidemiology of fragrance allergy

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

A fragrance formula may consist of ten to 300 or more different ingredients. The CosIng database lists 2587 ingredients used for perfuming[1], as well as several other materials classified as odour “masking” agents, which is equivalent with regard to allergy. A mixture of seven fragrance chemicals and one natural extract, which have been identified as major fragrance allergens in the past (34), are used for diagnosing contact allergy to fragrance

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

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

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

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

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

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

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

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

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

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

4.3.2. Clinical epidemiology

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

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

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

Fragrance Mix I ("Larsen Mix")

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

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

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

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

Note: $ Possibly included in (60).

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

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

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

Fragrance Mix II

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

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

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

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

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

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

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

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

Myroxylon pereirae (Balsam of Peru)

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

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

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

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

Oil of turpentine

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

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

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

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

4.3.3. Population-based epidemiology

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

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

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

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

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

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

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

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

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

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

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

4.4.1. Clinical relevance

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

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

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

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

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

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

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

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

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

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

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

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

Medical history

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

NACDG 2009 study (65)

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

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

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

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

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

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

Frosch 2002 (a) study (67)

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

Frosch 2002 (b) study (97)

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

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

Frosch 2005 study (35)

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

Danish Contact Dermatitis Group 2005-2008 (69)

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

Cosmetic products

Fragrance formulae from cosmetic products

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

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

IVDK "own perfumes" study (106)

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

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

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

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

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

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

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

4.4.3. Elicitation in diagnostic patch tests without clinical history

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

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

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

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

4.5. Socio-economic impact of contact allergy

4.5.1. Health related quality of life

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

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

4.5.2. Occupational restrictions

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

4.5.3. Costs to health care/health economics

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

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

4.6. Allergen avoidance

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.7. Conclusions

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

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

5. Activation of weak or non-sensitising substances into sensitisers - prehaptens and prohaptens

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

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

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

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

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

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

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

5.1. Prehaptens

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

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

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

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

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

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

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

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

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

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

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

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

Notes: § Bicentric or multicentre studies.
(ox.) Oxidised.
* Stabilised: according to the manufacturer contained additional substances aimed at limiting oxidation.

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

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

Reactions to ox. D- limonene and/or ox. L-limonene [a]	Pos. to ox. D-limonene: 41	14	34	11	27	11	27	(134)*
	Neg. to ox. D-limonene: 1771	113	6.4	91	5.1	62	3.5	
	Pos. to ox. L-limonene: 36	11	31	12	33	9	25	
	Neg. to ox. L-limonene: 1776	116	6.5	80	4.5	64	3.6	
Reactions to any of ox. linalool, myrcene, caryophyllene	Pos. to any of the tested ox. subst.: 31	12	39	6	31	12	39	(133)*
	Neg. to any of the tested ox. subst: 1480	93	6	63	4	46	3	

		Pos. to FM I		**Pos. to FM II**		**Pos. to MP**		**Pos. to coloph.**		
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Reactions to ox. linalool	Pos. at test conc. 4%: 30	8	26.7	5	16.7	10	33.3	5	16.7	(135)*
	Pos. at test conc. 6%: 55	12	21.8	8	14.5	11	20	8	14.5	
	Pos. at test conc. 11%: 72	14	19.4	9	12.5	14	19.4	9	12.5	
	Total pos. at any test conc: 75/1004	n.g.		n.g.		n.g		n.g.		
	Total neg. at any test conc: 929/1004	56	6.0	29	3.1	45	4.8	24	2.6	

Notes: * Bicentric or multicentre studies.
n.g. Not given.
(ox.) Oxidised.

5.2. Prohaptens

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

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

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

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

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

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

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

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

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

5.3. Conclusions

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

  It should be noted that the possibility to reduce the sensitisation potency by preventing air oxidation is also important for a direct acting hapten or prohapten, if a further activation by air oxidation to more allergenic compounds has been shown.
- In the case of prohaptens, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geranial (citral) and between cinnamyl alcohol and cinnamal.

  Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenol acetate, eugenyl acetate and geranyl acetate all of which are known to be used as fragrance ingredients.
- Further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is clearly needed to increase the safety for the consumer. Compounds suspected to act as prehaptens and/or prohaptens should be considered as allergens, unless it could be demonstrated that they do not become activated by one of the described pathways.

6. Retrieval of evidence and classification of fragrance substances

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

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

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

6.1. Retrieval of evidence

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

6.1.1. Search strategy for clinical data

Method of literature search:

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

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

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

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

6.1.2. Collection of experimental (LLNA) data

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

6.2. Grading of evidence

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

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

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

6.2.1. Quality of a clinical study

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

(i) Case reports or small case series, focusing on patients with positive (test) reactions to the target substance, sometimes including a set of non-exposed, possibly non-diseased "control patients"; these should present a concise summary of all relevant aspects of the patient's history, diagnostic procedures and possibly further outcomes.

(ii) Clinical series in which results of a group of patients patch tested with the target substance, often combined with other substances, are presented. In the latter type of report, usually only a minority of patients tested show a positive reaction to the test substance. This implies that the majority of patients can be used to illustrate the proportion of irritant, doubtful and negative reactions. The degree of detail on the patients' histories is usually limited in such studies, compared to case reports.

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

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

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

6.2.2. Quality of an experimental study

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

6.2.3. Quality of "other" evidence

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

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

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

6.3. Aggregating evidence for a final conclusion

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

6.3.1. Established contact allergen in humans

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

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

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

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

6.3.2. Established contact allergen in animals

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

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

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

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

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

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

To qualify as a *posible contact allergen*, if classification as "established …" or as "likely …" contact allergen is not applicable, *at least one* of the following criteria must be met:

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

[3] It should be noted that the SCCS considers such tests unethical (169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensiting cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:).

6.4. Conclusions

The present opinion includes (i) a well-defined search strategy for retrieving pertinent evidence; (ii) a definition of criteria used to evaluate available evidence; and, finally (iii) a set of rules to categorise the substances with regard to the relevant toxicological endpoint, i.e. sensitisation in man, based on the evidence.

7. Reported fragrance allergens from the clinical perspective

In this chapter, clinical evidence regarding sensitisation to individual fragrance chemicals and to natural extracts (essential oils) is tabulated. In this report “single chemicals” refers to chemicals of natural or synthetic origin whose chemical identity is fully known. The term “natural extracts” refers to plant or animal derived mixtures of natural chemicals, for example lavender oil, whose composition may be variable and may or may not have been fully or partly established. Full information, including possible synonyms, structural formulas (in the case of single chemicals only), a short summary of available evidence and further information, e.g. on regulatory status, is presented in Annex I.

7.1. Tabular summary of evaluated individual fragrance chemicals

Regarding nomenclature, INCI names are used wherever possible. If an INCI name is not available, the perfuming name as listed by CosIng is used. Detailed information on the publications identified and considered for this report can be found in Annex I. Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I, these substances have not been further evaluated and are thus not included in this chapter.

In this section, a tabular overview on the classification of substances considered is presented in four tables listing:

1. Established contact allergens in humans (→ *sufficient human evidence present*).
2. Substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans” (→ *limited human evidence present*).
3. Substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.
4. Substances eligible for inclusion (see beginning of chapter 6) for which no human data are available.

A critical point in understanding this scheme is that there is publication bias in reporting allergens. This is due to the fact that once a substance has been reported and accepted as a contact allergen in humans, further reports are less likely to be published unless they are part of a epidemiological survey or when there is a novel source of exposure. Moreover, the number of patients displaying positive test reactions obviously not only depends on the underlying prevalence of sensitisation, but also on how often a substance is patch tested. This implies that inclusion of an allergen or allergen mixture in the baseline patch test series (as for Fragrance Mix I and II, *Myroxylon pereirae* and HICC, and partly also other substances/mixtures) will yield the maximum possible number of cases. In contrast, patch testing in “special” series, e.g. as a break-down of single constituents of the respective mix in case of a positive reaction to the latter, or with application only in the case of strongly suspected fragrance intolerance, will mostly result in higher relative numbers than testing the same compound consecutively, but also in lower absolute numbers.

In Table 7-1, the single substances are listed with a semi-quantification of their impact which were categorised as established contact allergens in humans according to the criteria given in chapter 6.3.

Established contact allergens in humans, according to the criteria outlined in chapter 6.3.1, were categorised according to the number of patients reacting positively and to the number of patients tested, based on the publications considered (see annex I for references). The following categories were used:

+	Up to 10 positive test reactions reported
++	11 to 100
+++	101 to 1000
++++	> 1000

If a test allergen has been tested in less than 1,000 patients, “r.t.” (rarely tested) is added in the following tables. For this categorisation, absolute numbers of cases of sensitisation, and not the relative frequency of positive patch tests, were used, because relative frequencies depend heavily on the selection of patients for patch testing. Thereby, an important allergen tested routinely, in the baseline series, may yield 1 to 2% positive reactions (usually in several thousand patients), while an allergen tested in a selective fashion (in much fewer patients) may yield an even higher relative frequency. Moreover, case reports/series cannot be interpreted in terms of relative frequencies. The calculation of absolute numbers was based on all available literature, as detailed in the annex I to this opinion, i.e., regarding the 26 substances already listed in Annex III to the Cosmetics Directive includes data already evaluated in the previous opinion.

Table 7-1: Established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment: see text**
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL	122-40-7	++
AMYL CINNAMYL ALCOHOL	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISYL ALCOHOL	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL	100-51-6	++
BENZYL BENZOATE	120-51-4	++
BENZYL CINNAMATE	103-41-3	++
BENZYL SALICYLATE	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL (Lilial®)	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL	104-55-2	+++
CINNAMYL ALCOHOL	104-54-1	+++
CITRAL	5392-40-5	+++
CITRONELLOL	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN	91-64-5	+++
(DAMASCENONE)	23696-85-7	+ (r.t.)

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment: see text**
ROSE KETONE-4		
alpha-DAMASCONE (TMCHB)[#]	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE [#]	23726-92-3	+
delta-DAMASCONE [#]	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL	97-53-0	+++
FARNESOL	4602-84-0	+++
GERANIOL	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE *(HICC)*	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL	107-75-5	+++
ISOEUGENOL	97-54-1	+++
alpha-ISOMETHYL IONONE	127-51-5	++
(DL)-LIMONENE	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN[#]	92-48-8	++ (photo-allergy)
METHYL 2-OCTYNOATE	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment: see text**
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	++
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

Those substances which were categorised as +++ or more, i.e. those with the most reported cases, were also the top ranking substances in large series of patients tested with the 26 labelled fragrance ingredients ((44, 74) and additionally (170)). Geraniol is an exeption, as it was all negative in the Danish study (170), but was still among the top ten in the Dutch and German studies (44, 74), with prevalences of 0.5%-0.6% positives. Geraniol has, in addition, caused many cases of contact allergy in other areas of Europe (49).

The use of absolute numbers allows the pooling of studies with different selection criteria. Limonene and linalool were not tested in their oxidized forms in the three studies (44, 74, 170) and would not have been identified, if only these publications had been the basis of assessment.

It should be noted that oxidised fragrence terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrence terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available (see also chapter 5).

Table 7-2 lists those substances which gave rise to a few reported cases of contact sensitisation only, or where results have been reported from just one clinical department. Thus, the level of evidence concerning human data must be regarded as *limited*, according to the definitions given in chapter 6.3.

Table 7-2: Fragrance substances with positive human data, which are, however, not sufficient to categorise as "established contact allergen in humans". More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Comment**	**Ref.**
AMBRETTOLIDE	7779-50-2	3.4% positive reactions in 178 patients	(171)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (172))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(139)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(173)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(171)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(174)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(175).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(171)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(173)
ETHYL VANILLIN	121-32-4	1 occupational case	(176)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(97)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(173)
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, possibly "excited back syndrome" 0 / 95 in another study with <= 1/10 of above test conc.	(139) (70)
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(171)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(177)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(173)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(178)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(179)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(97) (70)
METHYLIONANTHEME	55599-63-8	1 case	(180)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(97)
METHYL OCTINE CARBONATE	111-80-8	1 case	(181)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(133)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(182)
NEROL	106-25-2	6.0% positive	(173)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(183)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly “excited back syndrome”	(139)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(139) (70)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(178) (184)
PHENYLPROPANOL	122-97-4	2 / 218	(173)
PHYTOL	150-86-7	1 case in human max. test	(185)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(186)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(187)

For a number of substances negative patch tests results were obtained, usually in rather small patient samples (max. 313 patients). For some of these substances exposure is substantial, according to data submitted from IFRA. It should be noted that a negative result does not rule out a notable sensitisation prevalence, as the study size has to be larger than, e.g. n=298 to yield a 95% CI which excludes a prevalence of 1% and larger than n=597 to exclude a prevalence of 0.5%.

Table 7-3: Fragrance substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Results / Comment**	**Ref.**
6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	21145-77-7	0 / 313 consecutive patients in 2 centres	(70)
AMYLCYCLOPENTANONE	4819-67-4	0 / 178	(171)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(70)
2-TERT-BUTYLCYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(70)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(70)
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(171)
3α,4,5,6,7,7α-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(70)
HEXYL SALICYLATE	6259-76-3	0 / 218 “top 100” substance and classified as R43	(173)
HIBISCOLIDE	6707-60-4	0 / 178	(171)
alpha-IONONE	127-41-3	0 / 205	(70)
beta-IONONE	79-77-6	0 / 205 “top 100” substance	(70)
ISOBORNYL ACETATE	125-12-2	0 / 107 “top 100” substance	(70)
METHYL ANTHRANILATE	134-20-3	0 / 91 “top 100” substance	(188)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 “top 100” substance	(70)
OXALIDE	1725-01-5	0 / 178	(171)
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 “top 100” substance	(70)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(139)
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(171)

For yet another subset of substances, no human data were publicly available. However, exposure to these substances is important as they are used in high volumes (this being the sole criterion for inclusion in this list) and, therefore their hazard with regard to contact sensitisation should be examined.

Table 7-4: Fragrance substances lacking human data and used in high volumes according to industry information.

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**
ANISALDEHYDE	123-11-5
BENZYL ACETONE	2550-26-7
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0
CITRONELLYL NITRILE	51566-62-2
CYCLAMEN ALDEHYDE	103-95-7
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0
DECANAL	112-31-2
DIHYDROMYRCENOL	18479-58-8
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	68039-49-6
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6
DIPHENYL ETHER	101-84-8
ETHYL 2-METHYLBUTYRATE	7452-79-1
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6
ETHYLENE BRASSYLATE	105-95-3
EUCALYPTOL	470-82-6
GERANYL ACETATE	105-87-3
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0
HEXYL ACETATE	142-92-7
IONONE isomeric mixture	8013-90-9
ISOAMYL ACETATE	123-92-2
ISOBERGAMATE [#]	68683-20-5
Longifolene	475-20-7
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0
METHYLBENZYL ACETATE	93-92-5
METHYL DECENOL	81782-77-6
METHYL beta-NAPHTHYL ETHER	93-04-9
METHYLUNDECANAL	110-41-8
OXACYCLOHEXADECENONE	34902-57-3
PENTADECALACTONE	106-02-5
PHENETHYL ACETATE	103-45-7
PHENOXYETHYL ISOBUTYRATE	103-60-6
PHENYLISOHEXANOL	55066-48-3
Tetrahydrolinalool	78-69-3
TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	63500-71-0

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
TRICHLOROMETHYL PHENYL CARBINYL ACETATE	90-17-5
TRICYCLODECENYL PROPIONATE	17511-60-3
TRIMETHYLHEXYL ACETATE	58430-94-7
gamma-UNDECALACTONE	104-67-6
VERDYL ACETATE	2500-83-6/ 5413-60-5

7.2. Tabular summary of evaluated natural extracts/essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic the demand for perfumes based on natural materials is considerable (189).

The three main methods used to concentrate plant fragrance substances (190); distillation, mechanical separation ("pressing"), and solvent extraction, yield very different extracts. Essential oils are obtained by water steam, water, ethanol, or water/ethanol distillation. Essence oils are essential oils that separate from the aqueous phase in the distillation receiver during the distillative concentration of fruit, usually citrus, juices. Citrus peel oils, apart from distilled lime oil, are prepared in a special way by pressing the peel to release mostly volatile substances from the pericarp in small oil glands, mostly highly volatile terpene hydrocarbons. However, they also contain small amounts of non-volatile compounds such as dyes, waxes and furocoumarins. The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concretes, an extract of fresh plant material with nonpolar solvents, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolutes, which are prepared by taking up concretes in ethanol; compounds that precipitate on cooling are removed by filtration, yielding a wax-free residue called absolute. Resinoids, used for their fixative properties, are prepared by extracting plant exudates with alcohols or nonpolar solvents. The products are usually highly viscous and thus sometimes diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are concentrates prepared from spices by solvent extraction (189).

An ISO norm exists regarding the nomenclature of aromatic natural raw materials (ISO/DIS 9235 Aromatic raw materials - vocabulary; International Standardisation Organisation, Geneva, Switzerland). This nomenclature has been considered in Annex I, whereas in the present opinion, nomenclature is according to the CosIng database. Concerning extraction processes for many essential oils, ISO standards exist; for detailed information see Annex I to this opinion.

Regarding clinical data in terms of contact allergy to essential oils and natural extracts, the main focus is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g. masseurs, physiotherapists (191, 192), aromatherapists (193-197), beauticians performing massages (198). For further details, e.g. PT results with various essential oils, see Annex I.

In this section, a tabular overview on the classification of substances considered is presented in three tables listing:

1. Extracts identified as *established contact allergens* in humans(→ *sufficient human evidence present*).

2. Extracts with positive human data, which are, however, not sufficient to categorise as *established contact allergen* in humans (→ *limited human evidence present*).
3. Extracts with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

In Table 7-5, essential oils with sufficient human evidence to categorise these as *established contact allergens* in humans are presented.

Table 7-5: Natural extracts classified as established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion, including variants of botanical nomenclature.

INCI name (or, if none exists, $perfuming name according to CosIng[4]) in bold; plant part / type of extract (partly indicative) in plain font	**CAS number**	**Comment: see text**
CANANGA ODORATA and Ylang-ylang oil	83863-30-3; *8006-81-3*	+++
CEDRUS ATLANTICA *BARK OIL*	*92201-55-3; 8000-27-9*	++
CINNAMOMUM CASSIA *LEAF OIL* ***CINNAMOMUM ZEYLANICUM*** *BARK OIL*	*8007-80-5* *84649-98-9*	++ (r.t.)
CITRUS AURANTIUM AMARA *FLOWER / PEEL OIL*	*8016-38-4; 72968-50-4*	++
CITRUS BERGAMIA *PEEL OIL EXPRESSED*$	*89957-91-5*	+ (r.t.)
CITRUS LIMONUM *PEEL OIL EXPRESSED* #	*84929-31-7*	++
CITRUS SINENSIS *(syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED*$	*97766-30-8; 8028-48-6*	++
CYMBOPOGON CITRATUS / SCHOENANTHUS *OILS*	*89998-14-1; 8007-02-1; 89998-16-3*	++
EUCALYPTUS SPP. *LEAF OIL*$	*92502-70-0; 8000-48-4*	++
EUGENIA CARYOPHYLLUS *LEAF / FLOWER OIL*	*8000-34-8*	+++
EVERNIA FURFURACEA *EXTRACT [5](Tree moss)*	*90028-67-4*	+++
EVERNIA PRUNASTRI *EXTRACT (Oak moss)* #	*90028-68-5*	+++
JASMINUM GRANDIFLORUM / OFFICINALE	*84776-64-7; 90045-94-6; 8022-96-6*	+++
JUNIPERUS VIRGINIANA	*8000-27-9; 85085-41-2*	++
LAURUS NOBILIS	*8002-41-3; 8007-48-5; 84603-73-6*	++
LAVANDULA HYBRIDA	*91722-69-9*	+ (r.t.)
LAVANDULA OFFICINALIS$	*84776-65-8*	++
MENTHA PIPERITA	*8006-90-4; 84082-70-2*	++
MENTHA SPICATA	84696-51-5	++
MYROXYLON PEREIRAE*(Balsam of Peru)* #	8007-00-9	++++

[4] http://ec.europa.eu/consumers/cosmetics/cosing/

[#] 76/768/EEC Annex III, part 1

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INCI name (or, if none exists, $perfuming name according to CosIng[4]) in bold; plant part / type of extract (partly indicative) in plain font	**CAS number**	**Comment: see text**
NARCISSUS SPP.	*diverse*	++
PELARGONIUM GRAVEOLENS	*90082-51-2; 8000-46-2*	++
PINUS MUGO/ PUMILA #	*90082-72-7 / 97676-05-6*	++
POGOSTEMON CABLIN	*8014-09-3; 84238-39-1*	++
ROSE FLOWER OIL (ROSA SPP.)	*Diverse*	++
SANTALUM ALBUM	*84787-70-2; 8006-87-9*	+++
TURPENTINE *(oil)* #	8006-64-2; 9005-90-7; 8052-14-0	++++
VERBENA absolute #	8024-12-2	++

Notes: r.t. Rarely tested.

Table 7-6 lists a number of essential oils, mostly tested in just one clinical department, and thus, or for other reasons, not satisfying the criteria for being categorised as *established contact allergen* in humans (i.e. *limited human evidence present*).

Table 7-6: Natural extracts with positive human data, which are, however, not sufficient to categorise as "established contact allergen in humans". More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	**CAS number**	**Comment**	**Ref.**
ACORUS CALAMUS *ROOT OIL*	*84775-39-3*	n=7 pos. reactions to "calamus"	(199)
CEDRUS DEODARA *WOOD OIL*	*91771-47-0*	Rudzki 1976/1986 found 3 / 3 positive reactions	(199, 200).
CITRUS AURANTIUM AMARA *LEAF OIL*	*72968-50-4*	Several cases in 2 series from 1 centre	(199, 200)
CITRUS TANGERINA *…*	*223748-44-5*	1 case	(201)
CYMBOPOGON NARDUS / WINTERIANUS *HERB OIL*	*89998-15-2; 91771-61-8*	Several cases in 2 series from 1 centre	(199, 200)
ILLICIUM VERUM *FRUIT OIL*	*84650-59-9*	Cases of active sensitisation; 34% consecutive patients pos. to 1%	(202)

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	**CAS number**	**Comment**	**Ref.**
LAVANDULA SPICA	*97722-12-8*	Several cases in 2 series from 1 centre	(199, 200)
LITSEA CUBEBA	*90063-59-5*	Several cases in 2 series from 1 centre	(199, 200)
PELARGONIUM ROSEUM	*90082-55-6*	2.1% pos. of 1483 patients	(203)
ROSMARINUS OFFICINALIS	*84604-14-8*	3 cases in 2 series from 1 centre	(199, 200)
SALVIA spp.	*Diverse*	Several cases in 2 series from 1 centre	(199, 200)
TAGETES PATULA	*91722-29-1*	1 case (aromatherapist)	(193)
THYMUS spp.	*84929-51-1*	4 / 84 pos	(199)
VETIVERIA ZIZANOIDES	*8016-96-4; 84238-29-9*	1 / 200 and 9 / 86 pos.	(199, 200)

The final table is an indicative list of natural extracts which lack published human data, but which are of interest: (i) as high-volume exposure; (ii) due to published positive animal experiments; or (iii) because they contain well-known (established) contact allergens.

Table 7-7: Indicative list illustrating natural extracts containing established human allergens or having R43-lable or positive LLNA, lacking published human data.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	**CAS number**	**Comment**
CITRUS PARADISI *PEEL OIL*	8016-20-4	high volume substance, classified as R43
CYMBOPOGON MARTINI *HERB EXTRACT*	*84649-81-0*	Pos. LLNA study by RIFM: EC3 value 9.6% (204).
MENTHA ARVENSIS	68917-18-0	high volume, classified as R43
OCIMUM BASILICUM	*84775-71-3*	Pos. LLNA study by RIFM: EC3 value < 2.5% (204).
PIMENTA RACEMOSA	85085-61-6	Contains, among other substances, the established contact allergen eugenol (42-56%)
SANTALUM SPICATA	*8024-35-9*	Contains, among other substances, the established contact allergens santalols (75%) and farnesol (10%)

7.3. Conclusions

- According to the criteria described in chapter 6.3 a total of 54 individual chemicals and 28 natural extracts (essential oils) can be categorised as *established contact allergens* in humans, including all currently regulated substances.
- Of the 54 individual chemicals which are established contact allergens in humans, 12 are considered to be of special concern due to the high number of reported cases, (> 100, i.e. category +++ or ++++ in Table 7-1). These are further considered in chapter 5 (limonene and linalool) and the remainder in chapter 11. In particular one ingredient stands out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1,500 reported cases since the 1999 opinion (see also chapter 4.3.1, chapter 11.3 and Annex I).
- For an additional 33 individual chemicals (Table 7-2) and 14 natural extracts (Table 7-6), positive patch test results have been reported. However, they do not qualify for the above category, i.e.only *limited human evidence* is present.
- For a number of fragrance substances (n=18, Table 7-3) patch testing did not yield positive results. However, numbers of patients tested are generally too small to rule out the existence of clinical contact sensitisation with sufficient confidence.No clinical evidence has been identified for 39 individual chemicals that have been reported to be frequently used (Table 7-4).
- For the substances (and, if possible, also for the main constituents of the natural mixtures) with limited or no human evidence, additional animal data and/or SAR considerations are taken into account. Aggregated data for these substances are presented in chapter 13.

8. Animal data

8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test[6] and the local lymph node assay (LLNA)[7]. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH[8]. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling[9], substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol "Xi" and the risk phrase "R43: May cause sensitisation by skin contact"; or, according to the recent regulation on classification, labelling and packaging (CLP[10]) "H317: May cause an allergic skin reaction".

As yet, there is no officially validated *in vitro* test method for skin sensitisation. Therefore, for cosmetic ingredients the LLNA, the GPMT and the Buehler test have also been used in risk assessment for regulatory purposes.

Positive results from the OECD guideline animal tests mentioned above which are sufficient to classify a substance as a skin sensitiser (R43) are:

- GPMT; at least 30% of the animals have a positive response.
- Buehler test; at least 15% of the animals have a positive response.
- LLNA; at least a 3-fold increase in lymph node cell proliferative activity is induced, compared to vehicle-treated controls (stimulation index SI ≥3). For positive LLNAs, an EC3 value is calculated which gives the estimated concentration of a chemical necessary to give a 3-fold increase in proliferative activity compared to vehicle-treated controls.

Further categorisation of substances classified with R43 into three groups according to allergen potency (extreme, strong and moderate) has been proposed by a European Commission expert group on skin sensitisation (161, 205), and proposed also in the ECHA guidance document on application of the CLP criteria (162). Such categorisation is based on EC3 values in the LLNA, on intradermal induction concentration in the GPMT, and topical induction concentration in the Buehler test. The potency categories and their default concentration values based on EC3 values in the LLNA as defined in (161): extreme sensitiser (EC3 value ≤ 0.2); strong sensitiser (EC3 > 0.2 - ≤ 2); and moderate sensitiser (EC3 value > 2). When LLNA EC3 values are available from more than one study, the lowest value should normally be used. Where multiple animal data sets lead to different categorisation of the same substance, the higher potency category should apply (161, 205).

The potency categorisation of substances based on the LLNA is applied by the SCCP in risk assessment of cosmetic ingredients, particularly hair dye substances (206).

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[6] OECD Guideline for testing of chemicals. Guideline 406: Skin Sensitisation. OECD, Adopted 12 May 1981, updated 17th July 1992.

[7] OECD Guideline for testing of chemicals. Guideline 429: Skin Sensitisation: Local Lymph Node Assay. OECD, Adopted 22 July 2010.

[8] Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

[9] Directives 67/548/EEC and 1999/45/EC.

[10] Regulation No. 1272/2008.

8.1.1. LLNA data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, IFRA submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report.

Table 8-1 displays the EC3 values for fragrance substances in the report submitted by industry (164). EC3 values for some additional fragrance substances in two published reviews (165, 166) have also been included in Table 8-1. Table 8-2 presents LLNA results for oxidised substances. Full data are given in Annex II. Table 8-3 summarises the distribution of fragrance substances, by potency category, according to EC3 values.

Additional EC3 values may be available in the scientific literature. Many more animal experiments may have been performed, but have not been published.

Table 8-1: Summary of local lymph node assay (LLNA) data on 66 fragrance substances, based on a report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009 (164)) and in published reviews by Gerberick et al. 2005 (165) and Kern et al. 2010 (166), respectively. EC3 values (% and M) are given. The order of substances is by decreasing sensitisation potency as assessed by LLNA EC3 values (lowest EC3 value indicating highest potency).

Substance	CAS no.	EC3 value		Reference
		%	M	
Hexyl salicylate	6259-76-3	0.18	0.008	(164, 166)
Cinnamal	104-55-2	0.2	0.015	(164)
Methyl 2-octynoate	111-12-6	<0.5	<0.032	(164, 166)
Isoeugenol	97-54-1	0.54	0.033	(164)
Citral	5392-40-5	1.2	0.079	(164)
2-Hexylidene cyclopentanone	17373-89-6	2.4	0.14	(164)
Methyl octine carbonate	111-80-8	2.5	0.15	(164)
Peru balsam absolute	8007-00-9	2.5	n/a	(164)
trans-2-Hexenal	6728-26-3	2.6	0.26	(164)
Benzyl Salicylate	118-58-1	2.9	0.23	(164, 166)
Butylphenyl methylpropional (BMHCA)	80-54-6	2.9	0.14	(164)
Phenylacetaldehyde	122-78-1	3	0.25	(164, 165)
Allyl phenoxyacetate	7493-74-5	3.1	0.16	(164)
Benzylideneacetone	122-57-6	3.7	0.25	(165)
3-Propylidenephthalide	17369-59-4	3.7	0.21	(164, 165)
Evernia prunastri extract oak moss	90028-68-5	3.9	n/a	(164)
Balsam oil, Peru *(Myroxylon pereirae Klotzsch)*	8007-00-9	4	n/a	(164)
Farnesol	4602-84-0	4.1	0.18	(164)
p-t-Butyl-dihydrocinnamaldehyde	18127-01-0	4.3	0.23	(164)
α-Methyl cinnamic aldehyde	101-39-3	4.5	0.31	(164, 165)

Substance	CAS no.	EC3 value		Reference
		%	M	
Eugenol	97-53-0	5.3	0.32	(164)
Hexyl cinnamal	101-86-0	5.3	0.25	(164)
Dihydrocoumarin	119-84-6	5.6	0.38	(165)
Geraniol	106-24-1	5.6	0.36	(164)
Carvone	6485-40-1	5.7	0.38	(164)
Diethyl maleate	141-05-9	5.8	0.34	(165)
2-Methoxy-4-methylphenol	93-51-6	5.8	0.42	(164, 165)
Anise alcohol	105-13-5	5.9	0.43	(164, 166)
Jasmine absolute *(Grandiflorum)*	8022-96-6	5.9	N/a	(164)
Dibenzyl ether	103-50-4	6.3	0.32	(164)
Cananga odorata leaf/flower oil ylang ylang “extra”	8006-81-3	6.8	N/a	(164)
Isocyclocitral	1335-66-6	7.3	0.48	(164)
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	7.5	0.50	(165)
Amyl cinnamal	122-40-7	7.6	0.38	(164)
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	8.1	0.54	(164, 165)
p-Isobutyl-α-methyl hydrocinnamaldehyde	6658-48-6	9.5	0.46	(164)
d-Limonene*	5989-27-5	<10	<0.73	(164)
Methylundecanal	110-41-8	10	0.54	(165)
Acetylcedrene	32388-55-9	13.9	0.57	(166)
Methylenedioxyphenyl methylpropanal	1205-17-0	16.4	0.85	(164, 166)
Benzyl benzoate	120-51-4	17	0.80	(165)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	17.1	0.81	(164, 165)
Benzyl cinnamate	103-41-3	18.4	0.77	(164, 166)
Hydroxycitronellal	107-75-5	19.3	1.12	(164)
Cinnamyl alcohol	104-54-1	21	1.57	(165)
α-iso-Methylionone	127-51-5	21.8	1.06	(164, 166)
Cyklamen aldehyde	103-95-7	22	1.64	(165)
4-Methoxy-α-methyl benzenpropanal	5462-06-6	23.6	1.32	(164)
Amyl cinnamyl alcohol	101-85-9	~25	~1.22	(164, 166)
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	25.1	1.07	(164)
Ethyl acrylate	140-88-5	28	2.8	(165)
Linalool*	78-70-6	30	1.94	(165)
Trimethylbenzenepropanol Majantol	103694-68-4	30	~1.68	(164)
Jasminum Sambac Flower CERA/Extract/Water	91770-14-8	35.4	N/a	(164)

Substance	CAS no.	EC3 value		Reference
		%	M	
Citronellol	106-22-9	43.5	2.78	(164, 166)
No EC3 value was established; higher concentrations should also have been tested				
6-Methyl-3,5-heptadien-2-one	1604-28-0	>5	>0.40	(164)
Camellia sinensis leaf tea leaf absolute	84650-60-2	>5	N/a	(164)
Cinnamyl nitrile	1885-38-7	>10	>0.77	(164)
Menthadiene-7-methyl formate	68683-20-5	>10	>0.51	(164)
Evernia furfuracea extract tree moss absolute	90028-67-4	>20	N/a	(164)
Isocyclogeraniol	68527-77-5	>25	>1.62	(164)
1-Octen-3-yl acetate	2442-10-6	>30	>1.76	(164)
Benzyl alcohol	100-51-6	>50	>4.62	(164)
Coumarin	91-64-5	>50	>3.42	(164)
Vanillin	121-33-5	>50	>3.3	(164)
No EC3 value calculated				
Benzaldehyde	100-52-7	-		(165)

Notes: * Material with low levels of oxidation according to (164)
n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

8.1.2. LLNA data on oxidised fragrance substances

For fragrance substances that can autoxidise upon air exposure, it is also important to investigate the sensitisation potency after air exposure. The oxidised compounds are clinically relevant as they represent what the consumers could come in contact with from perfumes and fragranced products. In Table 8-2 the LLNA data for some of the most commonly used fragrance substances, pure and after autoxidation, are presented. The EC3 values obtained for the pure substances are 5-10 times higher compared to those obtained for the same substances after air exposure. The experimental air exposure simulated air exposure that can take place during normal handling and storage. In the production process, some perfumes are “matured” aerobically, stirring included. During this process, some fragrance substances may be oxidised. It should be noted that, although only a few substances capable of oxidation have so far been investigated, structural alerts indicating possible autoxidation are common among the fragrance substances listed in this document (see chapter 9). It is important to further investigate this issue for increased understanding of the associated risk.

Table 8-2: Local lymph node assay (LLNA) data on four fragrance substances and one essential oil before and after air exposure, comparing the sensitisation potency of the pure (not oxidised) substance with the potency of the oxidised.

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
D-Limonene (ox. 10 w)	5989-27-5	1, 5, 25	3.0	(207)
D-Limonene (pure)	5989-27-5	25, 50, 100	30	

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
Linalool (ox. 10 w)	78-70-6	5, 10, 25	9.4	(127)
Linalool (ox. 45 w)	78-70-6	2.5, 10, 25	4.8	
Linalool (pure)	78-70-6	25, 50, 100	46.2	
Linalyl acetate (ox. 10 w)	115-95-7	0.5, 10, 40	3.6	(128)
Linalyl acetate (pure)	115-95-7	10, 30, 100	25	
Geraniol (ox. 10 w)	106-24-1	1, 3, 6, 10, 20	4.4	(119)
Geraniol (ox. 45 w)	106-24-1	0.5, 1, 3, 6, 10	5.8	
Geraniol (pure)	106-24-1	5, 10, 15, 20, 30	22.4	
Lavender oil (ox. 10 w)		1, 5, 10, 20, 50	11	(140)
Lavender oil (ox. 45 w)		1, 5, 10, 20, 50	4.4	
Lavender oil (not ox.)		5, 25, 100	36	

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.
Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.
Ox. x w: Oxidised by air exposure during x weeks.
* Acetone:olive oil.

8.2. Methodological considerations

EC3 mean values

In the submitted poster (163) and the report by IFRA (164), the LLNA weighted mean EC3 values (µg/cm^2) are presented. The SCCS considers it is misleading to present EC3 values as mean values from tests performed with different vehicles. It is generally agreed that the lowest EC3 value should be used if there is more than one study fulfilling the OECD guideline requirements (161, 205), and these have been introduced into Table 8-1. The EC3 values in the reviews by Gerberick et al. and Kern et al. (165, 166) were based on single representative experiments with a vehicle described in the OECD guideline 429 (see above), and preferably with acetone:olive oil. EC3 mean values, as in the submission by IFRA, were not presented in these two reviews.

Vehicle

The most frequently used *vehicle* in the submission by IFRA (164) was ethanol:diethyl phthalate (1:3), followed by acetone:olive oil (4:1). In some experiments, antioxidants were mixed with ethanol:diethyl phthalate. The vehicle was not reported in some of the references, and no rationale for using vehicles other than those recommended was given in the report (164). According to the OECD guideline 429 (see above), the recommended vehicles are acetone:olive oil (4:1), N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulphoxide, but others may be used if sufficient scientific rationale is provided. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus as an *additional control*, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.

Number of doses and animals

According to the OECD guideline 429 (see above), a minimum of three concentrations should be tested. The number of consecutive doses used in the reported data, was generally five, sometimes three and in few experiments two. The SCCS considers that too few concentrations were tested in four studies in which only two concentrations were used. Lower concentrations than those tested should have been used in experiments with five fragrance substances, in which the EC3 value could not be determined. Higher concentrations than those tested should also have been used in experiments with 12 substances, in which the EC3 value could not be determined.

The *number of animals* per dose group was generally four plus a non-exposed control group, sometimes five, and in few experiments six; the minimum according to the OECD guideline being four.

Units for concentrations

In the submission by IFRA (164) the EC3 values are given in weight per area unit (µg/cm^2). The SCCS considers that the EC3 values (%) are the values of primary interest in communicating risk assessment, as EU legislation, OECD guideline 429 and scientific literature refer to EC3 values (%). However, the SCCS recommends that molar (M) EC3 values should be considered, as they give the concentration based on the molecular weight of substances. They have thus been calculated and introduced into Table 8-1.

EC3 values (%) overestimate the intrinsic molecular sensitisation potency for low molecular weight compounds while compounds with a high molecular weight are underestimated. Regarding the differences in molecular weight between the studied fragrance substances, a variation is seen if the ranking list of the sensitisation potency is based on EC3 (%) or EC3 (M) since some substances have a molecular weight twice as high as others.

From comparisons in Table 8-1, we notice that, e.g. hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has an EC3 value of 17.1 %, or 0.81 M when the calculation includes its molecular weight, while for trans-2-hexenal the corresponding values are 2.6% and 0.26 M. The example shows that comparing the sensitisation potency between these two substances using the EC3 values in % exaggerates the sensitisation potency of trans-2-hexenal compared to that of HICC. When using the EC3 values in molar concentrations the difference is not so pronounced.

8.3. Summary of animal data by LLNA

The distribution of sensitising potency of fragrance substances compared to other substances, (e.g. biocides, dyes, plastic materials) taken from three references (164-166) as assessed by EC3 values in the LLNA, is shown in Figure 8-1 and Table 8-3.

For 10 substances, no EC3-value could be established. These should have been tested at higher concentrations – some of these would most probably have generated an EC3 value. However, we reported here “No EC3 value established”. 5 substances should have been tested also at lower concentration and in these cases the EC3 value could have been lowered, meaning a more severe potency category could have been achieved. In all, approx 150 experiments were reported in (164), listed in Annex II.

The median EC3 value of evaluable fragrance substances (5.9%) is similar to other substances tested (5.5%). However, very few fragrance substances have low EC3 values (≤ 2).

Substances with an EC3 value ≤2 may be categorised as strong or extreme sensitisers. Such potent sensitisers are comparatively rare among fragrance substances assessed in the LLNA. Nevertheless, fragrances are important allergens in humans, which points to repeated skin exposure to less potent sensitisers as a factor strongly determining sensitisation risk.

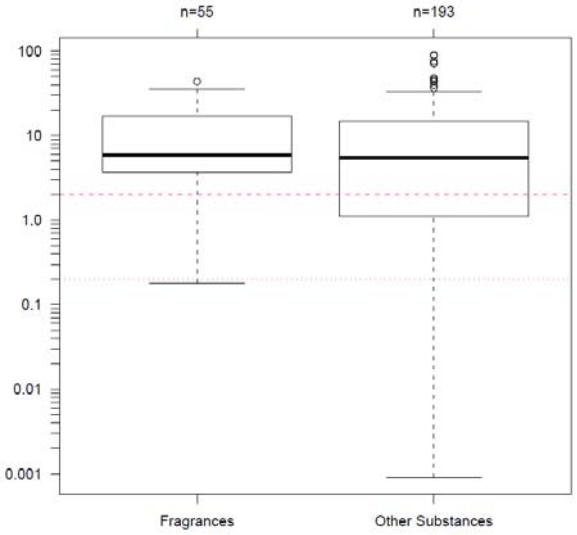


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in the box the median, and the top of the box the 3rd quartile (75% percentile). Outliers, i.e. below the 25% and above the 75% percentiles, are shown as whiskers. Beyond the 1.5-fold interquartile range, single values are shown as circles instead of whiskers. The difference in distribution is not significant (Wilcoxon test: p=0.061).

Note: EC3 values for the five oxidised fragrances additionally examined (Table 8-2) range from 3.0 to 4.8 (median 4.4) and are lower by a factor of around 7 than EC3 values of the respective non-oxidised material.

Table 8-3: Summary of EC3 values for fragrance substances in Table 8-1 and for other substances, all taken from the three references (164-166). The EC3 value intervals for potency categorisation (161, 205) were used for comparison of fragrances substances vs other substances.

EC3 value interval	Fragrance substances		Other substances	
	n	%	n	%
≤ 0.2	2	3%	28	11%
> 0.2 - ≤ 2	3	4%	38	15%
> 2	50	71%	127	49%
No EC3 value established *	10	14%	0	0%
No EC3 value calculated (NC)	5	7%	69	26%
All substances	**70**		**262**	

Note: * Substances should have been tested also at higher concentrations.

8.4. Conclusions

- In the event that human data are lacking, the LLNA provides important information on skin sensitising potential and potency.
- Animal data on fragrance substances submitted by IFRA (164) and assessed in this opinion were generated exclusively by LLNA. Other guideline methods are, however, also available.
- The vast majority of the submitted (164) and additional (165, 166) fragrance substances tested by the LLNA are skin sensitisers.
- Several studies in the IFRA report (164) were of insufficient quality, not following the OECD guideline.

- Fragrance substances that can be predicted to autoxidise upon air exposure should also be tested after air exposure, as oxidation may significantly increase their sensitising potency.
- It can be concluded that the skin sensitising potency, as assessed by the LLNA, is only one of several factors that are of importance for sensitisation to fragrance substances. This is illustrated by the fact that only a small fraction of sensitising fragrance substances can be categorised as an extreme allergen based on LLNA test results. Therefore, doses from repeated deposition onto skin must be considered a driving force of sensitisation risk.

9. Structure activity relationships (SAR): grouping of substances based on expert judgement

Whether or not a particular chemical will be a sensitiser, and how potent it will be if it is a sensitiser, depends on its ability, either directly or after activation, to react with appropriate proteins in the skin. This fundamental concept was intitially demonstrated by Landsteiner and Jacobs in 1936 (208) and subsequently validated by numerous studies with various types of chemicals (some key references: (209-213)). The ability to predict sensitisation potency, or lack of it, depends on being able to predict reactivity to skin proteins. This is the basis of SAR analysis for skin sensitisation. The prediction can often be made based on the chemical structure, recognising structural features (referred to as **structural alerts**) that are associated with reactivity.

The relationships between molecular structure and reactivity that form the basis for structural alerts are based on well established principles of mechanistic organic chemistry (214). Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and α,β-unsaturated carbonyl groups, C=C-CO- (alerting to the possibility of sensitisation via Michael addition of protein thiol groups). Major mechanistic reactivity domains have been discussed in detail by Aptula and Roberts (215). Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) is more complex compared to that of compounds that act as direct haptens without any activation. The autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers linalool and geraniol results in different major haptens/allergens. Moreover, the complexity of the prediction increases further for those compounds that can act both as pre- and prohaptens. In such cases, the impact on the sensitisation potency depends on the degree of abiotic activation (e.g. autoxidation) in relation to the metabolic activation.

These structural alerts can be applied by computerized expert systems, i.e. *in silico* or by estimations made by organic chemists (*in cerebro)* using their experience. When an organic chemist looks at a chemical structure, they recognise parts of the structure that they can associate with reactivity, the type of reactivity (i.e. assign the reaction mechanistic domain), and other features of the molecular structure that will affect the reactivity positively or negatively. Human experts should be aware of the complexities, and how structural modification can alter the reactivity associated with structural alerts, etc. Importantly, they can also recognise where there are unfamiliar structural features whose effects they cannot confidently predict. In such cases they can call for experimental chemistry work (*in chemico*) to be done to ascertain the presence or nature of, and degree of reactivity. *In chemico* methods include organic chemistry experimentation to identify chemical reaction products from oxidation and/or reaction with model nucleophiles, identification of mechanisms of reaction. In so called *in chemico* reactivity methods, the ability of a specific chemical to react with selected peptides is determined so as to predict the sensitisation potential of the chemical under investigation (216, 217). To make *in chemico* reactivity methods able to predict the activity of prohaptens, the addition of horseradish peroxidase and hydrogen peroxide oxidation system has been tested to model the enzymatic oxidation in the skin (218, 219).

Although computerized expert systems are derived from input by human experts, they are less well able to capture the subtleties of structure reactivity relationships, and they sometimes fail to detect aspects of chemistry that are obvious to organic chemists. Human experts should be aware of the complexities, as well as how structural modification can alter the reactivity associated with structural alerts, etc.

The SAR evaluation made in the section below is based on *in cerebro* alerts applied by organic chemists.

Depending on the type of reactivity (the **reaction mechanistic domain**), it is sometimes possible to make a quantitative prediction of potency in the LLNA, which can be used to predict potency in humans relative to related known human sensitisers. These predictions use quantitiative mechanistic models (**QMMs**) based on reactivity expressed quantitatively

by model parameters, and sometimes in combination with hydrophobicity. For example, potency of aliphatic aldehydes and ketones (the Schiff base domain) in the LLNA is modelled by a combination of reactivity and hydrophobicity (220), whereas the LLNA potency of DNCB analogues (the S_NAr domain) is well modelled by reactivity alone (221).

QMMs aiming not only to predict the potential to be a sensitiser but also to predict the potency, promise to be a useful tool in non-animal based risk assessment for skin sensitisation. However, in the field of fragrance substances there are major gaps in our present ability to apply QSAR/QMM. This is largely because many of the fragrance substances of interest have the potential to act via abiotic or metabolic activation (pre- and/or prohaptens, i.e. they themselves are only weak or non-sensitisers, but have the potential to be activated to form more potent sensitisers. Resulting sensitisation potency will depend on the extent of activation and the nature of the resulting products. It is possible to apply SAR analysis to identify these plausible possibilities, but QSAR modelling for these cases is not yet developed. However, much progress has been made in identifying structural alerts for the various activation mechanisms that have been recognised. This is reviewed by Karlberg et al. (122).

Chemicals with no structural alerts for direct reactivity, or for known activation mechanisms, and no unfamiliar structural features that might be associated with as yet unidentified activation mechanisms, can be predicted to be non-sensitising. Chemicals that do have alerts for reactivity (direct or via activation) are not necessarily sensitisers – they may be insufficiently reactive and/or insufficiently hydrophobic.

Substances meeting the inclusion criteria (see chapter 6), for which, however, no categorisation as established contact allergen in humans or established contact allergen in animals was possible, have been assessed for structural alerts. The results are presented in four tables based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitiser; structural alerts:
  Compounds containing structural alerts comprising direct reactive compounds and for compounds that after specific abiotic or metabolic activation (prohaptens and prehaptens) can be predicted to be sensitisers by structural comparison to known allergens.
- Possible sensitiser; structural alerts:
  Compounds containing structural alerts that by comparison to known allergens with similar structures were expected to be less reactive and hence less likely to be sensitising. Also compounds with structural alerts indicating a possible abiotic or metabolic activation (possible prehaptens or prohaptens) but with no structural data available for comparison, were included in this group. Consequently, a possible sensitiser may turn out to be a non sensitiser when tested in vivo.
- Predicted non-sensitiser (NS); no obvious structural alerts
- Not predictable due to insufficient/conflicting data

Table 9-1: Predicted sensitisers.

Substance (INCI) name	CAS number	Structural alerts
p-tert.-Butyldihydrocinnamaldehyde[§]	18127-01-0	Schiff base
Citronellal	106-23-0	Schiff base and possible prehapten
Citronellyl nitrile	51566-62-2	Possible prehapten
Decanal	112-31-2	Schiff base
3,7-Dimethyl-1,6-nonadien-3-ol	10339-55-6	Prehapten
Geranyl acetate	105-87-3	Prehapten and prohapten

Isoamyl salicylate	87-20-7	Acyltranfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehapten
Nerol	106-25-2	Prehapten and prohapten
Nerolidol (isomer not specified)	7212-44-4	Possible prehapten
Oxacyclohexadecenone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehapten

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

Substance (INCI) name	**CAS number**	**Structural alerts**
Ambrettolide	7779-50-2	Possible prehapten
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohapten via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehapten
Cuminaldehyde	122-03-2	Schiff base and possible prehapten
alpha-Cyclohexylidene benzeneacetonitrile	10461-98-0	Possible Michael acceptor
Cyclopentadecanone	502-72-7	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
trans-beta-Damascone	23726-91-2	Possible Michael acceptor
trans-trans-delta-Damascone	71048-82-3	Possible Michael acceptor and possible prehapten
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehapten
Dihydromyrcenol	18479-58-8	Possible prehapten
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehapten and possible prohapten
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehapten
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehapten
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehapten
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehapten
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohapten
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl	54830-99-8	Possible prehapten

Substance (INCI) name	CAS number	Structural alerts
acetate		
Hexahydro-methanoindenyl propionate	68912-13-0	Possible prehapten
Ionone isomeric mixture	8013-90-9	Possible Michael acceptor and possible prehapten
alpha-Ionone	127-41-3	Possible Michael acceptor and possible prehapten
beta-Ionone	79-77-6	Possible Michael acceptor
Isobergamate	68683-20-5	Possible prehapten
Isolongifoleneketone	33407-62-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Longifolene[§]	475-20-7	Possible prehapten
Methoxycitronellal	3613-30-7	Schiff base
Methyl decenol	81782-77-6	Possible prehapten
Methyl ionone (mixture of isomers)	1335-46-2	Possible Michael acceptor and possible prehapten
Methylionantheme	55599-63-8	Possible Michael acceptor and possible prehapten
5-Methyl-alpha-ionone	79-69-6	Possible Michael acceptor and possible prehapten
Myrtenol	515-00-4	Possible prehapten
Nopyl acetate	128-51-8	Possible prehapten
Phytol	150-86-7	Possible prehapten and/or prohapten
Rhodinol	6812-78-8	Possible prehapten
Terpineol acetate (isomer mixture)	8007-35-0	Possible prehapten
alpha-Terpinyl acetate	80-26-2	Possible prehapten
Tricyclodecenyl propionate	17511-60-3	Possible prehapten
Verdyl acetate	2500-83-6/ 5413-60-5	Possible prehapten

Note: [§] Classified as R43.

Table 9-3: Predicted non-sensitisers with no obvious structural alerts.

Substance (INCI) name	CAS number	Structural alerts
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	
Benzyl acetone	2550-26-7	Schiff base; the combination of reactivity and hydrophobicity may **not** be enough to confer sensitisation
2-tert.-Butylcyclohexyl acetate	88-41-5	
4-tert.-Butylcyclohexyl acetate	32210-23-4	
Cyclohexyl acetate	622-45-7	
Diphenyl ether	101-84-8	

Substance (INCI) name	CAS number	Structural alerts
Ethyl 2-methylbutyrate	7452-79-1	
Ethylene dodecanioate	54982-83-1	
Ethylene brassylate	105-95-3	
Eucalyptol	470-82-6	
Hexyl acetate	142-92-7	
Hibiscolide	6707-60-4	
Hydroxycitronellol	107-74-4	However, dehydration followed by autoxidation could give sensitising impurities
Isoamyl acetate	123-92-2	
Isobornyl acetate	125-12-2	
Methoxytrimethylheptanol	41890-92-0	
Methyl p-anisate	121-98-2	
Methyl anthranilate	134-20-3	
Methylbenzyl acetate	93-92-5	
Methyl dihydrojasmonate	24851-98-7	Schiff base; the combination of reactivity and hydrophobicity may **not** be enough to confer sensitisation
Oxalide	1725-01-5	
Pentadecalactone	106-02-5	
Phenethyl acetate	103-45-7	
Phenethyl alcohol	60-12-8	
Phenoxyethyl isobutyrate	103-60-6	
Phenylisohexanol	55066-48-3	
Phenylpropanol	122-97-4	
Tetrahydrolinalool	78-69-3	
Tetrahydro-methyl-methylpropyl)-pyran-4-ol	63500-71-0	
Trimethylhexyl acetate	58430-94-7	
Trimethyl-propylcyclohexanepropanol (tmch)	70788-30-6	
gamma-Undecalactone	104-67-6	

Table 9-4: Not predictable.

Substance (INCI) name	CAS number	Structural alerts
Anisaldehyde	123-11-5	Due to insufficient /conflicting data; structural similarities to benzaldehyde suggest certain activity in man
Trichloromethyl phenyl carbinyl acetate	90-17-5	Due to insufficient /conflicting data
Methyl beta-naphthyl ether	93-04-9	Due to insufficient /conflicting data

9.1. General results

From this work with the included SAR predictions, the following observations can be made.

- For substances for which sufficient experimental/clinical evidence is missing, SAR analyses have been performed to predict a probable or possible risk of allergenic (sensitising) effect. These predictions are based on chemical reactivity and the recognition of structural features in a substance that are in common with the structural features that have been shown to cause sensitisation from other substances. In cases where the SAR analysis indicates a sensitisation potential, the substance should be investigated further to confirm or reject the conclusion drawn from the SAR analysis.
- Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) becomes more complex compared to that of compounds that act as direct haptens without any activation.
- The complexity of the prediction increases further for those compounds that can act both as prehaptens and prohaptens.
- Prediction of the sensitisation potential of compounds that can act as prehaptens is further complicated by the fact that the autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers of linalool and geraniol results in different major haptens/allergens.

9.2. Conclusions

The SAR evaluation made in this section is based on *in cerebro* alerts applied by organic chemists.

- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods.
- SAR, as performed here, is only one consideration in the overall weight of evidence.

10. Exposure

Exposure to fragrance chemicals and other potential allergens is most commonly by direct skin contact. Exposures to fragrance chemicals occur from:

- Personal cosmetic use;
- Detergents and other household products;
- Medicaments;
- Occupation, i.e. personal hygiene, manufacturing ingredient(s), product in work process, plant materials;
- Secondary exposure from another individual (e.g. spouse, child);
- Toys;
- Oral intake;
- Airborne exposure.

Factors that are important for both the induction and elicitation of contact allergy are:

- Dose per unit area;
- Vehicle effects including penetration enhancers;
- Presence of skin irritants;
- Presence of other allergens (combination effects);
- Duration of skin exposure;
- Frequency of applications;
- Anatomical sites of exposure;
- Condition of the skin (barrier function impairment, pre-existing inflammation);
- Occlusion (e.g. in flexures, under clothing and personal protective equipment).

Fragrance mix ingredients are commonly present in cosmetic formulations (71, 222-224). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (225). The clinical significance of exposure to natural extracts is difficult to determine as there is often “hidden and variable” exposure to important and potent allergens in natural products.

10.1. Concentrations and quantities used

Consumers are exposed in daily life to fragrance chemicals from a large variety of products, such as cosmetics, toys, detergents and other cleaning products, etc. The fragrance exposure may be via dermal and/or inhalation route. With respect to “Terms of Reference” to the SCCS, only dermal exposure from cosmetics is addressed in this opinion. As cosmetics are the perfumed products most commonly used in daily life, potential fragrance allergens identified by the use of cosmetics also represent the exposures of these chemicals from other product categories. In recent years, it has become a trend to add fragrance chemicals to many other types of consumer products, such as children’s toys, toilet paper and nappies, which may contribute significantly to the fragrance exposure of the consumer by the dermal route.

Factors for the fragrance exposure assessment by the dermal route require knowledge on:

- Product types (categorisation of scented products) used by the consumer.
- Market survey (impression of the qualitative and quantitative contents of different allergens in consumer products).

- Hydrolysis, metabolism or oxidation of a fragrance material, which may generate a potential skin allergen.
- Chemicals in the product matrix, which may significantly enhance or reduce dermal absorption of a fragrance material.

Fragrance materials, both defined chemical substances and natural mixtures of chemicals (essential oils), are used in all types of cosmetic products: perfumes, eau de cologne, eau de perfume (EDP), and eau de toilette (EDT), aftershave lotion, deodorants, skin care products, skin cleansers, make-up cosmetics, hair care products, and oral care products, etc. However, some unscented cosmetic products have also reached the market in the last decade. Products containing the highest concentration of fragrance chemicals are perfumes, followed by eau de cologne, eau de perfume (EDP) and eau de toilette (EDT). Concentrations of fragrance chemicals in deodorant products are lower than those in EDT/EDP products, but still significant. Aftershave products also contain relatively high amounts of fragrance chemicals. Other cosmetic products contain relatively low amounts, 0.1-1% of fragrance compound, compared to up to 30% fragrance compound in EDT/EDP (226). The fragrance compound are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (226). For the exposure assessment, levels of fragrance chemicals in cosmetics containing significant amounts of fragrance materials (i.e. EDP/EDT/aftershave/deodorant) should be selected. It may not be possible to detect/measure the amounts of all fragrance chemicals when present in highly diluted form in a cosmetic product such as skin care products, make-up cosmetics etc. On the other hand, if a fragrance is evaluated safe for use when present in significant amounts in a product, it will also be safe for use in other products. Also the analysis of trend of the use of individual fragrance materials should be based on monitoring their contents in fine perfumes and deodorants.

Ninety of the 100 fragrance materials used in annual volumes > 175 tons in perfume formulations are fragrances and the remaining ten are used for other functions such as solvents or antioxidants (IFRA, personal communication 2010).

Among the 26 fragrances currently requiring individual labelling, amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone, and linalool are used in volumes greater than 175 ton. α-Amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri),* tree moss (*Evernia furfuracea*) are used in volumes less than 175 ton.

According to the information from the fragrance industry, 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products.

Since the implementation of the regulation of labelling of 26 fragrance substances in cosmetic products, qualitative information on fragrance exposure from cosmetics is provided in some market surveys performed on cosmetics (Table 10-1, (227)) and (Table 10-2, (228)) and on consumer products including cosmetics (Table 10-3, (229); Table 10-4, (115); and Figure 10-1, (105)). Thus, the implementation of the regulation of fragrance allergens in detergents (Directive 648/2004/EC), similar to that for cosmetics, has also added to the knowledge of fragrance exposure to the consumer. These market surveys revealed that fragrance ingredients which are potent allergens and frequently cause allergies in consumers are used as ingredients in consumer products including cosmetics. The results of these surveys further revealed that limonene and linalool were the most commonly used fragrance chemicals in cosmetics, while anisyl alcohol, cinnamal, α-amylcinnamyl alcohol, oak moss and tree moss were the least used fragrance ingredients in cosmetics and other consumer products. In general, the most potent allergens were also the most infrequently used ingredients. Prior to the regulation of the 26 allergens, analysis of 21 selected fragrance chemicals in deodorants also revealed additional 66 potential allergens in these products on the basis of structure activity relationship (230).

Table 10-1: Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (227).

Fragrance substance		% Products labelled to contain the fragrance substance
INCI name	**CAS number**	
Amyl cinnamal	122-40-7	8.2
alpha-Amylcinnamyl alcohol	101-85-9	2.9
Anise alcohol	105-13-5	0
Benzyl alcohol	100-51-6	9.6
Benzyl benzoate	120-51-4	9.1
Benzyl cinnamate	103-41-3	2.9
Benzyl salicylate	118-58-1	9.6
Butyl phenyl methyl propional	80-54-6	7.7
Cinnamal	104-55-2	1
Cinnamyl alcohol	104-54-1	6.7
Citral	5392-40-5	8.2
Citronellol	106-22-9	10.5
Coumarin	91-64-5	4.8
Eugenol	97-53-0	7.2
Farnesol	4602-84-0	2.9
Geraniol	106-24-1	12
Hexyl cinnamal	101-86-0	10.1
Hydroxycitronellal	107-75-5	6.3
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	31906-04-4	5.8
Isoeugenol	97-54-1	0.5
Alpha-isomethyl ionone	127-51-5	5.8
d-Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
Evernia prunastri/oak moss	90028-68-5	0
Evernia furfuracea/tree moss	90028-67-4	0

Table 10-2: Usage trends in deodorants of fragrance chemicals that are required to be labelled in cosmetics.

Fragrance substance		**88 products investigated in 2007 (228)**			**70 products investigated in 1998 (231)**	
INCI name	**CAS number**	**% Products labelled to contain the fragrance**	**Content in 23 selected products**		**Content in all 70 products**	
			% Products found to contain the fragrance	**Range(ppm)**	**% Products found to contain the fragrance**	**Range (ppm)**
Amyl cinnamal▪	122-40-7	10.2	17	2.3-165	31	1-617
alpha-amyl cinnamyl alcohol	101-85-9	-	-	-	n.a.	n.a.
Anise alcohol	105-13-5	2.3	9	1, 51	n.a.	n.a.
Benzyl alcohol	100-51-6	17.1	26	32-166	76	1-629*
Benzyl benzoate	120-51-4	25.0	48	3-4054	71	1-1075
Benzyl cinnamate	103-41-3	3.4	9	74, 143	n.a.	n.a.
Benzyl salicylate	118-58-1	39.8	48	136-5279	49	1-18758
Butyl phenyl methyl propional	80-54-6	48.9	70	1-5455	51	1-3732
Cinnamal▪	104-55-2	1.1	4	5	17	1-424
Cinnamyl alcohol▪	104-54-1	12.5	48	2-503	39	6-1169
Citral▫	5392-40-5	26.1	44	39-554	n.a.	n.a.
Citronellol▫	106-22-9	65.9	91	1-5848	81	1-5585
Coumarin▫	91-64-5	33.0	52	3.8-1255	57	1-1411
Eugenol▪	97-53-0	27.3	30	1-514	57	1-2355
Farnesol▫	4602-84-0	14.8	39	9-1791	n.a.	n.a.
Geraniol▪	106-24-1	48.9	87	1-399	76	1-1178

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
Hexyl cinnamal▫	101-86-0	33.0	48	1-4434	71	2-1684
Hydroxycitronellal▪	107-75-5	27.3	70	1-1746	50	1-1023
HICC▫	31906-04-4	33.0	74	1-4431	53	1-1874
Isoeugenol▪	97-54-1	9.1	35	1-138	29	1-458
Alpha-isomethyl ionone	127-51-5	46.6	65	6-2588	61	1-2765
D-Limonene◦	5989-27-5	53.4	70	1022-11386	n.a.	n.a.
Linalool◦	78-70-6	53.4	96	8-3447	97	9-1927
Methyl-2-octynoat◦	111-12-6	1.1	-	-	n.a.	n.a.
Evernia prunastri▪/oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
Evernia furfuracea▪/tree moss	90028-67-4	2.3	n.a.	n.a.	n.a.	n.a.

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.
- Fragrance not detected in any product.
n.a. Not analysed.
* Benzyl alcohol could not be determined in 49% of the products due to interference.

The most common fragrance allergens are contained in the two mixtures, which are used for diagnosing fragrance allergy, called Fragrance Mix I (▪) and Fragrance Mix II (▫), besides the oxidation product of terpens (◦), and tree moss extract are common allergens. Methyl-2-octynoate is an extreme, but rare allergen.

Table 10-3: Frequency of occurrence in consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (229).

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyral™	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evernia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evernia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0·3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

INCI, International Nomenclature of Cosmetic Ingredients; PCP, personal care products; MP, men's products; HP, household products; WP, women's perfumes; Cos, cosmetics; Deo, deodorants; Dent, dental products; BPMP, butyl phenyl methyl propional; Lyral™, hydroxy-isohexyl-3-cyclohexene carboxaldehyde.

Table 10-4: Frequency in 516 consumer products of the 26 fragrance substances that are required to be labelled in cosmetics* (115).

Fragrance substance INCI name	**% Product containing the chemical**
D-Limonene	48.3
Linalool	35.8
Butyl phenyl methyl propional	24.8
Geraniol	22.1
Alpha-isomethyl ionone	21.7
Hexyl cinnamal	21.3
Citonellol	21.1
Benzyl salicylate	18.6
Coumarin	17.0
Eugenol	15.7
Benzyl alcohol	15.3
Benzyl benzoate	14.7
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	12.8

Fragrance substance INCI name	% Product containing the chemical
Citral	11.6
Hydroxycitronellal	10.8
Amyl Cinnamal	7.9
Anise alcohol	7.0
Cinnamyl alcohol	6.4
Farnesol	3.9
Isoeugenol	3.1
Cinnamal	2.5
Benzyl cinnamate	2.3
Amylcinnamyl alcohol	1.9
Methyl-2-octynoate	1.0
Evernia prunastri▪/oak moss	0.8
Evernia furfuracea▪/tree moss	0.4

Note: * Consumer Products: Cosmetics and household products with labelling of the 26 fragrance allergens. The content of these fragrances was confirmed by chemical analysis.

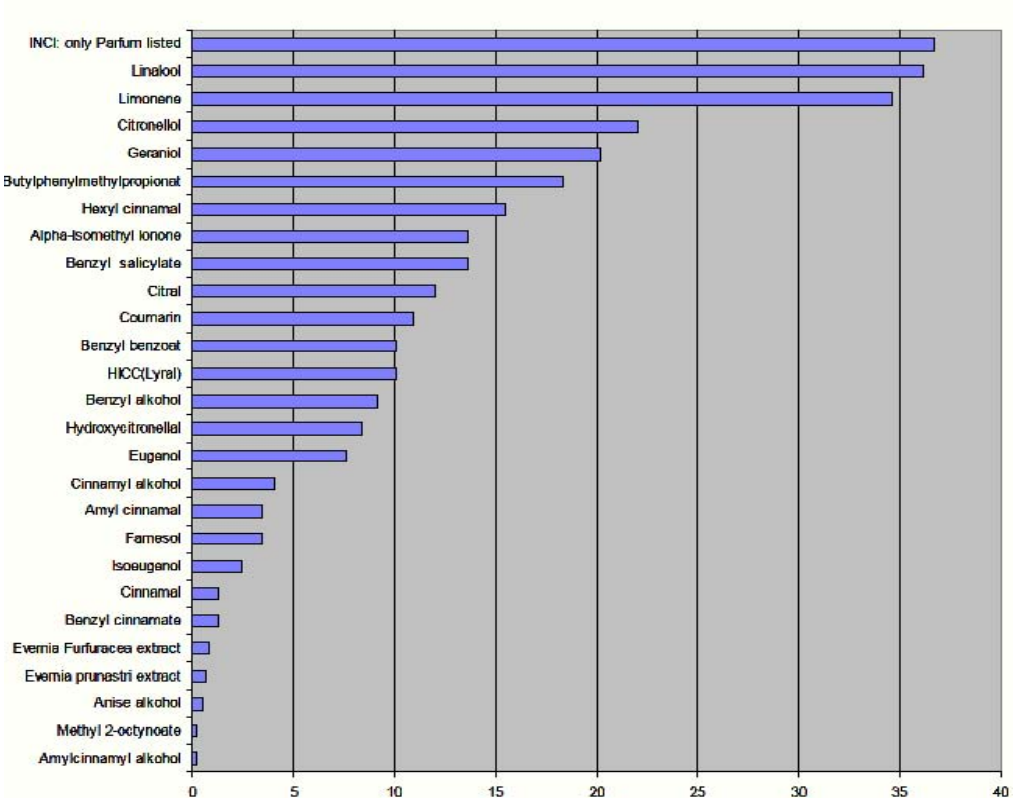


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

Contents of fragrance substances determined in cosmetic products have been described in several studies, both before and after the regulation of the 26 fragrance allergens. The studies prior to the regulation of the 26 fragrance allergens included many, but not all of these 26 allergens. On the other hand, these studies included some other possible fragrance allergens. The quantitative analysis of fragrance substances has been performed in prestige perfumes (5, 157, 232-234), deodorants (228, 231), children's cosmetics and cosmetic toys (115, 227, 235), products marketed as natural cosmetics (225) and in cosmetics used by patients with contact allergy to fragranced products (35, 71). Quantitative analyses have revealed that the consumer is exposed to most, but not all of the 26 fragrance allergens from the use of cosmetics. However, when fragrance exposure from other consumer products, for example detergents and other household products is also taken into consideration (Table 10-3, Table 10-4, Figure 10-1), (105, 115, 229, 236), exposure to all of the 26 allergens is foreseeable in daily life. Although from the data available, the exposure to α-amylcinnamyl alcohol, cinnamal, methyl-2-octynoate, *Evernia prunastri* (oak moss) and tree moss may appear to be low, these are very strong allergens.

The changes in the use of fragrance chemicals in cosmetic formulations, during last 12 years, i.e. before and after the regulation of the 26 fragrance allergens, is reflected in the studies concerning contents of fragrances substances in popular perfumes (5, 232). As described in Table 10-5, the content of FM I allergens in prestige perfumes was significantly reduced from 1996 to 2003. Whether this is also the case for the perfumes sold as natural cosmetics (Table 10-6) has not yet been investigated.

Table 10-5: Concentration of Fragrance Mix I ingredients in five prestige perfumes before and after the regulation of the 26 fragrance allergens.

Fragrance INCI name	**Concentration in the perfumes before regulation (5)**			**Concentration in the perfumes after regulation (232)**		
	In no. of perfumes	**Range % (w/w)**	**Mean % (w/w)**	**In no. of perfumes**	**Range % (w/w)**	**Mean % (w/w)**
Geraniol*	5	0.072-0.432	0.340	5	0.090-0.236	0.156
Cinnamal	2	0.002-0.002	0.002	0	-	-
Hydroxy-citronellal	5	0.222-0.979	0.615	5	0.015-0.478	0.169
Cinnamyl alcohol	4	0.068-0.232	0.147	0	-	-
Eugenol	5	0.032-0.738	0.337	2	0.001, 0.001	0.001
Isoeugenol	3	0.026-0.249	0.119	2	0.001, 0.004	0.003
Amyl cinnamal	1	0.019	0.019	0	-	-

Note: * Due to interference by linalyl acetate, concentration of geraniol+linalyl acetate is reported.

Table 10-6: Concentrations of Fragrance Mix I ingredients, hexyl cinnamal and coumarin in 22 perfumes marketed as natural cosmetics investigated in 1996.

Fragrance	In no. of perfumes	Concentration % (w/w)
Geraniol	14	1.191*
Cinnamal	3	0.089, 0.109, 2.101
Hydroxycitronellal	5	0.135-6.044
Cinnamyl alcohol	8	0.035-2.289
Eugenol	2	0.027, 0.139
Isoeugenol	8	0.194-3.039
Amyl cinnamal	9	0.105-7.706
Coumarin	11	0.046-6.043

Note: * Quantification was performed in one sample only, due to interference by a very large amount of linalyl acetate in other samples.

The trend in the use of most of the fragrance allergens in deodorants before and after their regulation is reflected by the two studies performed by Rastogi et al. (228, 231). The results of these studies cannot be directly compared, because the study from 1998 included randomly selected deodorants, while selection of the deodorants for the 2007 study was based on the labelling of the presence of known strong fragrance allergens in these products. The number of products analysed in the 1998 study were three times more than those analysed in 2007, but not all of the 26 fragrance allergens were analysed in the 1997 study. However, an indication of the change in the use of the fragrance allergens during 1998-2007 may be obtained by reviewing the results of these two studies. Among the 17 common fragrance substances studied in the two studies, the frequency of use of 16 of these substances in deodorants was reduced in 2007 compared to that in 1998 (Table 10-2). The frequency of use of butyl phenyl methyl propional in deodorants appeared to be unchanged. The contents of benzyl alcohol, benzyl salicylate, cinnamal, cinnamyl alcohol, eugenol, geraniol, isoeugenol and linalool were found to be lower in the deodorants analysed in 2007 compared to those in 1998. Citronellol, coumarin and alpha-isomethylionone contents in the deodorants were similar in both studies, but concentrations of benzyl benzoate, butyl phenyl methyl propional, hexyl cinnamal, hydroxyisohexyl-3-cyclohexene carboxyaldehyde and linalool were much higher in deodorants in 2007 compared to those in 1998. This analysis of trend of use of fragrance allergens in cosmetic products indicates that the regulated fragrance allergens are used less frequently, but exposures from some of the regulated fragrance allergens may be much higher compared to those before regulation.

Table 10-7: Atranol and chloroatranol content in eau de toilette/eau de perfume, investigated in 2004 and in 2007.

	2007 Study	**2004 Study**
No. of samples	22	17
Atranol present in no. of samples	15 (68%)	12 (70%)
Atranol content	ppb (ng/ml)	ppb (ng/ml)
Range	n.d.-880	n.d.-791
Mean±SD	157±249	97±224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Atranol content	ppb (ng/ml)	Ppb (ng/ml)
Range	0.9-208	1-175
Mean±SD	63±73	36±51
Median	22	10

Notes: n.d. Not detected.
*P <0.05 (chi-square test).
SD: Standard deviation.

Atranol (CAS no. 526-37-4) and chloroatranol (CAS no. 57074-21-2), constituents of oak moss and tree moss have been shown to be very potent fragrance allergens (237, 238). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (239). Two other commonly used fragrance chemicals, isoeugenol (240) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (71), have also been shown to be important contact allergens. The contents of atranol, chloroatranol, isoeugenol and hydroxyisohexyl-3-cyclohexene carboxyaldehyde in fine fragrances was determined for the exposure assessment of these fragrances (233). The results revealed that isoeugenol was present in 56%, HICC in 72%, atranol in 59%, and chloroatranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below 0.02% which is the maximum concentration recommended by the fragrance industry. HICC reached a maximum concentration of 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the EC Scientific Committee (241). The concentrations of atranol and chloroatranol in the products investigated in 2007 were comparable to those found in similar products in 2004 (Table 10-7, (233, 234). A significant decrease in the frequency of the presence of chloroatranol in the products was found in 2007 (Table 10-7).

10.2. Global exposure (household and occupational exposures)

Fragrances are used in cosmetics that the consumer applies to themself, as described in the previous section. In addition, exposure to fragrance substances is possible by a number of other exposure routes briefly outlined in this section.

Topical pharmaceutical products

In a study from Belgium, 370 of the 3,280 topical products marketed in Belgium have been found to contain one or more of 66 fragrance substances (242). This publication also contains a description of causative fragrance allergens in 127 patients reacting to 48 specific topical products. In a broader sense, exposure of the patient by extracts used in aromatherapy falls in this category as well.

Childrens products and toys

Children's products may contain fragrance allergens and high levels may be present (235). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (243).

Clothing

Washed fabrics have been reported to contain fragrances (244). Odour-neutralising agents are sometimes used for shoe insoles. In one case, an insole containing cinnamon, has been reported to lead to plantar vesicular contact dermatitis due to contact sensitisation to FM I and, in the breakdown, to cinnamal and cinnamyl alcohol (245).

Cleaning agents and other household products

Contact dermatitis from geraniol in washing-up liquid has been reported (246). Terpenes are used as solvents and cleansing agents (e.g. limonene) (247) and have been reported as cause of hand dermatitits (248, 249). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (250). In a review of 301 cosmetic and detergent consumer products in Sweden, in half of the cosmetics and one-third of the detergents, one or more of the 26 fragrances requiring labelling were identified (251). In the UK, a review of 300 consumer products showed that linalool and limonene were present in 63% of products. Dental products contained on average 1.1 fragrance substances that are presently required to be labelled and women's perfumes contained 12 of these fragrance substances (Table 4-1 and Table 4-3) (229).

Candles

The dermal hand transfer of three fragrance materials (cinnamic aldehyde, d-limonene and eugenol) from scented candles was determined in ten subjects (i.e. 20 hands) after grasping scented candles for five consecutive 20 second exposures/grasps. The total mean residues of cinnamal and eugenol transferred per grasp from the candles to the hands were 0.255 µg/cm(2) and 0.279 µg/cm(2), respectively (252).

Food

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (253)) or lichen planus-like lesions (e.g. due to cinnamal (254)) or contact gingivitis (e.g. due to eugenol (255)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (256) may cause allergic contact dermatitis by handling this food.

Occupational exposure

In a number of occupations, contact allergy to fragrances is more common than in others, including geriatric nurses, masseurs and physiotherapists, metal furnace operators and potters/glass makers, according to a multifactorial analysis (90). Moreover, hairdressers, beauty therapists and aroma therapists are examples of occupations where there is occupational exposure to fragrance-containing cosmetic and other products. Cleaners are exposed to fragrance-containing household products (e.g. detergents). Cooks and bakers are exposed to flavour chemicals and spices. Healthcare workers are also at risk of acquiring fragrance contact allergy. "Odour maskers" may contain important fragrance allergens (89, 90, 257-259). Occupational exposure and

occupational ACD to fragrances have been described in perfume bottlers (260). Industrial use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (259).

A number of fragrance chemicals are also used as biocides (see Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, published 11.12.2007 EN Official Journal of the European Union L 325/3 –L325/65), see Table 10-8 below.

Table 10-8: Parts of Annex I to (EC) No 1451/2007 (see above): "Active substances identified as existing", if use is 'perfuming' or 'masking' according to CosIng.

Biocide	EINECS	CAS number	Biocidal product group
Linalool	201-134-4	78-70-6	19
Geraniol	203-377-1	106-24-1	18, 19
Benzyl benzoate	204-402-9	120-51-4	2, 18
Eugenol	202-589-1	97-53-0	Not given
Farnesol	225-004-1	4602-84-0	Not given
(R)-p-mentha-1,8-diene	227-813-5	5989-27-5	12
Citriodiol/mixture of cis- and trans-p-menthane-3,8 diol	255-953-7	42822-86-6	1, 2, 19
Citral	226-394-6	5392-40-5	Not given
Pine ext.	304-455-9	94266-48-5	10
TANACETUM CINERARIIFOLIUM FLOWER EXTRACT	289-699-3	89997-63-7	18
Citrus oils (main component: limonene)	several	various	
Clove oil (main component: eugenol (83.8 %), caryophyllene (12.4 %)	/	8000-34-8	

Product groups(According to Biocide Directive 98/8/EC)

1 Human hygiene biocidal products
2 Private area and public health area disinfectants and other biocidal products
3 Veterinary hygiene biocidal products
10 Masonry preservatives
12 Slimicides
18 Insecticides, acaricides and products to control other arthropods
19 Repellents and attractants

The above illustrates that the consumer is exposed to fragrance substances from a wide variety of cosmetic products, other consumer products, pharmaceuticals and occupational exposures.

All these exposures are of importance in the context of contact allergy as it is not the source of exposure that is critical for both induction and elicitation, but the cumulative dose per unit area.

10.3. Exposures related to particular anatomical sites

Contact allergy to fragrances most often causes dermatitis of the hands, face and axillae. Axillary involvement has been shown to be statistically related to fragrance allergy (9). It is recognised that the axillary skin is a problematic area as it is moist, occluded and is easily irritated. Moreover, facial eczema is a common manifestation of fragrance allergy (3, 47). There is an association between fragrance allergy and hand eczema or aggravation of hand eczema (13-15). Vehicles may influence elicitation capacity of an allergen and the presence of detergents (surfactants) as in hand cleaning products may increase the clinical response by a factor of 4-6 (261). Men using wet shaving as opposed to electric razors have an increased risk of being fragrance allergic (17), most likely due to microtraumata and to the presence of surface active substances in shaving foam.

In use tests, the upper arm has been shown to be more sensitive than the forehead and lower arm (262). The axillae, neck and face are more sensitive than the upper arms (10). The threshold of elicitation may vary depending on the volatility of the substance (263). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (264).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (74). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (265). Following this, eczema may appear, or be worsened by, the use of a variety of product types including other cosmetics, household products, industrial products and flavours.

The association between contact allergy to fragrance ingredients and certain anatomical sites, which mirrors exposure to fragrance-containing products on these anatomical sites, has been described in several publications (266, 267), see above. However, due to the potential confounding effect of other factors, at least on some anatomical sites, an adjusted analysis will provide a more valid impression of the association between certain anatomical sites and contact allergy to fragrance ingredients. As an adjusted, multifactorial analysis relies on: (i) a substantial number of observations (patients tested); and (ii) an outcome prevalence not too close to 0%, such an approach has, hitherto, been limited to FM I.

In a paper published 2001, data from the IVDK in terms of patch test reactions to FM I and relevant clinical and demographic information of the patients tested (n=57,779) was studied by Poisson regression analysis (90). Risk was quantified by the prevalence ratio, which can be interpreted as an estimate of relative risk, i.e. the factor by which the risk of being sensitised to FM I (in this example) is to be multiplied (RR > 1: elevated risk; or RR < 1: reduced risk) if a certain “risk factor” is present, compared to those patients in whom this risk factor is not present (the reference category) (general aspects of such analyses are discussed in (268)). In the analysis, potential risk factors and confounders, respectively, including occupation, year of patch testing (to address a possible time trend), sex, age, past or current atopic dermatitis, in addition to anatomical site. The relevant part of Table 3 of (90) is reproduced below.

Table 10-9: Result of a Poisson regression analysis of patients tested with the Fragrance Mix between January 1992 and December 1998, considering two alternative outcomes – part I: non-occupational factors

Attribute	*Prevalence (%)*	*At least + (11.5%)*		*At least ++ (4.0%)*	
		PR	*95% CI*	*PR*	*95% CI*
Age:					
≤30	26.7	1.00	Reference	1.00	Reference
>30–44	23.8	1.42	1.31 to 1.53	1.61	1.40 to 1.84
>44–58	25.6	1.67	1.55 to 1.80	1.90	1.66 to 2.16
>58	23.9	1.93	1.77 to 2.10	2.07	1.79 to 2.39
Sex (female)	64.5	1.29	1.21 to 1.37	1.18	1.07 to 1.31
Main site:*					
Trunk	2.9	1.00	Reference	1.00	Reference
Hands	29.9	1.24	1.07 to 1.46	1.28	0.98 to 1.67
Arm	3.8	1.23	1.01 to 1.49	1.19	0.86 to 1.65
Face	15.2	1.20	1.03 to 1.42	1.13	0.86 to 1.48
Neck	1.4	1.39	1.10 to 1.75	1.31	0.88 to 1.94
Feet	2.8	1.26	1.02 to 1.55	1.19	0.84 to 1.68
Leg	8.7	1.59	1.36 to 1.89	1.50	1.14 to 1.99
Axilla	0.9	2.77	2.20 to 3.46	2.73	1.87 to 4.00
Other site	8.9	0.66	0.55 to 0.80	0.48	0.35 to 0.67

*Additionally controlled for several more sites—none of these associated with a significantly increased or decreased risk.

Compared to the trunk, which was arbitrarily chosen as the reference category, all other anatomical sites are associated with an increased risk of being sensitised to FM I (significantly if the lower limit of 95% CI is > 1). Most evidently, dermatitis of the axilla(e) is strongly associated with contact allergy to FM I, presumably due to the application of deodorants. Furthermore, the part of the table shown above illustrates a strong, positive age gradient, i.e. the older patients are, the more likely they are to be sensitised to FM I, the risk being almost double when comparing the oldest with the youngest age group. This observation is in concordance with a bivariate (unadjusted) association between age and contact allergy to FM I found in another study (89). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (269): (i) an even stronger age gradient; and (ii) no particular association to axillary dermatitis (included in the "other" category) was found (Table 10-10).

Table 10-10: Association between selected risk factors and positive patch test to *Myroxylon pereirae* resin. For full model see (269). Risk quantified with the prevalence ratio (PR) with accompanying 95% confidence interval (CI).

Factor	**PR**	**95% CI**
Atopic dermatitis, past or present	1.02	(0.95-1.10)
Female sex	1.13	(1.06-1.20)
Site		
Trunk	1.00	(reference)
Hand or Arm	1.03	(0.94-1.12)
Foot or Leg	1.76	(1.61-1.92)
Head or Neck	0.94	(0.86-1.03)
"Other" site	0.72	(0.64-0.81)
Missing site	1.07	(0.97-1.19)
Age		
30 years and younger	1.00	(reference)
31 to 44	1.92	(1.73-2.12)
45 to 58	2.87	(2.61-3.16)
58 or older	3.85	(3.49-4.25)

10.4. Conclusion

There are various modes of exposure to fragrances, including not only products used for their scent, such as perfumes and eau de toilette, after shaves, and deodorants, but also types of products where scent is an added feature, such as other cosmetic categories (including wipes), topical pharmaceuticals, household products, and products encountered in the occupational setting.

Consumer exposure can change over time, both qualitatively and quantitatively.

Different routes of exposure are reflected by certain anatomical sites affected: deodorants are associated with axillary dermatitis, the axillary skin being particularly vulnerable to sensitisation due to occlusion, maceration and irritation. However, while sensitisation and initial disease may follow a distinct pattern, later less specific exposures, e.g. via hand creams, cleaning lotions etc. may be sufficient to cause allergic contact dermatitis.

11. Dose-response relationships and thresholds

The dose-response relationship between exposure to contact allergens and induction of allergy, i.e. sensitisation, is well established in animal models and by experiments in healthy volunteers (270). It seems that not only the dose per unit area of allergen (271), but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (272). The induction of contact allergy is an immunological process (type IV-allergy), which is without any clinical symptoms. In the case of continued exposure or re-exposure with a sufficient dose of allergen, elicitation will occur. Elicitation is an inflammatory response (eczema) with clinical symptoms of erythema, induration and in some cases vesicles. Studies of the elicitation response are normally done in patients with an allergy to the substance in question. Different provocation models exist (see chapter 11.2.1). Elicitation experiments in healthy human volunteers following the induction have only rarely been performed (273) and may be considered a less valid model than patient studies. The reason is that following experimental induction, the level of sensitivity may not be at the same level as in a real life situation and that individuals who have actually acquired the disease are a more relevant endpoint to study.

Knowledge of the dose-response relationship provides an opportunity to establish levels of exposure which are safe for the majority of individuals. In the following chapter, the use of different data and models for the establishment of such safe levels in relation to fragrance ingredients are explored. The focus will be on those chemicals, which have been identified in chapter 7.1 as established contact allergens in humans and which have already given rise to a significant number of published cases (category 3 or more): cinnamal, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, isoeugenol. Limonene and linalool are considered in chapter 5 as their ability to cause sensitisation depends on air oxidation, and hydroxyisohexyl 3-cyclohexene carboxaldehyde is considered in chapter 4.3.2 and 11.4.

11.1. Induction

A model for dermal sensitisation quantitative risk assessment (QRA) has been developed and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments, mainly the local lymph node assay (LLNA) (see chapter 8.1). A set of safety factors are applied for inter-individual differences, for vehicle effects and for use considerations, stated to give rise to a safety margin from 10 to 1000 (274). In this way, a so-called “acceptable exposure level” is derived. The exposure to an allergen in different types of products should be below this level. The restrictions, which have been introduced by the fragrance industry based on the QRA model, are given in Table 11-1 for some important product categories.

The IFRA guidelines give concentration limits for 11 product categories (http://www.ifraorg.org/en-us/standards_1, last accessed 2011-11-02), three of which are mentioned in Table 11-1. These three products have the lowest concentrations except for lip products, which give a slightly lower concentration limit.

Table 11-1: Current IFRA restrictions based on induction experiments.

Fragrance chemicals	IFRA guideline[1]		
	Deodorant (%)	Hand cream (%)	Perfume (%)
Cinnamal	0.02	0.05	0.05
Cinnamyl alcohol	0.1	0.4	0.4
Citral	0.05	0.3	0.6
Coumarin	0.13	0.8	1.6
Eugenol	0.2	0.5	0.5
Farnesol	0.11	0.6	1.2
Geraniol	0.4	2.8	5.3
Hydroxycitronellal[2]	0.2	1.0	1.0
Isoeugenol[2]	0.01	0.02	0.02

Notes: 1) Exposure per mg/cm^2/day is based on 8.5 mg/cm^2/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.
2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

The SCCP evaluated this methodology (275) as well as its application to three model fragrance substances.

It was, among other things, concluded that:

> "The data provided show that the application of the dermal sensitisation QRA approach would allow increased exposures to allergens already known to cause allergic contact dermatitis in consumers. The model has not been validated and no strategy of validation has been suggested. There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."

and that:

> "Identification of safe levels of exposure to existing substances known to cause allergic contact dermatitis in the consumer should be based on clinical data and/or elicitation low-effect levels. Currently, these are the only methods which have proven efficient in reducing/preventing existing problems of sensitisation/allergic contact dermatitis in the consumer."

11.2. Elicitation

11.2.1. General considerations

A response in terms of elicitation of allergic contact dermatitis by application of the (suspected) allergen under standardised conditions is the outcome of interest of the routine diagnostic procedure for suspected contact allergy, the patch test. While the patch test procedure is largely standardised, it is optimised as a diagnostic tool for contact allergy. Thus exposure conditions are not comparable to actual exposures occurring in the daily life or working environment of the patient, which often involve long-term, repeated and low-dose contact with the allergen. Here, procedures such as the repeated open application test (ROAT) or provocative use test are often used, because they reflect actual exposure much better and can be used, for instance, to validate the current clinical relevance of a positive PT reaction.

Generally, exposure of a sensitised patient to a set of graded doses (quantity/area) of the suspected allergen, i.e. threshold testing, will allow not only quantitative diagnosis of the presence or absence of specific contact sensitisation but will additionally provide evidence on the intensity (degree) of sensitisation. This may have important individual consequences in terms of everyday or occupational exposures being capable (or not) of eliciting allergic contact dermatitis. However, beyond the individual perspective, clinical dose-response data collected from sensitised individuals provide a valuable estimate of the usual doses/unit area resulting in a positive, allergic response in a certain proportion of sensitised persons, e.g. 10, 50 or 90%. Maximum concentration levels can be derived, which are safe in terms of eliciting allergic reactions in only a defined low percentage of sensitised persons. As such data will always be based on small samples, the precision of the estimate should be considered, and therefore results are preferably given with confidence intervals.

A statistically significant relationship between threshold concentrations in the ROAT and patch test has been found, on analysing results from different allergens (see Table 11-2) (276), but the dose of allergen per unit area per application needed to elicit a reaction in the two study methods is not the same. A translation factor between the two methods has been suggested for non-volatile substances: EDxx(ROAT)=0.0296 *EDxx(patch test) based on testing nickel and methyldibromo glutaronitrile (276). Based on this the eliciting dose per application in an open test is 33 times lower than in the patch test. In practice it means that the cumulative dose in a ROAT (in µg/cm²) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in µg/cm^2) for a given number of responders (see Figure 11-1). For a given cut-off point the elicitation dose determined by patch testing will be higher than determined by ROATs.

Table 11-2: Spearman's rank correlation between the threshold concentration in the patch test and the repeated open application test for three allergens.

Allergen	Number of patients	Correlation coefficient	*P*-value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011

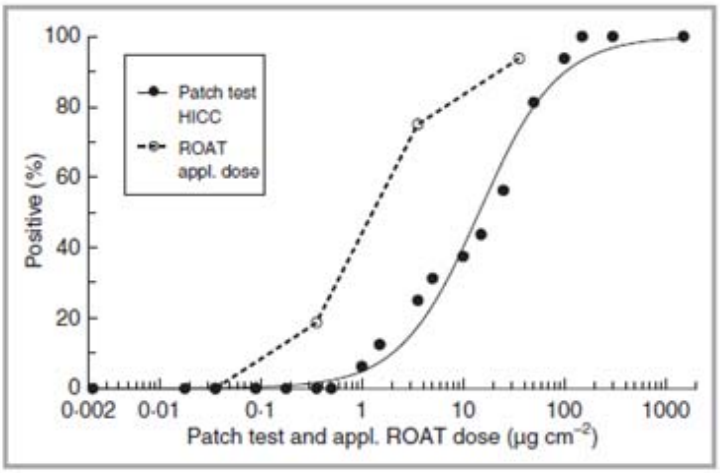


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded application as in the patch test.

In the translation between methods, evaporation needs to be taken into consideration for volatile substances. The experience, based on a study of the fragrance ingredient HICC and using the results from the literature on isoeugenol, is that if the same equation is used as for non-volatile substances, the response in the ROAT will be overestimated by a factor 3 to 4. Thus, the translation factor would be 0.1060 instead of 0.0296, but this needs to be confirmed by other fragrance allergens. This implies that for the fragrance ingredients tested, the eliciting dose per application in a ROAT was 9.4 times lower than the patch test compared to a 33 times lower dose for non-volatile substances (276). This needs to be confirmed by studying other fragrance allergens. Thus, according to these experiments, the dose ($\mu g/cm^2$) eliciting a response in threshold patch testing will be at most 33 times higher than established in the ROAT if an identical vehicle is used.

Volatility effects in skin sensitisation

The potency of volatile skin sensitisers can be underestimated, to an extent depending on how rapidly it evaporates, by assays such as the LLNA in which the test substance is applied topically to exposed healthy skin without occlusion. Such sensitisers present a greater sensitisation risk to consumers when the skin is occluded by clothing and/or compromised, than when healthy non-occluded skin is exposed.

Volatility at physiological temperature, say 40˚C, is represented by the vapour pressure p_{40} at that temperature. This is related to the boiling point T_B by the Clapeyron-Clausius equation, which can be written (277):

$$\text{Log } (p_{40}) = - (T_B - 40)Tr/2.303RT$$

Where p is in atmospheres, T_B is in ˚C, R is the gas constant, Tr is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to 22 $cal.deg^{-1}$ for many organic compounds) and T is physiological temperature in degrees absolute (= 313 for 40˚C).

It has been shown, in experiments where evaporation from a glass slide is measured under simulated LLNA conditions, that 2-hexenal (T_B = 146-149˚C, p_{40} = 17 mmHg) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal (T_B = 248˚C, p_{40} = 0.5 mmHg), more than 90% remains after 1 hour (278). In agreement with these findings, cinnamal fits a QSAR relating LLNA EC3 to reactivity, whereas the EC3 for 2-hexenal is higher (lower potency) than predicted from its reactivity.

The above is only a partial rationalisation, since different solubilities in different vehicles will influence the tendency to evaporate, according to Henry's law.

11.2.2. Studies on specific fragrance ingredients

Studies concerning chloroatranol/atranol, cinnamal, hydroxycitronellal, hydroxyisohexyl 3-cyclohexenecarboxaldehyde and isoeugenol have been identified. These are summarised in Annex III.

Overview of results

In four studies dummy deodorants spiked with a single fragrance allergen in realistic use concentrations have been used to study elicitation responses, unscented deodorants were used as control products in paired designs. The deodorants were used by patients sensitised to the fragrance allergen in question as well as a healthy control group

(without fragrance allergy) (102,103,104,279). Between 76 and 100% of the sensitised individuals reacted to the deodorants spiked with allergen, isoeugenol, cinnamal, hydroxycitronellal and hydroxyisohexyl 3-cyclohexene carboxaldehyde, and none of the controls (Table 11-4).

Table 11-3: Overview of results of deodorant provocation investigations with different allergens. Frequency in % of test groups, which reacted at different doses of allergen applied in a roll-on deodorant in the axilla, is given in the table.

Dose in ppm in deodorant	**Isoeugenol**	**Cinnamal (1)**	**Cinnamal (2)**	**Hydroxycitronellal**	**HICC**
0	0	0	0	0	0
63	23				
100			11		
200	69				64
320		25	55	57	
600					85
630	76				
1000		75	88	71	
1800					100
3200		100		100	
No. test persons	13	8	9	7	14
No. of control persons	10	20		7	10
% control persons, who reacted	0	0		0	0
Exposure according to study should be:	**< 63 ppm**	**<100 ppm**		**<320 ppm**	**< 200 ppm**
Reference	(279)	(103)		(104)	(102)

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Eleven studies concerning dose-response results of the five allergens listed above were identified, including the above mentioned studies of deodorants. An overview of the results of the studies concerning thresholds is given in Table 11-4. In Annex III the details of each study are given.

Table 11-4: Overview of threshold results from clinical studies.

"Observed" means that the proportion was actually observed in the study while "estimated" means that the value is derived from a fitted curve, i.e. is interpolated.

Chloroatranol			
ROAT			**Ref.**
In ethanol 92 % positive	0.025 µg/cm^2	observed	(238)
In ethanol 100% positive	0.125 µg/cm^2	observed	(238)
PATCH TEST			

ED10%	0.0004 µg/cm^2	estimated	(238)
ED50%	0.0045 µg/cm^2	estimated	(238)
Cinnamal			
ROAT			
In ethanol no effect	0.02%	observed	(101)
In ethanol 44 % positive	0.1%	observed	(101)
In ethanol 72 % positive	0.8%	observed	(101)
Deodorant matrix 11% positive	0.26 µg/cm^2 (0.01%)	observed	(103)
Deodorant matrix 41% positive	0.84 µg/cm^2 (0.032%)	observed	(103)
Deodorant matrix 82% positive	2.63 µg/cm^2 (0.1%)	observed	(103)
PATCH TEST			
ED50%	96 µg/cm^2	estimated	(101)
No effect level	0.4 µg/cm^2 (0.01%)	observed	(101)
No effect level	NG (0.002%)	observed	(103)
HICC			
ROAT			
In a cream base ED10%	4.9 µg/cm^2	interpolated	(105)
In a perfume (ethanol) ED10%	1.2 µg/cm^2	interpolated	(105)
In ethanol 61% positive	15.3 µg/cm^2 (3.4-22.2)	observed	(224)
In ethanol 89% positive	126.2 µg/cm^2 (40.5-226.2)	observed	(224)
In ethanol/water no response	0.0357 µg/cm^2	observed	(263)
In ethanol/water ED10%	0.064 µg/cm^2	estimated	(263)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm^2 (median)	observed	(102)
PATCH TEST			
ED10% (95% CI)	0.662 µg/cm^2 (0.052-2.35)	estimated	(263)
ED10%	0.75 µg/cm^2	estimated	(102)
ED10%	0.9 µg/cm^2 29 (7-69) ppm	estimated	(224)
ED50% (95% CI)	11.1 µg/cm^2 (3.41- 33.1)	estimated	(263)
ED50% (95% CI)	18.3 µg/cm^2 (3.41- 33.1)	estimated	(102)
ED50% (95% CI)	20 µg/cm^2 662 (350-1250) ppm	estimated	(224)
No effect level	<0.0022 µg/cm^2	observed	(263)
Hydroxycitronellal			
ROAT			
Deodorant matrix 57 % positive	0.94 µg/cm^2 (0.032%)	observed	(104)
Deodorant matrix 71 % positive	2.94 µg/cm^2 (0.1%)	observed	(104)
Deodorant matrix 100 % positive	9.40 µg/cm^2 (0.32%)	observed	(104)
PATCH TEST			

No effect level	<0.00012 % (=0.036 µg/cm^2)* (*calculated)	observed	(104)
Isoeugenol			
ROAT			
in ethanol 63% positive	5.6 µg/cm^2	observed	(100)
in ethanol 42% positive	2.2 µg/cm^2	observed	(264)
in ethanol 67% positive	9.0 µg/cm^2	observed	(264)
Deodorant matrix 23 % positive	0.167 µg/cm^2	observed	(279)
Deodorant matrix 69 % positive	0.53 µg/cm^2	observed	(279)
Deodorant matrix 77 % positive	1.67 µg/cm^2	observed	(279)
PATCH TEST			
ED50% (in petrolatum)	32 µg/cm^2	estimated	(100)
No effect (in ethanol)	<0.0005% (0.15 µg/cm^2)	observed	(264)
No effect (in petrolatum)	<0.4 µg/cm^2	observed	(100)

Summary of results for specific fragrance ingredients

Chloroatranol (constituent of *Evernia prunastri*)

In ROAT a dose of 0.025 µg/cm^2 to 0.125 µg/cm^2 in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm^2.

Cinnamal

In ROAT a dose of 0.26 µg/cm^2 gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm^2.

The ED50 in patch testing was 96 µg/cm^2.

HICC

In ROAT a dose of 0.0357 µg/cm^2 gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm^2 to 1.2 µg/cm^2. The dose in a cream base was 4.9 µg/cm^2.

In ROAT a dose of 15.3 µg/cm^2 to 126.2 µg/cm^2 in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm^2.

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm^2 gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm^2.

The no-effect level in patch testing was below 0.036 µg/cm^2.

Isoeugenol

In ROAT a dose of 2.2 µg/cm^2 a response in 42% and 9.0 µg/cm^2 in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm^2 caused a response in 23% and 77% reacted to 1.67 µg/cm^2.

The ED50 in patch testing was 32 µg/cm^2.

The no-effect in patch testing was below 0.15 µg/cm^2.

Elicitation levels have been studied for cinnamal, isoeugenol and hydroxycitronellal which are established contact allergens in humans and which already have given rise to a significant number of cases (> 100, see chapter 7). Further HICC has been studied extensively, but is considered in a separate section (chapter 11.3) of this opinion. It is however not possible to derive a safe threshold directly from the data of cinnamal, isoeugenol and hydroxycitronellal. The main reasons are that many of the test subjects reacted to all the tested doses in ROAT, which is a simulation of every day exposures. Thus it was not possible to determine the dose only eliciting responses in a few, e.g. 10% of the subjects and that only a limited number of exposure scenarios were studied.

The studies have covered few product types: hydro-alcoholic products, e.g. perfumes and deodorant roll-on matrix. The vehicle is one of many factors which influence the thresholds of allergic reactions. Also the presence of irritants and other allergens can influence the elicitation level. This means that the currently available studies do not cover all the relevant exposure scenarios. However, taking into account that dose-response investigations in sensitised patients are very complex to perform, it is not likely that much more data will become available in the near future. It is therefore necessary to exploit the full pool of elicitation data, also covering chemicals other than fragrance ingredients, to derive a more general threshold which could be used when no or insufficient data exist to set a specific threshold for a substance of concern.

General thresholds

The methodology of the different experiments has varied to some extent as different anatomical sites of exposure have been employed, different vehicles, exposure periods and cut-off points. The reason is that the studies have been performed to investigate various clinical and scientific aspects of allergic contact reactions and not for formal regulatory requirements. Some studies are small and for this reason the precision of the estimates of thresholds is limited. In spite of this, the results of the various experiments are reasonably uniform, except for chloroatranol which had very low threshold reactions, and show that low concentrations may elicit allergic reactions.

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent “meta-analysis” of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED10 at patch testing varied by a factor of 7 from the lowest to the highest value and the median was 0.82 µg/cm^2 if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 µg/cm^2 if included (see Table 11-6 and Figure 11-2 below: (280)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (280) and guinea pig QSAR evidence (281). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of 0.8 µg/cm^2 for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED10 value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions: 0.8 µg/cm^2 (280).

- Exposure doses and exposure areas from SCCS notes of guidance 7th revision (282) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (274).

Equation:

Safe concentration in product = (Generally safe exposure level (0.8 µg/cm^2)/daily exposure to product (µg/cm^2/day)) x 100 (for %).

Table 11-5: Concentration limits in different product types based on 0.8 µg/cm^2 allergen as a 'generally safe exposure level', if specific dose-response data are unavailable.

	Estimated daily exposure level (g) (Table 3 SCCS NoG)	**Mean exposed skin surface (cm^2) (Table 2 SCCS NoG)**	**Exposure /cm^2/day in grams**	**Exposure /cm^2/day in µg (1g= 1x10^6 µg)**	**Concentration limit in product % in product: (GEL/daily exposure) x 100**
Body lotion	7.82 g	15,670 cm^2	0.000499	499	0.16%
Face cream	1.54 g	565 cm^2	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 cm^2	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221[1)]	2210	0.04%

Note: 1) 2.21 mg/cm^2/day from Technical dossier Quantitative Risk Assessment.

The estimated daily use of the various product categories in Table 11-5 are based on the SCCS Notes of Guidance (see above), except for perfume, for which no value is given. This value is taken from the Technical Dossier on Quantitative Risk Assessment from RIFM.

Generally the estimated use of different products is higher in the IFRA/RIFM assessments than in SCCS Notes of Guidance.

Table 11-6: Overview of dose-response studies and thresholds for eight allergens, after (280).

ED10 patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (283), MCI/MI (Kathon ™ CG) (284), nickel (285), methyldibromo glutaronitrile (MDBGN) (286), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (102, 224, 263), isoeugenol (264, 279) and formaldehyde (287). The shaded values were considered as outliers.

Study	**Number of patients**	**ED_{10} (µg/cm^2)**	**95 % interval**
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59

Study	Number of patients	ED_{10} (µg/cm^2)	95 % interval
Nickel 1999	26	7.49	2.42–14.5
Nickel 2005	13	0.74	0.066–2.38
Nickel 2007	20	0.82	0.13–2.37
Cobalt 2005	11	0.44	0.033–1.3
Chromium	17	1.04	0.0033–5.55
Isoeugenol 2001	24	1.48	0.22–4.74
Isoeugenol 2005	13	0.23	0.0073–1.32
HICC 2003	18	0.85	0.062–3.26
HICC 2007	14	1.17	0.043–5.05
HICC 2009	17	0.66	0.052–2.35
MDBGN 2004	19	0.025	0.00021–0.19
MDBGN 2008	18	0.50	0.052–1.69

Note: The ED10 value is the concentration which elicits an allergic reaction in 10% of a group of sensitised individuals under patch test conditions.

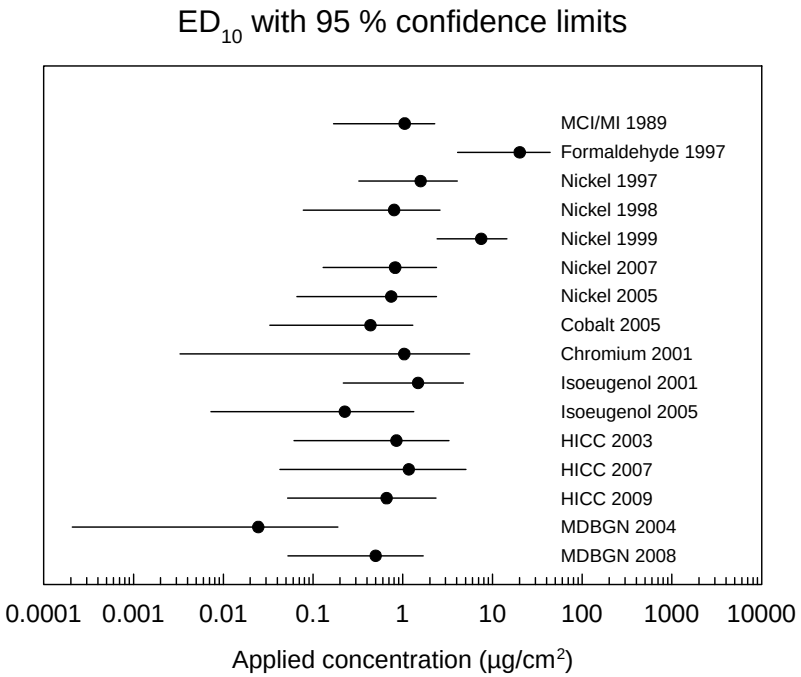


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

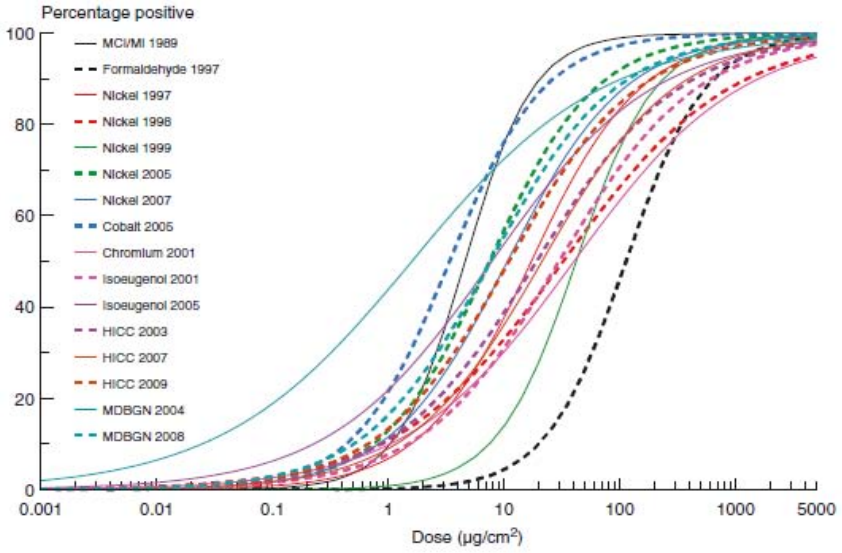


Figure 11-3: The fitted dose-response curves from the studies in Table 11-6**,** which are the basis for estimation of the ED10 value, after (280).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was 0.8 µg/cm^2. In the model data for the fragrance substances isoeugenol and HICC was included. The two studies on isoeugenol and the three studies on HICC gave an average ED10 value of 0.85 µg/cm^2 and 0.89 µg/cm^2 with a range 0.23-1.48. This means that even if the model was used for these substances individually the result would be very similar to the general threshold value.

The data from cinnamal and hydroxycitronellal studies was not incorporated in the model because: (i) serial dilution patch testing was done in petrolatum for cinnamal, making the dosing less exact; (ii) and only seven patients participated in the hydroxycitronellal study, while a criteria for inclusion in the model was ten participants (280).

According to the above calculations, a limit of 0.8 µg/cm^2 for the product types of most importance for fragrance allergy corresponds to concentrations of 100 to 400 ppm (0.01-0.04%) for deodorants, perfume spray, hand and face lotions. For body lotion the general threshold was 0.16%. However, it does not seem meaningful in the context of contact allergy to distinguish between different types of creams, as a body cream would be applied with the hands and the relevant parameter in contact allergy is dose per area skin and not total dose.

A general threshold would have to take into consideration the uncertainties in quantification of exposure and safe thresholds as well as the possibilities of aggregate exposures and exposure to chemically similar substances. Therefore in setting one general threshold the product category carrying the highest risk of sensitisation and elicitation, which is deodorants, was chosen to drive the generation of the threshold. This means that a threshold of 0.8 µg/cm^2 is equal to 0.01% or 100 ppm (see Table Table 11-1 and the related text), the lowest of the threshold values derived.

The approach taken by the SCCS is based on scientific evidence published in peer-reviewed journals (283)(284)(285)(286)(102, 224, 263)(264, 279)(287) in the past 20 years. The meta-analysis deriving the general threshold limit at 0.8 ug/cm² limit has been published (280) in a peer-reviewed journal. The use of threshold limits based on elicitation data is a well established methodology which has been applied (with success) in EU to prevent further cases of induction and elicitation (primary and secondary prevention) in the case of nickel allergy, chromium in cement, chromium in shoes in

Germany, dimetyl fumurate in consumer items and also in part in IFRA guidelines e.g. concerning HICC.

The elicitation threshold model is based on 16 studies of 8 allergens, two of which are fragrance ingredients. It includes data from moderate to extreme allergens with a median EC3 value of 1.2.

The 11 fragrance allergens to which the limit is suggested to apply range from extreme to moderate with median EC3 value of 4.8, although in the case of coumarin an EC3 value could not be established.

Thus in general the potency profile of the fragrance substances of concern is not very different from those included in the model to provide the suggested general safe threshold.

The approach is targeting the relevant end-point, namely, allergic contact dermatitis. The mere consideration of potency of the allergen, according to the LLNA (EC3), is insufficient in identifying the size of the problems of contact allergy/allergic contact dermatitis. Additional information is needed from clinical and epidemiological studies, exposure assessment and dose-elicitation studies. For instance, the elicitation thresholds of e.g. HICC (EC3: 17.1) and isoeugenol (EC3: 0.54) are very similar (0.85 µg/cm² and 0.89 µg/cm², respectively) despite very different potencies. Both are frequent causes of contact allergy.

It should be noted that the general threshold is only suggested to be used for substances of concern if no specific data of sufficient quality exist to set an individual safe threshold. In cases where specific data of sufficient quality are available, these data should be used to set an individual safe threshold.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (288) and in a reduction in morbidity, i.e. elicitation (289). Another example is restriction of chromium VI in cement (290).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri and Evernia furfuracea*, should not be present in consumer products because they are extremly potent allergens (239). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most

frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (291)) (for more studies see chapter 4.3.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (69). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (105). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (102).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (292). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: “The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material” (293). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (293).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/ antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcoholics for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcoholics for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02

Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.
QRA Quantitative risk assessment.
* Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the pre-consultation version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany (294)) and 2003-2011 (Danish contact dermatitits group (295)), respectively. The first analysis identified a slight decrease, which was considered “not overwhelming in absolute terms”, namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

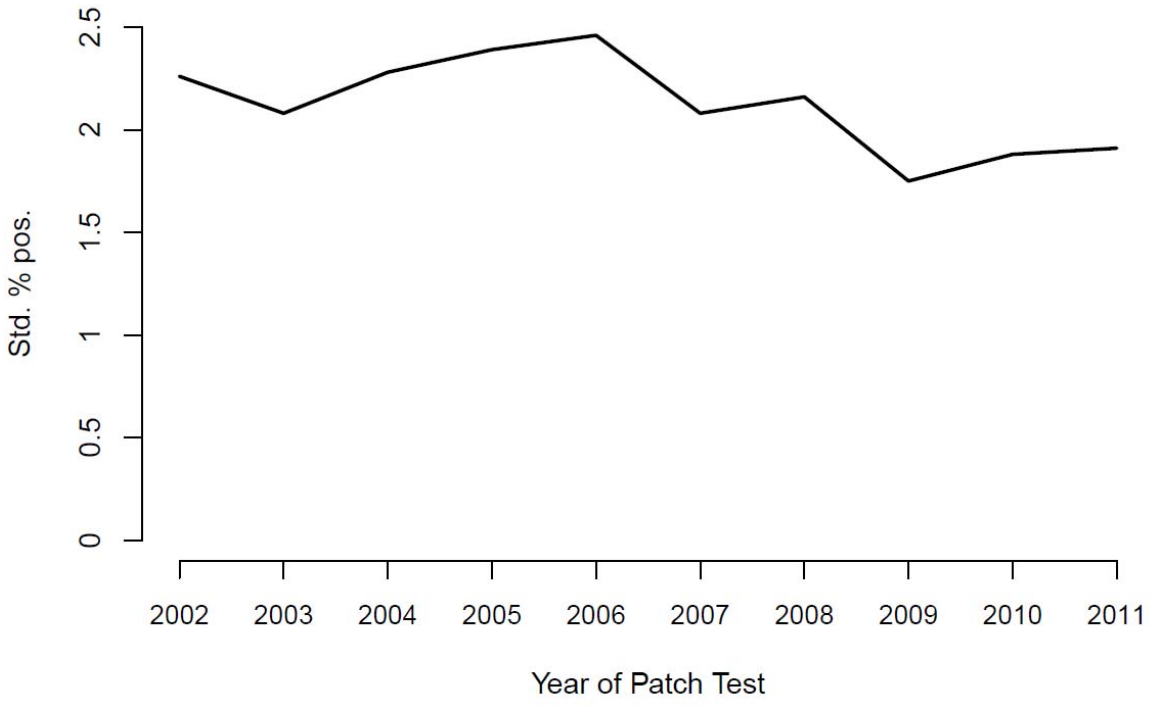


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

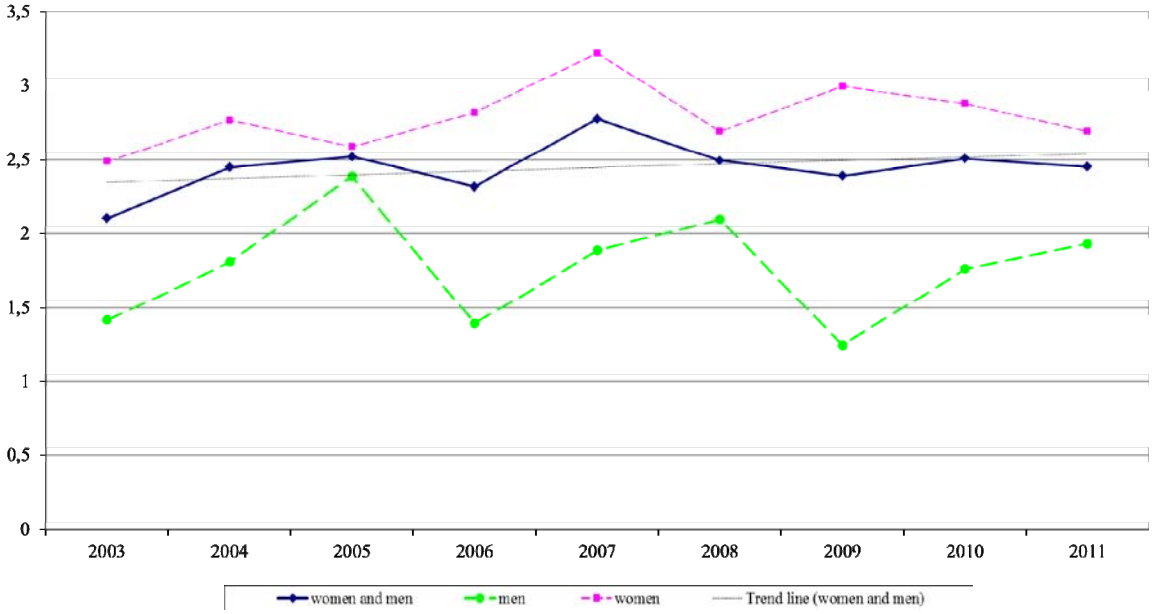


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: “There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer.”
- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, “safe use” means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of 0.8 µg/cm^2 was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.

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

- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.

- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.

- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri and Evernia furfuracea*, should not be present in consumer products because they are extremly potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents, despite efforts to reduce the allergen content (296).

12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up 10% of the European population carry mutations, which impairs the skin barrier and which seem to increase the risk of fragrance allergy. Data are needed to qualify and quantify the increase in risk of susceptible groups in order to provide a better protection of all consumers.
- Aberrant enzyme activity in certain individuals, often related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations. More research into the role of relevant traits is needed.
- Dose-response data from clinical studies are available for only a few allergens. To establish individual safe levels such data are required for all established allergens of concern and covering an appropriate range of product types. This would also consolidate the basis of the use of a general threshold for safe use of fragrance allergens.
- Data on human exposure to fragrances from the use of different product categories is very scarce and therefore does not provide an optimal basis of risk assessment, e.g. exposure data on use for perfume/eau de cologne are lacking.
- Most experimental studies are done on individual fragrance ingredients, while exposure to allergens in cosmetic products is usually to mixtures of allergens. The risk of sensitisation and elicitation may depend on the mixture of substances, but very few studies on this exist. It is necessary to improve the knowledge base on cocktail effects on sensitisation/elicitation to improve the basis of risk assessment and management.
- Screening in dermatitis patients should be performed with air exposed samples of such fragrance substances that in experimental studies have been demonstrated to act as prehaptens, i.e. autoxidise and form oxidation mixtures containing allergenic oxidation products.
- Patch testing should if possible, be performed with the isolated true haptens formed from prehaptens and prohaptens to increase the possibility to diagnose allergy from these type of substances.
- There is a need for more experimental research to further establish the impact of the behaviour of fragrance substances when applied on the skin (including factors such as volatility, autoxidation, skin penetration, reactivity in skin and bioactivation).

12.2. Non-human studies

- Several studies in the industry submission (164) were of insufficient quality, not following the OECD guidelines.
- In some cases it was found that either very few concentrations points had been used in LLNAs, or concentrations were insufficient for achieving a 3-fold increase of the SI.

  A sufficient number of doses (concentrations) should be applied in LLNAs (at least 5) so that interpolation (for deriving an EC3 value) can rely on more than two or three actual data points to be more reliable. SCCS therefore suggests a change in the OECD guideline 429. (It is important to remember that the production of unreliable data is a waste of animals.) Moreover, the maximum concentration should be high enough to achieve a > 3-fold increase in SI, as far as this is possible with the substance/vehicle combination chosen.
- Data on experimental results are often not published, but available only on file in the companies having performed the tests. Access to such results would be important for the scientific community, e.g. in the context of REACH, or independently, either to the public domain, or to a Public Trustee.
- The OECD guideline 429 recommends several vehicles. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus, as an additional control, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.
- As long as no validated *in vitro* method exists, more research is needed. Until one or more method(s) have been decided to fulfil the requirements for substituting *in vivo* testing, the *in vivo* testing for prediction of skin sensitisation has to be used.
- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances. Therefore, further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is needed to increase the safety for the consumer, i.e. experimental studies which include air oxidation and bioactivation.
- Further experimental investigations of the sensitisation potential of fragrance substances are needed to determine the impact of the volatility of the substance as well as the effect of the vehicle on skin penetration/absorption and reactivity.
- From a clinical perspective it is important for the individual who is sensitised to one fragrance substance to know if they must also avoid other fragrance substances that can cause allergic contact dermatitis due to cross-reactivity with the original sensitiser. Prediction of risks for cross-reactivity requires sound application of theoretical principles in combination with well-designed experimental studies. This is a field that has not been studied very much so far and needs to be focused on much more in the future.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods as possible. Prediction of different activation pathways should be included.
- Effect estimates such as proportions of sensitised humans or animals, or mean stimulation indices, EC3 values and other derivations should ideally be accompanied by an interval estimate (confidence interval) to address precision (297).

13. Opinion

Contact allergy to fragrances is a common, significant and relevant problem in Europe. The studies since the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 26 fragrance allergens, identified by the SCCNFP, are still relevant fragrance allergens for consumers because of their exposure from cosmetic products. Additional exposure to many of these 26 fragrance allergens also occurs from the use of other consumer products, such as detergents, toys, etc. Some of these fragrance substances are also used as preservatives.

The overall trend of fragrance contact allergy appears to have been stable for the last 10 years, as some causes of fragrance allergy have decreased and others increased. From the few population-based studies, it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. This is based on the limited testing with eight common fragrance allergens (FM I) out of the approximately 2500 fragrance ingredients listed in CosIng and indicative of the substances that may be present in fragrance compounds. However, the real prevalence of contact allergy to fragrance substances may be higher if the testing were to be performed with the full spectrum of fragrance allergens, including oxidised substances, where relevant.

Among eczema patients in the European population, around 16% are sensitised to fragrance ingredients. The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Contact sensitisation, and its clinical manifestation, allergic contact dermatitis, can be prevented if the exposure to known contact allergens is reduced or abolished (primary prevention). Experiences so far, have indicated that not all substances that later turned out to be significant contact allergens after human exposure, were predicted by experimental studies, e.g. the preservative methyldibromo glutaronitrile and the fragrance chemical HICC. Thus, a significant exposure of the population may occur before a substance is established as an important contact allergen in man.

Elicitation of allergic contact dermatitis occurs when a consumer sensitised to a certain substance is re-exposed to the substance in question. Prevention at this stage, termed secondary prevention, can be achieved if use of the allergen in products is eliminated or reduced to a tolerable level (general prevention), or if the patients succeed in avoiding all sources of exposure (individual prevention). Ingredient listing of individual fragrance allergens has been shown to be an important tool to enable consumers with an identified allergy to reduce/avoid relevant exposures. Moreover, ingredient listing is also of great importance to ensure that an adequate diagnosis of fragrance contact allergy can be made without undue delay. If the information given on the presence of fragrance allergens is incomplete, diagnosis of fragrance contact allergy may be missed.

The SCCNFP, in its 1999 opinion, identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the European Cosmetics legislation (298) as ingredient labelling of these 26 fragrance substances (Annex III, entries 67-92). However, safe use concentrations for these substances in cosmetic products have not yet been determined and much new evidence concerning fragrance allergy has been published since 1999. The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature up to October 2010. This review addresses the issue of contact allergy to fragrance substances, including natural extracts and updates the list of fragrance allergens relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of: (i) established fragrance allergens; (ii) likely fragrance allergens; and (iii) possible fragrance allergens. The review also includes fragrances, which on modification by oxidation or by enzyme mediated processes, can produce allergens. Available dose-response data have been

examined to answer whether safe thresholds can be established for the most frequent fragrance allergens.

13.1. Question 1

Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?

In order to answer this question, the SCCS has used clinical and epidemiological data to identify known fragrance allergens. These were categorised as *established contact allergens in humans* (see Table 13-1).

Where sufficient animal evidence was present, these substances were categorised as established contact allergens in animals (Table 13-2). For a number of other fragrance substances, combinations of limited clinical data together with SAR considerations have been applied to indicate likely fragrance allergens in man (Table 13-3). Finally, SAR has also been applied to substances that lack human data to identify fragrance chemicals that have the structural potential to be contact allergens. Substances with insufficient human data were also considered as possible fragrance allergens. For these further tests (experimental/clinical data) are required (Table 13-4).

Table 13-1: Established contact allergens in humans.

For categorisation of importance (+ to ++++) see chapter 7.1. Allergens of special concern are substances where between 100 and 1,000 cases (+++) and more than 1,000 (++++) have been published. These are set in bold. Fragrance substances identified as allergens in the 1999 opinion of SCCNFP (1) are marked with an asterisk.
“ox.” = oxidised; “non-ox.” = non-oxidised; “r.t.” = rarely tested (see chapter 7)

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Human evidence: see text**
Individual chemicals		
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL*	122-40-7	++
AMYL CINNAMYL ALCOHOL*	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISE ALCOHOL*	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL*	100-51-6	++
BENZYL BENZOATE*	120-51-4	++
BENZYL CINNAMATE*	103-41-3	++
BENZYL SALICYLATE*	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL *	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +

INCI name (or, if none exists, perfuming name according to CosIng)	**CAS number**	**Human evidence: see text**
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL*	**104-55-2**	**+++**
CINNAMYL ALCOHOL*	**104-54-1**	**+++**
CITRAL*	**5392-40-5**	**+++**
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN*	**91-64-5**	**+++**
(DAMASCENONE) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL*	**97-53-0**	**+++**
FARNESOL*	**4602-84-0**	**++ - +++**
GERANIOL*	**106-24-1**	**+++**
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE *(HICC)**	**31906-04-4 / 51414-25-6**	**++++**
HYDROXYCITRONELLAL*	**107-75-5**	**+++**
ISOEUGENOL*	**97-54-1**	**+++**
alpha-ISOMETHYL IONONE*	127-51-5	++
(DL)-LIMONENE*	**138-86-3**	**++ (non-ox.);** **+++ (ox.)**
LINALOOL*	**78-70-6**	**++ (non-ox.)** **+++ (ox.)**
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-	67801-20-1	++ (r.t.)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CYCLOPENTENYL)PENT-4-EN-2-OL		
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	+
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++
Natural extracts		
CANANGA ODORATA and Ylang-ylang oil	**83863-30-3; 8006-81-3**	**+++**
CEDRUS ATLANTICA BARK OIL	92201-55-3; 8000-27-9	++
CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
CITRUS AURANTIUM AMARA FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
CITRUS BERGAMIA PEEL OIL EXPRESSED	89957-91-5	+ (r.t.)
CITRUS LIMONUM PEEL OIL EXPRESSED	84929-31-7	++
CITRUS SINENSIS (syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED	97766-30-8; 8028-48-6	++
CYMBOPOGON CITRATUS / SCHOENANTHUS OILS	89998-14-1; 8007-02-1; 89998-16-3	++
EUCALYPTUS SPP. LEAF OIL	92502-70-0; 8000-48-4	++
EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL	**8000-34-8**	**+++**
EVERNIA FURFURACEA EXTRACT*	**90028-67-4**	**+++**
EVERNIA PRUNASTRI EXTRACT*	**90028-68-5**	**+++**
JASMINUM GRANDIFLORUM / OFFICINALE	**84776-64-7; 90045-94-6; 8022-96-6**	**+++**
JUNIPERUS VIRGINIANA	8000-27-9;	++

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
	85085-41-2	
LAURUS NOBILIS	8002-41-3; 8007-48-5; 84603-73-6	++
LAVANDULA HYBRIDA	91722-69-9	+ (r.t.)
LAVANDULA OFFICINALIS	84776-65-8	++
MENTHA PIPERITA	8006-90-4; 84082-70-2	++
MENTHA SPICATA	84696-51-5	++
MYROXYLON PEREIRAE	**8007-00-9;**	**++++**
NARCISSUS SPP.	diverse	++
PELARGONIUM GRAVEOLENS	90082-51-2; 8000-46-2	++
PINUS MUGO/PUMILA	90082-72-7 / 97676-05-6	++
POGOSTEMON CABLIN	8014-09-3; 84238-39-1	++
ROSE FLOWER OIL (ROSA SPP.)	Diverse	++
SANTALUM ALBUM	**84787-70-2; 8006-87-9**	**+++**
TURPENTINE (oil)	**8006-64-2; 9005-90-7; 8052-14-0**	**++++**
VERBENA ABSOLUTE	8024-12-2	++

Table 13-2: Fragrance substances categorised as established contact allergens in animals.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Individual chemicals			
Allyl phenoxyacetate	7493-74-5	none	3.1
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0	none	4.3
CYCLAMEN ALDEHYDE	103-95-7	none	22
Dibenzyl ether	103-50-4	none	6.3
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	limited	7.5
trans-2-Hexenal	6728-26-3	none	2.6
2-Hexylidene cyclopentanone	17373-89-6	none	2.4
HEXYL SALICYLATE	6259-76-3	negative	0.18
p-Isobutyl-α-methyl hydrocinnamaldehdye	6658-48-6	none	9.5

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Isocyclocitral	1335-66-6	none	7.3
α-Methyl cinnamic aldehyde	101-39-3	none	4.5
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0	none	16.4
METHYLUNDECANAL	110-41-8	none	10
2-Methoxy-4-methylphenol	93-51-6	none	5.8
4-Methoxy-α-methyl benzenpropanal	5462-06-6	none	23.6
METHYL OCTINE CARBONATE	111-80-8	limited	2.5
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	none	8.1
PHENYLACETALDEHYDE	122-78-1	limited	3
Natural extracts			
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	none	35.4

Table 13-3: Fragrance substances categorised as likely contact allergens by combination of evidence.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
AMBRETTOLIDE	7779-50-2	limited	none	+
CARVACROL	499-75-2	limited	none	+
Citrus paradisi §	8016-20-4	none	R43	n.a.
CUMINALDEHYDE	122-03-2	limited	none	+
CYCLOPENTADECANONE	502-72-7	limited	none	+
trans-trans-delta-DAMASCONE	71048-82-3	limited	none	+
2,4-dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	none	R43	+
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	limited	none	+
ETHYL VANILLIN	121-32-4	limited	none	+
HELIOTROPINE	120-57-0	limited	none	+
ISOAMYL SALICYLATE	87-20-7	limited	none	++
ISOLONGIFOLENEKETONE	33407-62-4	limited	none	+
Longifolene §	475-20-7	none	R43	+
Mentha arvensis §	68917-18-0	none	R43	n.a.
METHOXYCITRONELLAL	3613-30-7	limited	none	+
METHYL CINNAMATE	103-26-4	limited	none	++
METHYLIONANTHEME	55599-63-8	limited	none	+

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
5-METHYL-alpha-IONONE	79-69-6	limited	none	+
MYRCENE	123-35-3	limited	none	++
MYRTENOL	515-00-4	limited	none	+
NEROL	106-25-2	limited	none	++
Nerolidol (isomer not specified)	7212-44-4	limited	none	++
NOPYL ACETATE	128-51-8	limited	none	+
PHYTOL	150-86-7	limited	none	+
RHODINOL	6812-78-8	limited	none	+
trans-ROSE KETONE-5	39872-57-6	limited	none	++

§ Substances/natural mixtures were classified as R43, according to the submission by IFRA. The evidence on which this classification was based was not available to the SCCS, so the validity of classification cannot be assessed. Nevertheless, the four substances/substance mixtures should be treated as *likely contact allergens*.

n.a.: not applicable (natural mixture)

Table 13-4: Fragrance substances categorised as possible contact allergens.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
Individual chemicals				
CYCLOHEXYL ACETATE	622-45-7	limited	none	0
ETHYLENE DODECANEDIOATE	54982-83-1	limited	none	0
HYDROXYCITRONELLOL	107-74-4	limited	none	0
METHOXYTRIMETHYLHEPTANOL	41890-92-0	limited	none	0
METHYL p-ANISATE	121-98-2	limited	none	0
METHYL DIHYDROJASMONATE	24851-98-7	limited	none	0
PHENETHYL ALCOHOL	60-12-8	limited	none	0
PHENYLPROPANOL	122-97-4	limited	none	0
AMYLCYCLOPENTANONE	4819-67-4	negative	none	+
BENZYL ACETATE	140-11-4	negative	none	+
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	negative	none	+
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	negative	none	+
alpha-IONONE	127-41-3	negative	none	+
beta-IONONE	79-77-6	negative	none	+
METHYL IONONE (mixture of	1335-46-2	negative	none	+

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
isomers)				
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	negative	none	+
alpha-TERPINYL ACETATE	80-26-2	negative	none	+
CITRONELLYL NITRILE	51566-62-2	none	none	++
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0	none	none	+
DECANAL	112-31-2	none	none	++
DIHYDROMYRCENOL	18479-58-8	none	none	+
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6	none	none	++
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6	none	none	+
GERANYL ACETATE	105-87-3	none	none	++
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0	none	none	+
IONONE isomeric mixture	8013-90-9	none	none	+
ISOBERGAMATE	68683-20-5	none	none	+
METHYL DECENOL	81782-77-6	none	none	+
TRICYCLODECENYL PROPIONATE	17511-60-3	none	none	+
OXACYCLOHEXADECENONE	34902-57-3	none	none	++
VERDYL ACETATE	2500-83-6/ 5413-60-5	none	none	+
trans-beta-Damascone	23726-91-2	none	none	+
gamma-Damascone	35087-49-1	none	none	+
Citronellal	106-23-0	none	none	++
Phenethyl salicylate	87-22-9	none	none	++
Natural extracts				
ACORUS CALAMUS ROOT OIL	84775-39-3	Limited	none	
CEDRUS DEODARA WOOD OIL	91771-47-0	Limited	none	
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Limited	none	
CITRUS TANGERINA …	223748-44-5	Limited	none	
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Limited	none	
ILLICIUM VERUM FRUIT OIL	84650-59-9	Limited	none	
LAVANDULA SPICA	97722-12-8	Limited	none	

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
LITSEA CUBEBA	90063-59-5	Limited	none	
PELARGONIUM ROSEUM	90082-55-6	Limited	none	
SALVIA spp.	Diverse	Limited	none	
TAGETES PATULA	91722-29-1	Limited	none	
THYMUS spp.	84929-51-1	Limited	none	
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	Limited	none	

Regarding the above categorisation of fragrance substances, the following aspects need to be considered when interpreting an outcome other than established contact allergen in humans:

- If human evidence is negative, there is still a potential sensitisation risk, as in this set of substances the number of (consecutive) patients tested was low, i.e. up to a few hundred.
- If EC3 values are given as higher (>) than a certain value (see 8.3), an exact EC3 could not be established, as the substance had been tested in too low concentration(s). In these cases, the substances have not been categorised as 'established contact allergen in animals'.
- For SAR, the categories of prediction are: non-sensitiser (0); possible-sensitiser (+); predicted sensitiser (++); and not predictable (n.p.). (For details see Table 9-3 and Table 9-4). SAR predictions are only considered when human and animal data are limited or missing.
- Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I to this opinion, these substances have not further been evaluated.

Fragrance ingredients listed in Table 13-1 clearly have caused disease in man, and based on the clinical experience alone, these 82 substances were classified as established contact allergens in humans, 54 individual chemicals and 28 natural extracts (mixtures of chemicals), including all 26 fragrance allergens identified by SCCNFP in 1999. For a number of other substances, no patch test data were available, but positive animal data, obtained by a validated guideline method (LLNA) addressing hazard, indicate that a – yet not quantified – risk for humans is very likely to exist, given sufficient exposure. In other cases only in a relatively small number of patients has been tested positively ('limited human evidence'). Here, combination with SAR analyses corroborates the conclusion that these substances, too, are sufficiently qualified to be regarded as 'likely fragrance allergens'.

Of those 82 substances identified as established contact allergens in humans, 12 chemicals (listed in Table 13-5) and eight natural extracts are considered of special concern as they have given rise to at least 100 reported cases. These substances pose a particularly high risk of sensitisation to the consumer and are further considered in the answer of question 2. One substance, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), was shown to be the cause of allergic contact dermatitis in more than 1500 reported cases since 1999. The number of cases is only those reported in scientific publications, and therefore the actual number of cases is severely under-estimated.

Table 13-5: Established fragrance contact allergens of special concern (single chemicals only).

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)

*including their respective esters

The established contact allergens in animals (Table 13-2) and the likely contact allergens, identified based on a combination of limited evidence from man together with positive SAR predictions (Table 13-3), are predicted to cause disease in man given sufficient exposure.

Information on the presence of all the substances given in Table 13-1, Table 13-2 and Table 13-3 in cosmetic products is important in order to enable aimed testing of patients with contact dermatitis and to diagnose fragrance allergy without delay. Further, this information is important to the sensitised consumer as it will enable them to avoid cosmetic products, which they may not tolerate.

Substances given in Table 13-4 are possible contact allergens and further data are required to judge if these are contact allergens in humans and give rise to contact allergy in consumers.

Conclusions - Question 1

The studies since the SCCNFP Opinion on fragrance allergy in consumers (1) have confirmed that the fragrance allergens currently listed in Annex III, entries 67-92 are still relevant fragrance allergens for the consumers from their exposure to cosmetic products.

The review of the clinical and experimental data shows that many more fragrance substances than those identified in the SCCNFP opinion of 1999 have been shown to be sensitisers in humans. A comprehensive list of established contact allergens in humans is given in Table 13-1.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking.

Additionally, limited human and/or animal evidence together with structure activity relationship analysis suggests that other fragrance ingredients may be a cause of concern with regard to their potential of causing contact allergy in humans.

Ingredient listing is important in clinical practice for the management of patients who are allergic to one or more of the listed fragrance chemicals. It is also important for the

patients in order to avoid future exposure to fragrance contact allergens which they may not tolerate.

The SCCS considers that those substances itemised in Table 13-1, Table 13-2 and Table 13-3 represent those fragrance ingredients that the consumer should be made aware of when present in cosmetic products.

Substances known to be transformed (e.g. hydrolysis of esters) to known contact allergens should be treated as equivalent to these known contact allergens. The combined concentration of the alcohol and its ester must be considered regarding exposure. Important indicative, but not exhaustive, examples include isoeugenol and its esters, geraniol and its esters, eugenol and its esters, and linalool and its esters.

13.2. Question 2

Can the SCCS establish any threshold for their safe use based on the available scientific data?

Dose-response relationships exist between exposure to contact allergens and the proportion of consumers who will become sensitised to an allergen (i.e. induction), as well as the proportion who will suffer from allergic contact dermatitis (elicitation). For a number of recognised contact allergens in man, dose-elicitation studies on sensitised individuals are available. These studies indicate that it is in principle possible to derive exposure levels that the majority of sensitised individuals will tolerate. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy and limit the risk of induction.

Among the established chemical fragrance allergens, 12 were identified as posing a high risk of sensitisation to the consumer (Table 13-5), i.e. more than 100 reported cases. For these substances, limitation of exposure would help to protect sensitised consumers from developing allergic contact dermatitis.

In cases where specific data of sufficient quality on threshold levels for a particular allergen are available, these data should be used to set an individual safe threshold. However, when such quality data are not available and a substance has been identified to pose a high risk of sensitisation to the consumer, a general threshold limit can be applied.

Dose-response studies have been performed with only four of these fragrance substances (HICC, isoeugenol, cinnamal and hydroxycitronellal). In addition, such a study has also been performed on chloroatranol, a potent allergen in *Evernia prunastri and Evernia furfuracea*. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.

If no adequate data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 µg/cm^2 has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 µg/cm^2 under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

The model providing the general threshold of 100 ppm has been based on single substances only and no general safe level for the natural extracts of concern can be

identified, but the maximum use concentration applies to the identified fragrance allergens also when present in the natural extract.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of more than 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I), which will severely underestimate the actual prevalence in the population. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. The SCCP concluded in 2003 that 200 ppm of HICC would be tolerated by the majority of sensitised individuals and this level of exposure would have a low potential to induce sensitisation (241). Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Chloroatranol and atranol are the main allergenic components of *Evernia prunastri* and *Evernia furfuracea*. The SCCS concluded in 2004 (239) that these should not be present in cosmetic products, due to their exceptionally high sensitisation potential. Attempts to effectively reduce the content of these compounds in “oak moss abs.” (300) have largely failed to reduce contact allergy to *Evernia prunastri* and *Evernia furfuracea* and the data presented in this opinion show that the number of cases remains high.

Conclusions - Question 2

There are two components to the safety of fragrance ingredients in terms of contact allergy. First, the need to eliminate or reduce induction of contact allergy (primary prevention), which, when it occurs, is life long. Secondly, the need to eliminate or reduce elicitation reactions (secondary prevention) on the skin of those individuals who are already sensitised. Human dose elicitation experiments have hithereto been performed only for a very small number of substances. It is unlikely that more of these studies will be performed due to experimental and subject recruitment difficulties.

For individual substances, no levels that could be considered safe for the majority of consumers could be established from the available data.

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 $\mu g/cm^2$ (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

Such a threshold based on elicitation levels in sensitised individuals will be sufficiently low to protect both sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy.

The SCCS is of the opinion that for substances idendified as posing a high risk to the consumer and for which no individual thresholds could be derived (Table 13-5), the general threshold of 0.01% would limit the problem of fragrance allergy in the consumer significantly.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the above identified fragrance allergens also when present in the natural extract. This will also reduce the risk of sensitisation and elicitation from natural extracts.

It is important to stress that this general threshold, although limiting the problem, does not preclude that the most sensitive segment of the population may react upon exposure to these levels. Hence, this threshold does not remove the necessity for providing information to the consumer concerning the presence of the fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, in 2003 the SCCP suggested that levels of up to 200 ppm would be tolerated by the majority of sensitised individuals. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized. The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri and Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to allergenic constituents, despite efforts to reduce the allergen content (296). The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

13.3. Question 3

Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation, and thus increasing the risk of sensitisation.

Experimental and clinical studies have shown that there are fragrance substances that act as prehaptens, i.e. their sensisitation potency is markedly increased by air exposure due to oxidation (autoxidation). Non/low-sensitising compounds are thereby transformed into more potent sensitisers. Limonene, linalool, linalyl acetate, alpha-terpinene and geraniol have all been identified as prehaptens. These fragrance substances are common in scented cosmetics as well as in household products. The clinical studies show that the exposure to allergens formed due to autoxidation causes significant contact allergy in consumers. Patch testing with oxidised limonene and oxidised linalool shows that these substances rank among the most common contact allergens.

In the SAR analyses performed in this work by the SCCS, fragrance compounds with structural alerts that indicate that they are possible prehaptens have been identified (Table 9-1, Table 9-2). In such cases further thorough investigations are needed. It is also important to investigate the stability of the primary oxidation products (the hydroperoxides) formed from various structures of fragrance compounds. The stability of these compounds can have great impact on the sensitisation potency of the oxidised compound as they are strong sensitisers. However, the secondary oxidation products (aldehydes and epoxides) can also be important sensitisers depending on the overall structure of the compound as was demonstrated for oxidised geraniol.

Air oxidation of prehaptens can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autoxidation rate depends not only on the compound itself, but also on its purity. The prevention of autoxidation using antioxidants

needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation. As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

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

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 µg/cm^2 (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

Compounds that are bioactivated by metabolising enzymes to haptens are referred to as prohaptens. Established prohaptens of clinical importance are cinnamyl alcohol, geranial, geraniol, eugenol, isoeugenol and alpha-terpinene.

Table 13-6: Known prehaptens and prohaptens.

Fragrance substance	Activation by air oxidation	Bioactivation (oxidation)	Bioactivation (hydrolysis)
Cinnamyl alcohol		x	
Eugenol		x	
Eugenyl acetate		x	x
Geranial	x	x	
Geraniol	x	x	
Geranyl acetate	x	x	x
Isoeugenol		x	
Isoeugenyl acetate		x	x
Limonene	x		
Linalool	x		
Linalyl acetate	x		
alpha-Terpinene.	x	x	

When bioactivation occurs, the risk of cross-reactivity should be considered. An increased complexity in the cross-reactivity pattern is obtained when a compound could act both as a prehapten and a prophapten. For instance, it is known that cinnamyl alcohol and cinnamal can cross-react due to the formation of common sensitising substances. The same applies to geraniol and citral.

In case derivatives of a fragrance substance are used, it must be taken into account that the derivative could be transformed into the parent or a cross-reacting compound. For such derivatives the same rules as for the corresponding parents should apply, unless the

stability of the derivative has been demonstrated. In particular, hydrolysis of esters to the corresponding alcohols can cause cross-reactions. Acetate esters of eugenol, isoeugenol and geraniol are frequently used in cosmetics.

To be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests.

Activation of individual compounds to various haptens increases the risks of cross-reactivity between chemicals and also causes difficulties in prediction of these risks. Prediction of risks requires sound application of theoretical principles in combination with well designed experimental studies. Based on the acquired knowledge, qualified suggestions using structure activity relationship (SAR) regarding many fragrance substances have been made (Table 9-1 to Table 9-3). However, as the stability of formed oxidation products (mainly hydroperoxides) is important for the sensitisation potency, the SAR hypotheses must be followed by experimental investigations for the actual compounds.

Conclusions - Question 3

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation. Fragrances with published data showing the formation of sensitising compounds by autoxidation, bioactivation or both include the following (see also Table 13-6).

Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation are limonene, linalool, and linalyl acetate.

Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation are cinnamyl alcohol, eugenol, isoeugenol and isoeugenyl acetate.

Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation are geraniol and alpha -terpinene.

A fragrance substance that sensitises without activation but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is geranial (one isomer of citral).

In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

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

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

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

The substances presented above are based on current knowledge and should be seen as indicative and illustrative of the general problem. As substances with structural alerts for acting as pro- and/or prehaptens are quite common among the fragrance substances listed (see Tables 9-1 and 9-2), the possibility for activation to generate new potent allergens should be considered.

The SCCS is of the opinion that substances known to be transformed (e.g. by oxidation either via air oxidation or via bioactivation) to known contact allergens should be treated as equivalent to these contact allergens, i.e the same restrictions and other regulatory requirements should apply, unless specific data exist that allow for an individual assessment. Important indicative examples include limonene, linalool, linalyl acetate, geraniol, geranial, alpha-terpinene, eugenol, isoeugenol and cinnamyl alcohol.

14. List of abbreviations

ACD	Allergic contact dermatitis
alc.	Alcohol (as vehicle)
CI	Confidence interval
CLP	Classification, labelling and packaging
coloph.	Colophonium
DCs	Dendritic cells
EC	European Commission
ESSCA	European Surveillance System on Contact Allergies
EDT	Eau de toilette
EDP	Eau de perfume
EU	European Union
FM	Fragrance mix
GC	Gas chromatography
GPMT	Guinea pig maximisation test
HICC	Hydroxyisohexyl 3-cyclohexene carboxaldehyde
HRIPT	Human repeat insult patch test
IFRA	International Fragrance Association (www.ifraorg.org)
IVDK	Information Network of Departments of Dermatology (www.ivdk.gwdg.de)
INCI	International Nomenclature on Cosmetic Ingredients
LCs	Langerhans cells
LLNA	Local lymph node assay
MPR	*Myroxylon pereirae* resin
NACDG	North American Contact Dermatitis Group
OECD	Organization of Economic Co-operation and Development
ox.	oxidised
pet.	Petrolatum (as vehicle)
ppm	parts per million (10000 ppm = 1%)
PPV	Positive predictive value
PR	Prevalence ratio
PT(ed)(ing)	Patch test(ed) (ing)
QMM	Quantitative mechanistic model
QRA	Quantitative risk assessment
(Q)SAR	(Quantitative) structure activity relationship
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
RIFM	Research Institute for Fragrance Materials (www.**rifm**.org/)

ROAT	Repeated open application test
SC	Single constituents (of one of the fragrance mixes)
SCCS	Scientific Committee on Consumer Safety
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SCCP	Scientific Committe on Consumer Products
UK	United Kingdom
US(A)	United States (of America)
UV	Ultraviolet

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Annex I - Catalgoue of fragrance allergens

Contents

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Single chemicals

Often, results with the single constituents of the FM I or, yet more rarely, FM II, are presented in one paper. As the main ordering of this annex is by allergen, core information on these studies is presented in a tabular format and referenced by a unique acronym in the single sections, to avoid redundancy. Regarding nomenclature, terms which are often not officially an INCI Name but Perfuming Name as listed by CosIng are used. “Current Regulation” refers to the EU Cometics Directive only.

Table 55: Background information on studies reporting results with (all) single constituents of the FM I (**amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, isoeugenol, EVERNIA PRUNASTRI**)

Reference	Country	Study period, Patients	Comments by reviewers
Larsen 2002 c (1)	7 industrial countries worldwide	Prior to 2002 n=218 patients with known contact allergy to fragrance ingredients	Test concentrations identified as non-irritating in serial dilution testing in 20 healthy volunteers
Utrecht 1999 (2)	Utrecht, The Netherlands	1994-1998 n=757 patients with suspected ACD to cosmetics	All patients tested with FM I and single constituents
Sheffield 1999 (3)	UK	1994-1995 n=744, 40 of these positive to FM I and tested with single constituents	
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
Hungary 2002 (5)	Hungary, multicentre study,	1998-1999, n=3604 patients	recruitment not clear, presumably consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients
IVDK 2010 (7)	Germany, Switzerland and one centre in	2005-2008 n=36961 tested with FM I, n=4167 with FM II and	

	Austria	all SC	

Table 56: Results of PTing with single constituents of the FM I in patients positive to the FM I (as percent)

N(pos) to FM I, ref.	*Evernia prun.*	Isoeug.	Hydroxy citron.	Cinnamal	Cinnamyl alcohol	Eugenol	Gera-niol	Alpha-amyl cinnamal
N=160 (5)	13.1%	14.8 %	2.5%	8.1%	20.6%	8.8%	7.5%	5.0%
N= 991 (8)	18.4%	11.2 %	10.1%	6.1%	6.1%	6.6%	4.6%	2.4%
N=50 (2)	19.6%	14.3 %	8.9%	8.9%	7.1%	5.4%	2.7%	0%
n=40 Sheffield 1999 (3)	30%	20%	2.5%	12.5 %	10%	5%	0%	0%
N=226 Coimbra 2000 (9)	22.1%	19.9 %	6.6%	13.3 %	7.9%	14.6 %	8.4%	4.4%
N=655 IVDK 2010 (7)	29.8%	18.0 %	12.8%	11.6 %	9.6%	6.7%	4.7%	2.8%

Table 57: Background information on studies reporting results with (all) single constituents of the FM II (**citronellol, citral, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), Farnesol, alpha-Hexyl-cinnamic aldehyde**)

Reference	Country	Study period, Patients	Comments by reviewers
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
EU 2005 (10)	6 European centres	10/2002 – 06/2003, n=1701	Applied in consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients

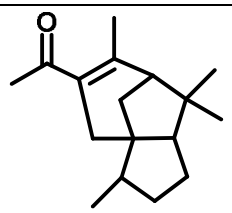
IVDK 2010b (11)	Germany, Switzerland and one centre in Austria	2005-2008 n=35633 tested with FM II, n=2217 with all SC	

Table 58: Background information on studies reporting results with several fragrance compounds not, or only partly, corresponding to mixes (later created) or with essential oils

Reference	Country	Study period, Patients
deGroot 2000 (12)	The Netherlands (multicentre)	09/1998-04/1999 n=1825 consecutive patients
An 2005 (13)	South Korea (multicentre)	04/2002 – 06/2003 n=422 consecutive patients
Sugiura 2000 (14)	Nagoya, Japan	1990-1998 n=1483 patients with suspected cosmetic dermatitis
Frosch 1995 (15)	11 European depts.	Prior to 1995 n=1069 consecutive patients
Frosch 2002 a (16)	6 European depts.	10/1997-10/1998 n=1855 consecutive patients
Frosch 2002 b (17)	6 European depts.	Prior to 2002 n=1606 consecutive patients
Coimbra 2000 (9)	Portugal	07/1989-06/1999 n=226 with FM I SC n=67 also with other fragrances
Larsen 1977 (18)	US	1977 n=20 “perfume-sensitive patients”
Larsen 2001 (19)	worldwide multicentre	? (prior to 2001) n=178 patients with known contact allergy to fragrance ingredients
Belsito 2006 (20)	North American (5 US, 1 Canadian) depts.	2003 n=1603 patients
NACDG 2009 (21)	US and Canada	2005-2006 n= 4454 patients
Wöhrl 2001 (22)	“FAZ” clinic Vienna	1997-2000 n=747 of 2660 consecutive patients tested with special series
EECDRG 1995 (15)	European, multicentre	Different fragrances, tested in 2 concentrations, in sets of about 100 patients each in different centres
Goossens 1997 (23)	Leuven, Belgium	1978-1987 n=111 “Japanese perfume series” (highly selected patients)

Reference	Country	Study period, Patients
Malten 1984 (24)	Dutch multicentre	N=182 patients with suspected cosmetic dermatitis tested with 22 fragrance compounds
DeGroot 1985 (25)	Dutch	N=179 patients with suspected cosmetic dermatitis tested with 16 fragrance compounds
Rudzki 1976 (26)	Warsaw, Poland	N=200 consecutive patients
Rudzki 1986 (27)	Warsaw, Poland	N=86 patients of 299 (of 5315) patients with positive reaction to FM I tested with essential oils series
Santucci 1987 (28)	Rome, Italy	N=1500 consecutive patients; n=63 reacting positively to FM I re-tested with extended fragrance series
Nakayama 1974 (after (29))	Japan	N=183 patients with cosmetic dermatitis
IVDK 2010c (30)	Germany, Switzerland and one centre in Austria	15682 patients tested with at least one essential oil in different test series
Trattner/David (31)	Tel Aviv, Israel	N=641 consecutive patients

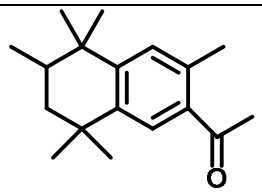
Catalogue of single chemicals evaluated

ACETYLCEDRENE	
CAS # 32388-55-9	
EC # 251-020-3	
1-[(3R,3aR,7R,8aS)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl]-ethanone	
Other names 1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-, [3R-(3α,3aβ,7β,8aα)]-Ethanone; 1H-3a,7-Methanoazulene, Ethanone deriv.; Acetyl-α-cedrene; Lixetone; Vertofix	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, a total of 0.2% had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction to 1% and and none to 5% “Vertofix ®” in pet., tested in 100 consecutive patients in Stockholm, were observed (15). In a case report, a 28-year-old patient with axillary dermatitis after using 2 different deodorants tested positive not only to HICC, but also to acetyl cedrene (tested 10.8% in diisopropylene glycol (20 healthy controls negative) (32). In this case report it is stated that “Acetyl cedrene (Vertofix Coeur) is a complex reaction mixture of which a principal constituent is methyl cedryl ketone”.

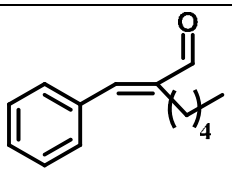
Additional information:
Acetyl cedrene (Vertofix®, IFF) is a complex mixture obtained from cedar wood oil by the acetylation of terpenes. The principal component of acetyl cedrene is methyl cedryl ketone (CAS 32388-55-9). It is a “top 100” substance (IFRA, pers. comm.2010)

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	
CAS # 21145-77-7	
EC # 216-133-4 / 244-240-6	
1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone	
AHMT (perfume), AHTN, Extralide, Fixolide, Musk tonalid, NSC 19550, Tentarome, Tetralide, Tonalid, Tonalide.	

Current regulation: Annex III, part 1, entry 182

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Tonalide ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

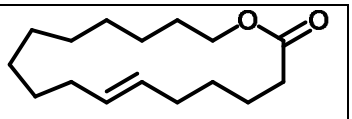
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; Jasmine aldehyde; NSC 6649; Pentylcinnamaldehyde; α-Amyl-β-phenylacrolein; α-Amylcinnamal; α-Amylcinnamaldehyde; α-Pentylcinnamaldehyde	

Current regulation: Annex III, part 1, entry 67

Clinical data:
In the "background information" section of the 1999 opinion (33), amyl cinnamal (synonymous: alpha amyl cinnamaldehyde) has been classified as frequently reported contact allergen because it has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=4, i.e., 0.2% (95% CI: 0.1 – 0.5%) positive reactions to this compound (1% pet.) in 2062 consecutively PTed patients (4). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed (6). The Larsen 2001 study yielded 2.3% positive reactions in 178 patients with known contact allergy to fragrance ingredients (test concentration: 5% pet.) (19). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to amyl cinnamal (22). The IVDK 2010 study, 0.26% (95% CI: 0 – 0.60%) of 1214 consecutively tested patients reacted to the compound, while 0.61% (95% CI: 0.36 – 0.86%) of 4375 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7).

Additional information:
It is a "top 100" substance and classified as R43 (IFRA, pers. comm. 2010).

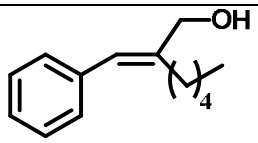
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

Current regulation: /

Clinical data:
The Larsen 2001 study, using omega-6-hexadecenlactone (HDL, 5% pet.) as test concentration, diagnosed 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Ambrettolide is 1 of 2 components of Ambrette seed oil (obtained from *Hibiscus abelmoschus* L., *Malvaceae*) responsible for the musk odour. In Surburg/Panten, the compound has the chemical name (Z)-7-hexadecen-16-olide (or Hexadec-7-en-16-olide according to CosIng), CAS 123-69-3 (34).

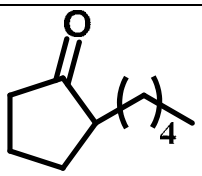
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol,	
2-Benzylidene- (6CI,8CI)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

Current regulation: Annex II, Part 1, entry 74

Clinical data:
In the "background information" section of the 1999 opinion, amyl cinnamyl alcohol is mentioned to cross-react with amyl cinnamal. Moreover, this compound has been identified as a cause of allergic reactions in a notable number of persons with eczema from the use of cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.4% (95% CI: 0.1 – 0.7%) positive reactions in 1977 consecutively PTed patients (4). The IVDK 2010 study, 0.79% (95% CI: 0.54 – 1.04%; percentages standardised for age and sex) of 5650 patients PTed reacted to the compound (7). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6).

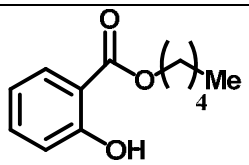
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

AMYLCYCLOPENTANONE	
CAS # 4819-67-4	
EC # 225-392-2	
2-Pentylcyclopentanone	
2-Pentyl-1-cyclopentanone; 2-Pentylcyclopentanone; 2-Pentylcyclopenten-1-one; 2-n-Amylcyclopentanone; 2-n-Pentyl cyclopentanone; Delphone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

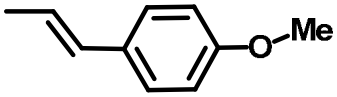
AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

Current regulation: /

Clinical data:

In the Frosch 2002 a study, a total of n=3 (0.2%) had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% amyl salicylate and 1 positive reaction to 5% amyl salicylate were observed in 100 consecutive patients patch tested in Stockholm (15).

Additional information:
A RIFM review is available (36). It is a “top 100” substance (IFRA, pers. comm.2010)

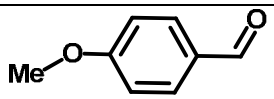
trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, EINECS); *E*-Anethole (INCI); 1-Methoxy-4-[(1E)-1-propenyl]benzene; (E)-1-Methoxy-4-(1-propenyl)-benzene (CosIng); NSC 209529; trans-1-(4-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)propene; trans-1-p-Anisylpropene; trans-4-(1-Propenyl)anisole; trans-Anethol; trans-Anethole; trans-p-Anethole; trans-p-Methoxy-β-methylstyrene	

Current regulation: /

Clinical data:
A case of a 64 year old patient, who developed severe cheilitis and a loss of taste has been described (37). Both were reversible after the cessation of use of previous toothpastes. The patch test was strongly positive to anethole (isoform not given) 5% pet.; this was found an ingredient of the causative toothpaste. Two cases of occupational allergic contact dermatitis occurring in a traditional cake factory due to anise oil have been described, both testing (strongly) positive to anise oil (5% o.o.) and anethole (5% pet.) (38).

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). trans-Anethole can be purified from star anise oil (34, 39), see 3.2., and is the main component of anise, star anise

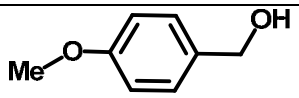
and fennel oils (38)

ANISALDEHYDE	
CAS # 123-11-5	
EC # 204-602-6	
4-Methoxy-benzaldehyde	
p-Methoxybenzaldehyde; p-Anisaldehyde; 4-Anisaldehyde; Aubepine; Crategine; NSC 5590; Obepin; p-Anisic aldehyde; Anisic aldehyde; p-Formylanisole.	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

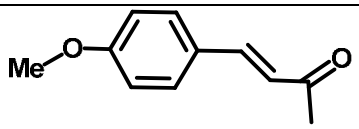
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy-α-hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methyloxy)phenyl]methanol; p-(Methoxyphenyl)methanol; p-Anisalcohol; p-Anisyl alcohol; p-Methoxybenzyl alcohol	

Current regulation: Annex III, part 1, n° 80

Clinical data:
In the “background information” section of the 1999 opinion, anisyl alcohol is classified as “less frequently reported allergen”; 2 studies were identified where 3 and 4 cases, respectively, with cosmetic dermatitis due to contact allergy to anisyl alcohol had been reported (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2004 consecutively PTed patients, patch test concentration: 1% pet. (4). Similar results were obtained in the following period, with n=1 (and n=3 irritant and n=6 doubtful) reactions in 986 patients tested with 1% in pet. (30). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6).This test concentration has been regarded as relatively high by Hostynek and Maibach (40). The test concentration of Anisyl Alcohol has been further validated by Bruze et al. and 10% in pet was recommended as a non-irritant concentration for routine investigations (40a).

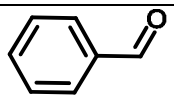
Additional information: /

ANISYLIDENE ACETONE	
CAS # 943-88-4	
EC # 213-404-9	
4-(4-Methoxyphenyl)-3-Buten-2-one	
1-(p-Methoxyphenyl)-1-buten-3-one; 4-(4-Methoxyphenyl)-3-buten-2-one; 4-(p-Methoxyphenyl)-3-buten-2-one; 4-Methoxybenzalacetone; 4-Methoxybenzylideneacetone; 4-Methoxystyryl methyl ketone; 4'-Methoxybenzylideneacetone; Anisalacetone; Methyl p-methoxystyryl ketone; NSC 31752; NSC 7946; p-Anisalacetone; p-Methoxybenzalacetone; p-Methoxybenzylideneacetone; p-Methoxystyryl methyl ketone	

Current regulation: Annex III, part 1, n° 443

Clinical data:
In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to anisylidene acetone 2% pet. (24)

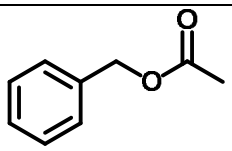
Additional information: /

BENZALDEHYDE	
CAS # 100-52-7	
EC # 202-860-4	
Benzaldehyde	
Artificial Almond Oil; Benzaldehyde FFC; Benzenecarbonal; Benzenecarboxaldehyde; Benzoic acid aldehyde; Benzoic aldehyde; NSC 7917; Phenylformaldehyde; Phenylmethanal	

Current regulation: /

Clinical data:
In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions to benzaldehyde 5% pet. (22). The IVDK 2010 study, 6 weak positive reactions were observed, i.e., 0.16% (95% CI: 0.03 – 0.29%; percentages standardised for age and sex) of 2820 patients PTed reacted to the compound (7). A review is available in the Int. J. Toxicol. (41). In the case of a 19 year old pastry maker, Seite-Bellezza et al. report on immediate reactions to MP, cinnamal and benzaldehyde (tested at 5% pet.) subsiding after a few hours, in line with the patient's history (42).

Additional information: /

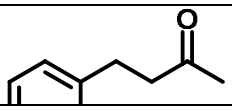
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl actetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl aceteate in pet., tested in 100 consecutive patients in Odense, DK, were observed (15). Benzyl acetate is a component of several natural mixtures, for example a major constituent of Narcissus abs., and a minor constituent of Jasmine abs. (17).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

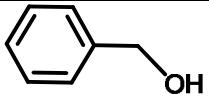
BENZYL ACETONE	
CAS # 2550-26-7	

EC # 219-847-4	
4-Phenyl-2-butanone	
4-Phenylbutan-2-one (REACH, EINECS); Benzylacetone; Methyl 2-phenylethyl ketone; Methyl phenethyl ketone; NSC 44829; NSC 813M; Phenethyl methyl ketone; 1-Phenyl-3-butanone; 2-Phenylethyl methyl ketone	

Current regulation: /

Clinical data: /

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (43).

BENZYL ALCOHOL	
CAS # 100-51-6	
EC # 202-859-9	
Phenylmethanol	
Benzyl alcohol; (Hydroxymethyl)benzene; Benzenecarbinol; Benzylic alcohol; NSC 8044; Phenylcarbinol; Benzenemethanol; Phenylmethyl alcohol; Sunmorl BK 20; TB 13G; α-Hydroxytoluene; α-Toluenol	
Current regulation: Annex III, part 1, n° 45; Annex VI, part1, n ° 34	

Clinical data:
In the “background information” section of the 1999 opinion, benzyl alcohol is classified as allergen frequently causing allergic reactions. It has been found to cause allergic reactions in 1.2 to 15% of patients with eczema from cosmetic products (33). A CIR expert panel review is available in the Int. J. Toxicol. (44).

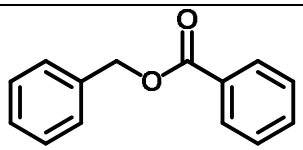
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

Both in terms of case reports (45-47) and clinical epidemiology data (0.22 % [95% CI: 0.16 – 0.28%] positive tested with benzyl alcohol in the context of a “topical drugs” series, n=26448 (7)) the relevance of this alternative exposure is highlighted. In a study from Alicante, Spain, 86 selected patients were tested with benzyl alcohol, yielding 2 positive reactions (48).

After application of saline soaks preserved with benzyl alcohol onto his stasis dermatitis, a 53 year old patient developed a rash, which was, according to test results obtained by J. D. Guin and J. Goodman, at least partly due to an immediate hypersensitivity to benzyl alcohol, as verified by an intense urticarial reaction at the test site lasting several days (49). According to 2 cases reported by A. A. Fisher, PT-proven, relevant delayed type hypersensitivity is not associated with immediate reactions in scratch or intradermal tests (50). D. W. Shaw describes a patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications (51). Another contribution points to covert exposures to benzyl alcohol even in products labelled “fragrance free” (52) probably because benzyl alcohol is used as preservative, or an essential oil containing benzyl alcohol is used as cosmetic ingredient.

Additional information:

Benzyl alcohol is a component of several natural mixtures, including Myroxylon pereirae resin, which have been used for extraction, but is nowadays synthesised (53). It is permitted in certain foodstuffs (liquors: < 100 mg/l, sweets and cakes: < 250 mg/kg) under the coding "E 1519" (http://www.zusatzstoffe-online.de/zusatzstoffe/317.e1519_benzylalkohol.html, last accessed 2009-11-27). In addition to being a fragrance compound (which may be used, even in relatively high concentration, to scent topical medications (54)), benzyl alcohol is used as antioxidant in topical therapeutics or cosmetics. The German "Rote Liste" (http://www.rote-liste.de, last accessed 2009-11-11), for instance, lists 205 specialties containing benzyl alcohol. Benzyl alcohol may be used up to 1.0% as a preservative in cosmetic products according to the Cosmetic Directivr 76/768/EEC

BENZYL BENZOATE	
CAS # 120-51-4	
EC # 204-402-9	
Benzyl benzoate	
Benzyl ester benzoic acid; Ascabin; Ascabiol; Benylate; Benzyl benzenecarboxylate; Benzyl benzoate; Benzyl phenylformate; Benzylets; Colebenz; NSC 8081; Nicca Sunsolt LM 7EX; Novoscabin; Pelemol B66; Peruscabin; Phenylmethyl benzoate; Scabagen; Scabanca; Scabcare BB; Scabide; Scabiozon; Scobenol; Vanzoate; Venzonate	

Current regulation: Annex III, part 1, n° 85

Clinical data:

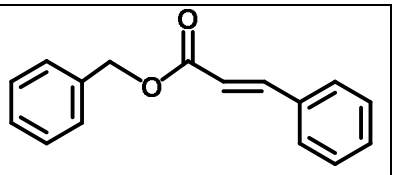
In the "background information" section of the 1999 opinion, benzyl benzoate is classified as "less frequently reported allergen"; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2003 consecutively PTed patients, test concentration 1% pet. (4). In the subsequent period (2005-2008), n=1062 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). Thus, the pooled proportion of positive patch test reactions is 1 / 3385 (0.03%, exact upper 1-sided 95% CI: 0.14%)

Additional information:

Benzyl benzoate naturally occurs in MP resin and ylang-ylang oil. Nowadays it is synthesised and used for a variety of purposes (53). These include use as a scabicide (one brand specialty on the German market, using a concentration of 10% for children and 25% for adults), possibly with some differences among European countries. In France, a combination of benzyl benzoate 10% and sulfiram 2% is reported to be used most often (55). Hausen et al. review the older literature and mention a study identifying 1 sensitised patient in 73 patients treated for scabies (details not given) (53). According to the mandatory factsheet (see PDF "benzylbenzoate_infosheet_DE.pdf") dermatitis after anti-scabies treatment is "rare", in a range between 1:1000 and 1:10000.

It is a "top 100" substance (IFRA, pers. comm.2010).

BENZYL CINNAMATE	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

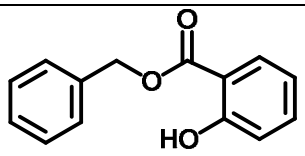
Current regulation: Annex III, part 1, n° 81

Clinical data:

In the “background information” section of the 1999 opinion, benzyl cinnamate (synonymous: benzyl 3-phenyl-2-propenoate, cinnamein) is classified as “less frequently reported allergen”; one study of patients with contact allergy to cosmetic products was identified and further a study where benzyl cinnamate associated with contact sensitisation to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2042 consecutively PTed patients, test concentration 5% pet. (4). The IVDK 2010 study, n=4 weak positive were observed, amounting to 0.12% (95% CI: 0 – 0.25%; percentages standardised for age and sex) of 2872 patients PTed reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, using the same test concentration, were observed in 320 patients (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22).

Additional information: A RIFM review is available (56).

BENZYL SALICYLATE	
CAS # 118-58-1	
EC # 204-262-9	
Benzyl 2-hydroxybenzoate	
Salicylic acid, Benzyl ester; Benzoic acid, 2-Hydroxy-, phenylmethyl ester; Benzyl o-hydroxybenzoate; NSC 6647	

Current regulation: Annex III, part 1, n° 75

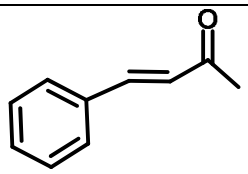
Clinical data:

In the "background information" section of the 1999 opinion (33), benzyl salicylate is classified among the frequent allergens, with 0.2 to 10% of patients with eczema from cosmetic products testing positively. In one study, benzyl salicylate accounted for 75% of reactions to commercial products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=2, i.e. 0.1% (95% CI: 0.01 – 0.4%) positive reactions in 2041 consecutively PTed patients (test concentration 1% pet.) (4). The IVDK 2010 study, 2 of 3775 patients PTed reacted weakly positive to the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at 2% pet. (6). In the deGroot 2000 study, 10 of 1825 consecutive patients tested positive to benzyl salicylate (2% pet.), of these, 3 were not detected by the FM I (12). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22). Trattner/David found 2 positive cases in 641 consecutive eczema patients (31). In a study from Alicante, Spain, 86 selected patients were tested with benzyl salicylate, yielding 2 positive reactions (48).

Additional information:

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010). A RIFM review is available, including internal results on, e.g. HRIPT, and a review of LLNA results, where benzyl salicylate is classified as "weak" allergen (57).

BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; Acetocinnamone; Benzalacetone; Benzylideneacetone; Methyl 2-phenylvinyl ketone; Methyl phenylvinyl ketone; Methyl styryl ketone; Methyl β-styryl ketone; NSC 5605; Styryl methyl ketone	

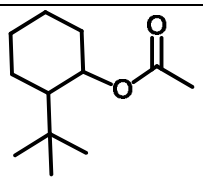
Current regulation: Annex II, n° 356

Clinical data:

In the Malten 1984 study, none of 182 patients displayed a positive PT reaction to

benzylidene acetone 0.5% pet. (24).

Additional information: /

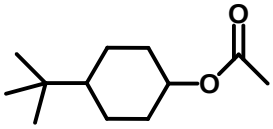
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Verdox ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (58).

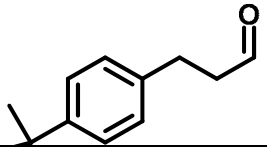
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinol A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; NSC 163103; Oryclone, Oryclone special, Oryclon extra; p-t-BCHA; p-tert-Butylcyclohexyl acetate; para-tert-Butylcyclohexyl acetate; PTBCHA; Velvetone; Verbeniax; Vertenex; Vertinate; Vertopol; Ylanate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Vertenex ®” in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (59).

p-tert -Butyldihydrocinnamaldehyde	
CAS # ***18127-01-0***	
EC # 242-016-2	

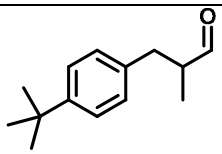
4-(1,1-Dimethylethyl)-benzenepropanal	
p-tert-Butyl-hydrocinnamaldehyde; 3-(4-tert-Butylphenyl)propanal; Bourgeonal; p-tert-Butyldihydrocinnamaldehyde	

Current regulation: III/155

Clinical data: /

Additional information:
It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=39132

BUTYLPHENYL METHYLPROPIONAL (Lilial®)	
CAS # 80-54-6	
EC # 201-289-8	
3-(4-tert-Butylphenyl)-2-methylpropanal	
p-t-Butyl-alpha-methylhydrocinnamic aldehyde; 2-(4-tert-Butylbenzyl)propionaldehyde (REACH, EINECS); 4-(1,1-Dimethylethyl)-α-methyl-benzenepropanal; Hydrocinnamaldehyde, p-tert-Butyl-α-methyl-; (±)-2-Methyl-3-(4-tert-butylphenyl)propanal; 2-Methyl-3-(4-tert-butylphenyl)propanal; 2-[(4-tert-Butylphenyl)methyl]propanal; 3-(4-tert-Butylphenyl)-2-methylpropanal; 3-(p-tert-Butylphenyl)-2-methylpropionaldehyde; 3-(p-tert-Butylphenyl)isobutylaldehyde; 4-(1,1-Dimethylethyl)-α-methylbenzenepropanal; 4-tert-Butyl-α-methylhydrocinnamic aldehyde; Lilestralis; Lilial; Lysmeral; NSC 22275; lilestral; p-tert-Butyl-α-methylhydrocinnamaldehyde; p-tert-Butyl-α-methylhydrocinnamic aldehyde; pt-Bucinal; α-Methyl-p-tert-butylhydrocinnamaldehyde; β-Lilial	

Current regulation: Annex III, part 1, n° 83

Clinical data:

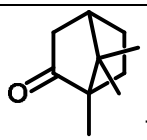
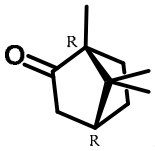
In the “background information” section of the 1999 opinion, lilial is classified as “less frequently reported allergen”; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were considered to possibly have been false positive (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002a study yielded 0.2% positive reactions to Lilial® (10% pet.) among the 1855 consecutive patients tested (16). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2004 patients consecutively tested (4). The IVDK 2010 study, 0.62% (95% CI: 0.04 – 1.21%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In the deGroot 2000 study, 9 of 1825 consecutively tested patients had a positive reaction to lilial® (5%

pet.) (12). Lilial® has been identified as constituent of perfumes used by a patient, causing ACD (60).

Additional information:

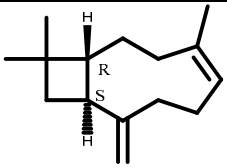
It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2  464-49-3
CAS # 76-22-2 / 464-49-3	
EC # 207-355-2 / 200-945-0	
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (76-22-2) **(1R,4R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one** (464-49-3)	
76-22-2: DL-Bornan-2-one (REACH, EINECS); 2-Bornanone; Bornan-2-one, INCI name according to CAS; CAMPHOR/DL-bornan-2-one; Camphor; (±)-Camphor; DL-Camphor; 1,7,7-Trimethylnorcamphor; 2-Camphanone; Alphanon; Borneo camphor; Root bark oil; Spirit of camphor **464-49-3**: (1R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one; (1R,4R)-(+)- Camphor; (+)-2-Bornanone; (+)-Camphor; (1R)-(+)-Camphor; (1R)-Camphor; (1R,4R)-(+)-Camphor; (R)-(+)-Camphor; (R)-Camphor; Camphor; D-Camphor; D-(+)-Camphor; Alcanfor; Japanese camphor.	

Current regulation: /

Clinical data:
From the UK, a case of allergic contact dermatitis after application of Earex ® ear drops due to rectified camphor oil (tested 10% pet.) was reported (61). Application of a liquid rubefacient of Asian origin caused allergic contact dermatitis in a 58-year-old patient, according to the positive PT result with 10% camphor (“alcaonfor”) in pet. due to this ingredient (62). In the US, a case of contact dermatitis due to “Vics VapoRub” has been reported (63).

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010).

beta-CARYOPHYLLENE	
CAS # 87-44-5	
EC # 201-746-1	
(1R,4E,9S)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	
(E)-(1R,9S)-(-)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; [1R-(1R*,4E,9S*)]-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; (-)-(E)-Caryophyllene; (-)-Caryophyllene; (-)-E-Caryophyllene; (-)-trans-Caryophyllene; (-)-β-Caryophyllene; (E)-Caryophyllene; Caryophyllene; Caryophyllene B; NSC 11906; l-Caryophyllene; trans-Caryophyllene; β-Caryophyllen; β-Caryophyllene; (-)-β-Caryophyllene	

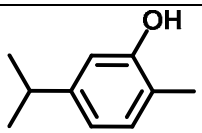
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% positive reactions to caryophyllene (5% pet.) in 1606 consecutive were observed (17).

Additional information:

beta-Caryophyllene autoxidizes at air exposure. As the primary oxidation products, the hydroperoxides, are very unstable and immediately form epoxides with low sensitizing capciaty, the increase in allergenic activity caused by autoxidation is comparably low (64). A multicenter study identified 0.5% positive reactions to oxidized *beta*-caryophyllene (3.0% pet.) in 1511 consecutive patients (65). Of these, 2 patients (0.1%) reacted to the major oxidation product (caryophyllene oxide) (3.9% pet.).

CARVACROL	
CAS # 499-75-2	
EC # 207-889-6	
2-Methyl-5-(1-methylethyl)-phenol	
2-Hydroxy-1-methyl-4-(1-methylethyl)benzene; 2-Hydroxy-p-cymene; 2-Methyl-5-(1-methylethyl)phenol; 2-Methyl-5-isopropylphenol; 3-Isopropyl-6-methylphenol; 5-Isopropyl-2-methylphenol; 5-Isopropyl-o-cresol; 6-Methyl-3-isopropylphenol; Antioxine; Dentol; Isopropyl o-cresol; Isothymol; NSC 6188; p-Cymen-2-ol	

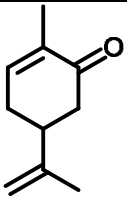
Current regulation: /

Clinical data:

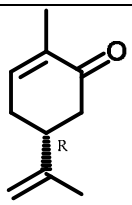
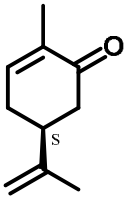
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an “excited back syndrome” and are thus of limited evidence (25). Meynadier et al. [11] patch tested 28 patients with contact allergy to fragrance ingredients using 2% carvacrol in pet. Positive reactions were observed in 3 of 28 patients (after (66)).

Additional information:

Carvacrol is derived from p-cymene by sulfonation followed by alkali fusion. Carvacrol can also be derived from savory, thyme, marjoram, oregano, lovage root, and Spanish origanum oil (66). Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001, according to (66).

CARVONE	 99-49-0
CAS # 99-49-0 / 6485-40-1 / 2244-16-8	
EC # 202-759-5 / 229-352-5 / 218-827-2	
2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (99-49-0)	

[11] Meynadier, J. M., J. Meynadier, J. L. Peyron, and L. Peyron. 1986. Clinical forms of skin manifestations in allergy to perfume. *Ann. Dermatol. Venerol.* 113:31–39.

(5R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (6485-40-1) **(5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one** (2244-16-8)	 6485-40-1  2244-16-8
99-49-0: p-Mentha-6,8-dien-2-one; (±)-Carvone; 2-Methyl-5-isopropenyl-2-cyclohexenone; 5-Isopropyl-2-methyl-2-cyclohexen-1-one; Carvone; DL-Carvone; Karvon; Limonen-6-one; NSC 6275; p-Mentha-1(6),8-dien-2-one **6485-40-1**: R)-(-)-p-Mentha-6,8-dien-2-on); (-)-(5R)-Carvone; (-)-(R)-Carvone; (-)-Carvone; (-)-p-Mentha-6,8-dien-2-one; (4R)-(-)-Carvone; (R)-(-)-Carvone; (R)-Carvone; L-(-)-Carvone; L-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; l-Carvone **2244-16-8**: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone	

Current regulation: /

Clinical data:

Cases of allergic contact cheilitis due to L-carvone in toothpastes have been reported (67-69). In an earlier study, 15 of 541 (2.8%) of consecutive PT patients tested also with L-Carvone (5% pet.) exhibited positive reactions, which were (i) associated with positive PT results to *Compositae* mix and (ii) mostly were not considered clinically relevant. Upon re-testing with lower concentrations (2% and 1% pet.) only 2 of 8 patients thus tested were positive (70).

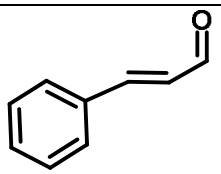
“Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis on the head. She had used a hair conditioner with a “mint” scent, and the dermatitis resolved when she discontinued using this product. While the manufacturer would not confirm carvone as an ingredient, the clinical course, patch-test results, and ingredient list strongly suggest that this was a relevant allergen in this case of allergic contact dermatitis”[12]

Additional information:

D-Carvone occurs in caraway seed oil and dill oil in a concentration of up to 60%. L-Carvone is a component of the oil from Mentha spicata (spearmint).

R-Carvone is identified as a secondary oxidation product in autoxidized limonene (71). However, it is not a major allergen in this oxidation mixture and only one of 30 patients with known contact allergy to oxidized R- limonene reacted when tested with carvone (3% pet.) (72). Experimental findings in guinea pigs show no cross reactivity between R- and S carvone, but both enantiomers were found to be equally strong sensitizers (73).

[12] http://www.ncbi.nlm.nih.gov/pubmed/20233552

CINNAMAL	
CAS # 104-55-2	
EC # 203-213-9	
3-Phenyl-2-propenal	
Cinnamaldehyde; 3-Phenyl-2-propen-1-al; 3-Phenyl-2-propenaldehyde; 3-Phenylacrolein; 3-Phenylacrylaldehyde; 3-Phenylpropenal; Abion CA; Benzylideneacetaldehyde; Cassia aldehyde; Cinnacure; Cinnamal; Cinnamic aldehyde; Cinnamite; Cinnamyl aldehyde; NSC 16935; NSC 40346; Phenylacrolein; Zimtaldehyde; β-Phenylacrolein	

Current regulation: Annex III, part 1, n° 76

Clinical data:

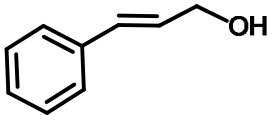
In the “background information” section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.0% (95% CI: 0.6 – 1.6%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.6% (95% CI: 0.5 – 3.6%) had positive reactions to cinnamal (6). In a study by the North American Contact Dermatitis Group, no significant trend of cinnamal contact sensitisation in the consecutive patients analysed was observed between 1984 (5.9% pos.) and 2000 (3.6% pos.); tested at 1% pet. (74). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded 1.7% positive reactions. In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.9% positive reactions (22). The NACDG study found 3.1% positive reactions in 4435 patients tested (21). The IVDK 2010 study, 1.43% (95% CI: 0.67 – 2.18%) of 1214 consecutively tested patients reacted to the compound, while 2.64% (95% CI: 2.16 – 3.13%) of 4527 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=7 reacted positively to cinnamal (48).

While, in addition to typical ACD due to contact sensitisation, immediate reactions to some fragrance compounds (and MPR, see below) are observed not infrequently, such immediate type reactions may rarely be very severe (anaphylaxis) and possibly immunologically mediated, as illustrated by the case of a 42 year old nurse with anaphylaxis (maximum grade of contact urticaria syndrome) 20 min after application of cinnamal (75). Following industrial use as “odour masking” agent, cinnamal caused occupational ACD in an exposed worker (76).

Additional information:

A specific RIFM review is available (77); another RIFM review addresses several cinnamic compounds (78).

CINNAMYL ALCOHOL	
CAS # 104-54-1	
EC # 203-212-3	
3-Phenyl-2-propen-1-ol	

Cinnamyl alcohol; 1-Phenyl-3-hydroxy-1-propene; 1-Phenylprop-1-en-3-ol; 3-Hydroxy-1-phenylprop-1-ene; 3-Phenyl-2-propenol; 3-Phenylallyl alcohol; Cinnamic alcohol; NSC 623440; NSC 8775; Styrone; Styryl alcohol; Styryl carbinol; γ-Phenylallyl alcohol	

Current regulation: Annex III, part 1, n° 69

Clinical data:

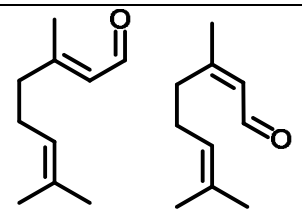
In the "background information" section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to cinnamyl alcohol, tested at 2% pet., i.e., twice the commonly used concentration (6). As test concentrations of up to 5% are apparently non-irritating (de Groot et al. after (33)), the latter data can be regarded as valid. In the An 2005 study, 13 of 422 consecutive patients, i.e., 3.1%, had positive reaction (13) (test concentration 2%). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 0.73% (95% CI: 0.17 – 1.30%) of 1214 consecutively tested patients reacted to the compound, while 2.36% (95% CI: 1.89 – 2.83%) of 4502 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to cinnamyl alcohol (48).

Additional information:

In a recent experimental study protein-cinnamal adducts were detected in skin homogenates treated with cinnamal and cinnamyl alcohol but not with alpha-amyl cinnamal. This suggests that there is a common hapten involved in cinnamal and cinnamyl alcohol sensitization, in line with the observation of a marked concordance upon patch testing (7, 79), and that metabolic activation (to cinnamal) is involved in the latter. Conversely, there does not appear to be a common hapten for cinnamal and alpha-amyl cinnamal (80), again in line with the observations in the IVDK 2010 study (7).

A RIFM review is available (81)

CITRAL	
CAS # 5392-40-5	
EC # 226-394-6	
3,7-Dimethyl-2,6-octadienal	
3,7-Dimethyl-2,6-octadien-1-al; Citral; Citral PQ Extra; Lemarome N; Lemsyn GB; NSC 6170	**Citral** = isomeric mixture of Geranial and Neral

Current regulation: Annex III, part 1, n° 70

Clinical data:

In the "background information" section of the previous opinion (33), citral is classified as frequent allergen, causing about 1% allergic reactions in consecutive PT patients, and being a proven cause of contact allergic reactions in 2.6% patients with eczema from

cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002 a study yielded 1.1% positive (and 1.3% doubtful) reactions among the 1855 consecutive patients tested (16). In a study on 586 consecutive patients with hand eczema it has been noted that citral (2% pet.) not only caused (mostly weak) positive PT reactions, but far more often irritant reactions (n=82 vs. n=28). It was hypothesised that this very property could contribute to citral's sensitising potential (82). In the EU 2005 study, 12 of 1701 patients (0.7%, 95% CI: 0.4 – 1.2%) reacted positively to 2% citral in pet. (10). The IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2021 consecutively PTed patients; 10 of 13 citral positive patients also reacted positively to geraniol (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 multicentre study, 19 of 1825 consecutive patients tested positively to citral (2% pet.), 4 of whom did not react positively to the FM I (12). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reaction (13) (test concentration 2%). In the Malten 1984 study, neral at 1% in pet. yielded 2.6% positive reactions in 182 patients (24). In a study from Alicante, Spain, 86 selected patients were tested with citral, yielding 2 positive reactions (48).

Citral in a lip salve has been reported to have caused longstanding, recurrent allergic contact cheilitis in a 30 year old female patient, diagnosed by a strong positive reaction to the FM II, followed by a strong positive reaction to citral (83).

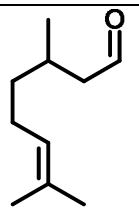
Additional information:

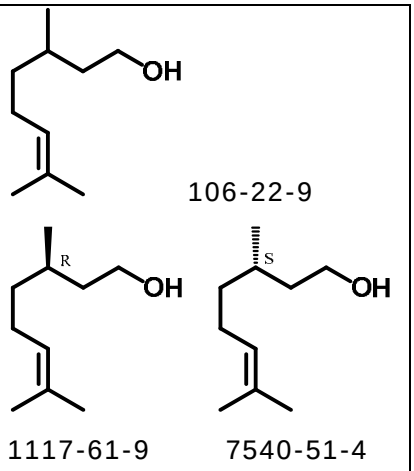
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

Geranial forms oxidation product with increased sensitizing capacity both via spontaneous autoxidization at air exposure and via metabolic oxidation (Hagvall L. Thesis 2009: http://hdl.handle.net/2077/18951).

Geranial and neral have been identified as secondary oxidation products when geraniol autoxidizes (84). They have also been identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLAL	
CAS # 106-23-0	
EC # 203-376-6	
3,7-Dimethyl-6-octenal	
(±)-Citronellal; 2,3-Dihydrocitral; 3,7-Dimethyloct-6-en-1-al; Citronellal; NSC 46106; Rhodinal; dl-Citronellal; β-Citronellal	
Current regulation: /	
Clinical data: /	
Additional information: A compound of essential oils of citrus fruits, namely grapefruit, but also contained in “citronella oil” and oil of Melissa.	

CITRONELLOL	
CAS # 106-22-9 / 1117-61-9 / 7540-51-4	
EC # 247-737-6 / 214-250-5 / 231-415-7	
3,7-Dimethyl-6-octen-1-ol (106-22-9); **(3R)-3,7-Dimethyl-6-octen-1-ol** (1117-61-9); **(3S)-3,7-Dimethyl-6-octen-1-ol** (7540-51-4)	
106-22-9: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronellol 950; DL-Citronellol; Dihydrogeraniol; NSC 8779; Rodinol; dl-Citronellol; β-Citronellol **1117-61-9**: (R)-3,7-Dimethyl-6-octen-1-ol; (R)-(+)-3,7-Dimethyl-6-octen-1-ol; (+)-(R)-Citronellol; (+)-Citronellol; (+)-β-Citronellol; (3R)-(+)-β-Citronellol; (R)-(+)-Citronellol; (R)-(+)-β-Citronellol; (R)-Citronellol; (R)-β-Citronellol; D-Citronellol; d-Citronellol **7540-51-4**: (-)-3,7-Dimethyl-6-octen-1-ol; (-)-(S)-Citronellol; (-)-Citronellol; (-)-β-Citronellol; (S)-(-)-Citronellol; (S)-(-)-β-Citronellol; (S)-3,7-Dimethyl-6-octen-1-ol; (S)-Citronellol; (S)-β-Citronellol; L-Citronellol; l-Citronellol	106-22-9 1117-61-9 7540-51-4
Current regulation: Annex III, part 1, n° 86	

Clinical data:

In the “background information” section of the 1999 opinion, citronellol is classified as “less frequently reported allergen”; with few cases of contact allergy reported in the literature (33).

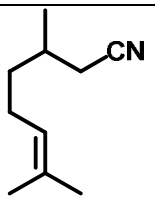
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronellol“ (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%, 95% CI: 0.06 – 0.6%) reacted positively to 1%

citronellol in pet.; at the same concentration, n=23 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2003 patients consecutively PTed (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at only 2% pet. (6). The Larsen 2001 study yielded 5.6% positive reactions to l-citronellol (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Citronellol autoxidizes spontaneously in contact with air in the same way as linalool forming allergenic primary oxidation products, hydroperoxides (AT Karlberg, personal communication, 2011).

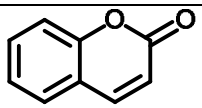
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLYL NITRILE	
CAS # 51566-62-2	
EC # 257-288-8	
3,7-Dimethyl-6-octenenitrile	
3,7-Dimethyl-6-octenonitrile (REACH, EINECS, INCI); Agrunitril; Agrunitrile; Citronellyl nitrile	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010)

COUMARIN	
CAS # 91-64-5	
EC # 202-086-7	
2H-1-Benzopyran-2-one	
1,2-Benzopyrone; 2-Chromenone; 2-Propenoic acid, 3-(2-hydroxyphenyl)-, δ-lactone;5,6-Benzo-2-pyrone; Benzo-α-pyrone; Coumarinic anhydride; NSC 8774; Rattex; Tonka bean camphor; cis-o-Coumarinic acid lactone; o-Hydroxycinnamic acid lactone	

Current regulation: Annex III, part1, n° 77

Clinical data:

In the "background information" section of the previous opinion (33), coumarin is classified as frequent allergen, causing allergic reactions in about 0.4 – 0.8% in consecutive PT patients, and causing contact allergic reactions in 0.8-10% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the EU 2005 study, none of the

1701 patients reacted positively to 5% coumarin in pet., while 7 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2020 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 study, 13 of 1825 consecutive patients reacted positively to coumarin (5% pet.) (12).

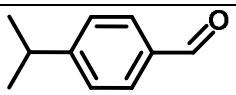
V. Mutterer et al. present the case of a 44 year old patient in whom coumarin was identified as culprit allergen by controlled ROAT testing with 1%, after having caused dermatitis by the use of a deodorant containing coumarin at 0.23% and an EdT (90).

Additional information:

Coumarin is found in several plant families, including *Melilotus* and *Galium*, e.g., *Galium odoratum* (sweet woodruff), however, also in oil of lavender, lovage and others (53).

Researchers from INSERM and "Rhodia Organique, Lyon , France" observed that pure coumarin is not an allergen in the LLNA, however, commercially available materials, containing "contaminants" (3,4-dihydrocoumarin, 6-chlorocoumarin and 6,12-epoxy-6H,12H-dibenzo[b,f][1,5] dioxocin, were identified as weak and moderate sensitisers, resp. (91).

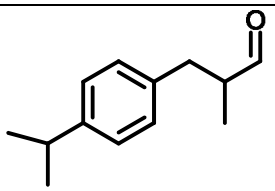
Coumarin is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CUMINALDEHYDE	
CAS # 122-03-2	
EC # 204-516-9	
4-(1-Methylethyl)-benzaldehyde	
4-Isopropylbenzaldehyde; p-Isopropylbenzaldehyde; 4-(Propan-2-yl)benzaldehyde; 4-Isopropylphenylcarboxaldehyde; Cumaldehyde; Cumic aldehyde; Cuminal; Cuminaldehyde; Cuminic aldehyde; Cuminyl aldehyde; NSC 4886; p-Cumic aldehyde; p-Isopropylbenzaldehyde; p-Isopropylbenzenecarboxaldehyde	

Current regulation: /

Clinical data:
The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 15% PT preparation of cuminaldehyde (25).

Additional information: …

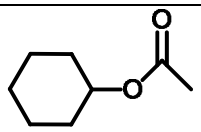
CYCLAMEN ALDEHYDE	
CAS # 103-95-7	
EC # 203-161-7	
α-Methyl-4-(1-methylethyl)-benzenepropanal	
p-Isopropyl-α-methyl-hydrocinnamaldehyde; 2-Methyl-3-(4-isopropylphenyl)propionaldehyde; 2-Methyl-3-(p-isopropylphenyl)propionaldehyde; 3-(4-Isopropylphenyl)-2-methylpropanal; 3-(p-Isopropylphenyl)-2-	

methylpropionaldehyde; 3-p-Cumenyl-2-methylpropionaldehyde(REACH, EINECS); 4-Isopropyl-α-methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl-α-methylhydrocinnamaldehyde; α-Methyl-4-(1-methylethyl)benzenepropanal; α-Methyl-p-isopropylhydrocinnamaldehyde	

Current regulation: …

Clinical data:
/

Additional information: It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

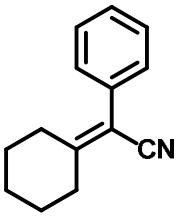
CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.5% positive reactions among 218 patients with know contact allergy to fragrance ingredients were observed (1).

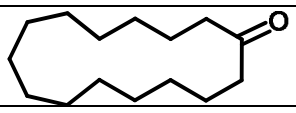
Additional information: A RIFM review is available (92).

alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	
CAS # 10461-98-0	
EC # 423-740-1	
α-Cyclohexylidenebenzeneacetonitrile	
alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ1α-Phenyl-α-Cyclohexaneacetonitrile; 2-Cyclohexylidene-2-phenylacetonitrile; NSC 408284; Peonile (REACH)	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

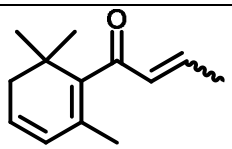
CYCLOPENTADECANONE	
CAS # 502-72-7	

EC # 207-951-2	
Cyclopentadecanone	
CPE 218; Exaltone; NSC 63900; Normuscon; Normuscone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, n=3, i.e., 1.7% positive reactions were observed to the compound, tested 5% pet., in 178 pateints with known contact allergy to fragrance ingredients (19).

Additional information: …

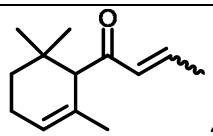
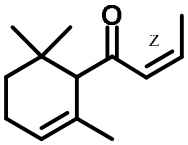
DAMASCENONE ***ROSE KETONE-4*** (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-(2-butenoyl)-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-crotonyl-1,3-cyclohexadiene; Rose ketone # 4	

Current regulation: Annex III, part1, n° 160 (max. conc. 0.02%)

Clinical data: /

Additional information:

RIFM reviews are available (93, 94), quoting 1 negative, and 2 positive (2 of 37, 1 of 50 volunteers) HRIPTs with damascenone based on 2 LLNA, the EC3 values were calculated as 1.24% and 1.22%, respectively (94).

alpha-DAMASCONE (TMCHB)	 43052-87-5  23726-94-5
CAS # 43052-87-5 / 23726-94-5	
EC # x / 245-845-8	
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-2-enone (43052-87-5); **(2Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one** (23726-94-5)	
43052-87-5: 2,6,6-Trimethyl-1-crotonyl-2-cyclohexene; α-Damascone **23726-94-5**: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)-α-Damascone; cis-α-Damascone	

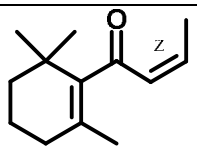
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

Clinical data:
In the Frosch 2002 b study, n=8 (0.5%) mostly strong positive PT reactions to consecutive patients were noted using a mixture of alpha and beta damascene, 0.1% pet. each (17). In human sensitisation experiments, after epicutaneous induction with 30% 1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enone **(**TMCHB, CAS # 43052-87-5) with adjuvant, to enhance response to this weak sensitiser, 8 of 30 patients were elicited by a challenge with 3% TMCHB 2 weeks later (95).

Additional information:

The former CAS # refers to alpha-Damascone or 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one. The latter CAS # refers to the identified ingredient cis-alpha-Damascone or (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, the content of which is restricted (SCCS-opinion 0392/00).

A RIFM review is available on alpha-damascone (96), quoting a number of partly positive HRIPT and other human studies, as well as different animal experiments. In 1 LLNA reported, an EC3 value of 3.3% was found. Another RIFM review is available for cis-alpha-damascone (97), supplying, however, no data on sensitisation.

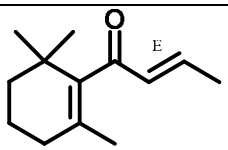
cis-beta-DAMASCONE	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)-β-Damascone	

Current regulation: Annex III, part 1, n° 162 (max. conc. 0.02%)

Clinical data:
Regarding results of the Frosch 2002 b study, see under alpha-damascone.

Additional information:

A RIFM review is available (98), citing several negative and one positive HRIPTs, and a number of – mostly positive – animal experiments.

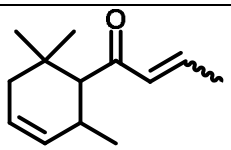
trans-beta-DAMASCONE	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)-β-Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans-β-Damascone; β-Damascone	

Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (99), citing 2 negative HRIPT and 1 negative maximisation test, and a number of positive animal experiments (the EC3 value, based on 1 LLNA, was found to be 2.4%).

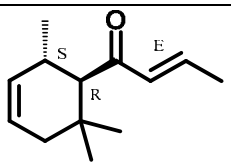
delta-DAMASCONE	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ-Damascone	

Current regulation: Annex III, part 1, n° 161 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (100), citing several positive HRIPT and 1 negative HRIPT. Cross sensitisation to alpha- and beta-damascone was demonstrated in 3 sensitised subjects. 2 LLNA studies are reported on, yielding EC3 values of 5.19% and 9.6%, resp.

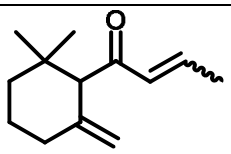
trans-trans-delta-DAMASCONE	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1α(E),2β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans-δ-Damascone; δ-Damascone; trans, trans-δ-Damascone	

Current regulation: Annex III, part 1, n° 165 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (101), citing 1 positive HRIPT (2/15 with 1%).

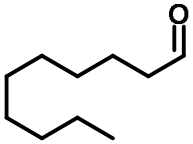
gamma-DAMASCONE	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

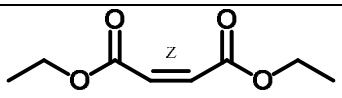
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

DECANAL	
CAS # 112-31-2	
EC # 203-957-4	
n-Decanal	
Capraldehyde; Capric aldehyde; Caprinaldehyde; Caprinic aldehyde; Decaldehyde; Decanaldehyde; Decyl aldehyde; Decylic aldehyde; NSC 6087; n-Decaldehyde; n-Decyl aldehyde	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

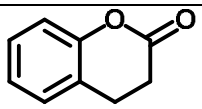
DIETHYL MALEATE	
CAS # 141-05-9	
EC # 205-451-9	
(**2Z)-Diethyl but-2-enedioate**	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

Current regulation: Annex II, n° 426

Clinical data:

In the Malten 1984 study, 3.2% of 182 patients displayed a positive PT reaction to diethyl maleate 0.1% pet. (24). In this study, it has been noted that “in the max. test and clinically this is a strong sensitiser having caused patch test sensitisation (42%)”

Additional information: /

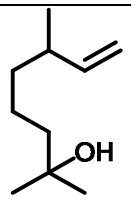
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ-lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

Current regulation: Annex II, n° 427

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dihydrocoumarine 5% pet. (24).

Additional information: /

DIHYDROMYRCENOL	
CAS # 18479-58-8	
EC # 242-362-4	
(±)-2,6-Dimethyloct-7-en-2-ol	
1,1,5-Trimethyl-6-heptenol; 2,6-Dimethyl-7-octen-2-ol; 3,7-Dimethyl-1-octen-7-ol; 2,6-Dimethyl-7-octen-2-ol (INCI)	

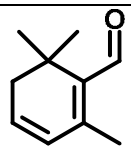
Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (103), listing 2 negative HRIPTs and 1 negative human maximisation test.

It is a "top 100" substance (IFRA, pers. comm.2010).

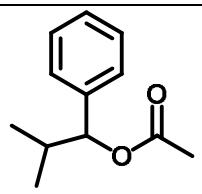
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

Additional information:

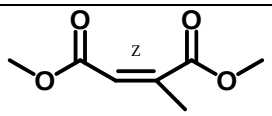
A RIFM review quotes one positive HRIPT (5 of 53) and one negative HRIPT (0 of 54) (93).

DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	
CAS # 151-05-3	
EC # 205-781-3	
2-Methyl-1-phenylpropyl acetate	
Benzeneethanol, α,α-dimethyl-, acetate; Phenethyl alcohol, α,α-dimethyl-, acetate; 1,1-Dimethyl-2-phenylethyl acetate; 2-Methyl-1-phenyl-2-propyl acetate; 2-Methyl-1-phenylpropan-2-yl acetate; Benzyldimethylcarbinol acetate; Benzyldimethylcarbinyl acetate; Dimethylbenzylcarbinol acetate; Dimethylbenzylcarbonyl acetate; NSC 46123; α,α-Dimethylphenethyl acetate	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% positive PT reactions to consecutive patients were noted (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and one to 5% DMBCA in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

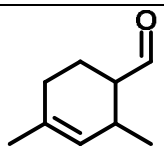
DIMETHYL CITRACONATE	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

Current regulation: Annex II, n° 431

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dimethylcitraconate 12% pet. (24). In this paper, a human maximisation test positive in "4/44" is quoted.

Additional information: …

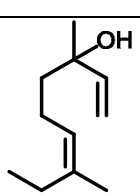
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

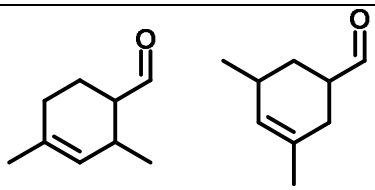
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyllinalool	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (104), citing 1 negative human maximisation test (n=25).

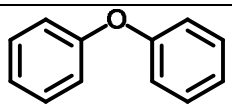
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde **3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde**	2,4- 3,5-
Hivertal; Vertocitral	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 2.3% positive PT reactions were observed with the isomer mixture, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

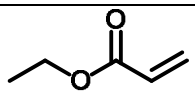
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryl JK-EB; Diphenyl ether; Diphenyl oxide; NSC 19311; Oxybisbenzene; Phenoxybenzene; Phenyl oxide	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ETHYL ACRYLATE	
CAS # 140-88-5	
EC # 205-438-8	
Ethyl 2-propenoate	
Acrylic acid ethyl ester (6CI,8CI); 2-Propenoic acid ethyl ester; Ethyl 2-propenoate; Ethyl acrylate; Ethyl acrylic ester; Ethyl propenoate; NSC 8263	

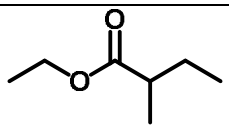
Current regulation: Annex II, n° 435

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In the NACDG 2009 multicentre study, 0.9% of

consecutive patients (n=4428) had a positive PT reaction (21).

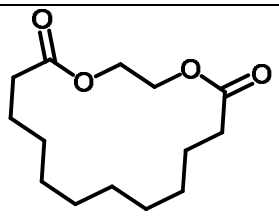
Additional information: /

ETHYL 2-METHYLBUTYRATE	
CAS # 7452-79-1	
EC # 231-225-4	
Ethyl 2-methylbutyrate	
Butyric acid, 2-methyl-, ethyl ester (6CI,7CI,8CI); (±)-Ethyl 2-methylbutanoate; 2-Methylbutanoic acid ethyl ester; 2-Methylbutyric acid ethyl ester; Ethyl 2-methylbutanoate; Ethyl 2-methylbutyrate; Ethyl α-methylbutyrate; NSC 1103	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

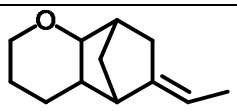
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

Current regulation: /

Clinical data:

In the Larsen 2002 c study on 218 patients with known contact allergy to fragrance ingredients, this compound caused 0.9% positive PT reactions at 5% pet. (1).

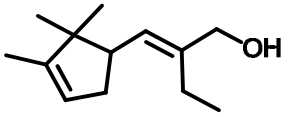
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	
CAS # 93939-86-7	
EC # 300-376-9	
6-Ethylideneoctahydro-5,8-methano-2H-1-benzopyran	

Current regulation: /

Clinical data:
In the Larsen 2001 study, no positive PT reactions were observed with this compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

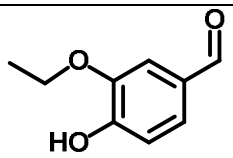
Additional information: /

2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	
CAS # 28219-61-6	
EC # 248-908-8	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol; 2-Ethyl-4-(2',2',3-trimethylcyclopent-3'-enyl)but-2-enol; Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

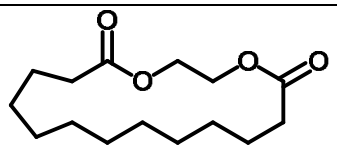
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; 3-Ethoxy-4-hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde ethyl ether; Quantrovanil; Rhodiarome; Vanillal; Vanirom	

Current regulation: /

Clinical data:

The case of a 28-year-old metal grinder with allergic contact dermatitis to a “cutting oil reodorant” has been reported, who tested positively not only to the cutting fluid, the reodorant, but also to several ingredients of the latter product, including “Vanillal S 10026”, 5% pet. (105).

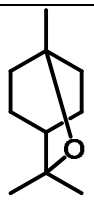
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ETHYLENE BRASSYLATE	
CAS # 105-95-3	
EC # 203-347-8	
1,4-Dioxacycloheptadecane-5,17-dione	
Tridecanedioic acid, cyclic ethylene ester; Ethylene glycol, cyclic tridecanedioate; Astratone; Cyclic ethylene glycol tridecanedioate; Cyclic ethylene tridecanedioate; Emeressence 1150; Ethylene brassylate; Musk T; NSC 46155	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

EUCALYPTOL	
CAS # 470-82-6	
EC # 207-431-5	
1,3,3-Trimethyl-2-Oxabicyclo[2.2.2]octane	
1,8-Epoxy-p-menthane; 1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane; 1,8-Cineol; 1,8-Cineole; 1,8-Epoxy-p-menthane; 2-Oxa-1,3,3-trimethylbicyclo[2.2.2]octane; Cajeputol; Cineol; Cineole; Eucalyptol; Eucalyptole; Eucalytol; Eucapur; Eukalyptol; NSC	

6171; Terpan; p-Cineole	

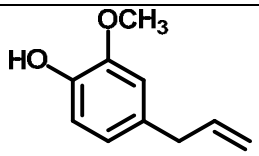
Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010).

See also ***EUCALYPTUS SPP.*** *LEAF OIL;* eucalyptol is the major ingredient there (up to 85%), but found in significant quantities also in a number of other essential oils (see 3.2).

EUGENOL	
CAS # 97-53-0	
EC # 202-589-1	
2-Methoxy-4-(2-propen-1-yl)-phenol	
Other names: 4-Allyl-2-methoxy-phenol; 1-Allyl-4-hydroxy-3-methoxybenzene; 2-Hydroxy-5-allylanisole; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-(2'-propenyl)phenol; 2-Methoxy-4-[2-allyl]phenol; 2-Methoxy-4-allylphenol; 3-(3-Methoxy-4-hydroxyphenyl)propene; 3-(4-Hydroxy-3-methoxyphenyl)-1-propene; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; Allylguaiacol; Bioxeda; Caryophyllic acid; Dentogum; Eugenic acid; Eugenol; NSC 209525; NSC 8895; p-Allylguaiacol; p-Eugenol	

Current regulation: Annex III, part 1, n° 71

Clinical data:

In the “background information” section of the previous opinion (33), eugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.2% in consecutive PT patients and accounting for 4 to 16% of reactions to the FM I. Allergic reactions had been observed in 0.7 – 20% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). F. Giusti et al. examined 1754 consecutive patients tested with eugenol 1% pet. in addition to the baseline series, 09/1998 - 01/2000. 21 patients (1.2%) reacted positively to eugenol (106). In the An 2005 study, 8 of 422 consecutive patients, i.e., 1.9%, had positive reaction (13) (test concentration 2%). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 2.5% positive reactions (22). The IVDK 2010 study, 0.44% (95% CI: 0.04 – 0.84%) of 1214 consecutively tested patients reacted to the compound, while 1.57% (95% CI: 1.19 – 1.95%) of 4801 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to eugenol (48).

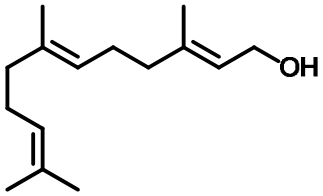
Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material was reported from India (108); however, occasional cases are also reported from Europe (109). Occupational exposure to eugenol / zinc oxide type dental restorative material, which is apparently less frequently used nowadays, may lead to occupational sensitisation to eugenol, as illustrated by a case report (110).

Additional information:

Eugenol is the main component (80-95%) of clove oil, but also found in citronella oil, pimento leaf oil and cinnamon bark oil (see section 3.2).

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

FARNESOL



CAS # 4602-84-0

EC # 225-004-1

3,7,11-Trimethyl-2,6,10-Dodecatrien-1-ol

Farnesol; 3,7,11-Trimethyl-2,6,10-dodecen-1-ol; FCI 119a; Farnesyl alcohol; NSC 60597; Nikkosome

Current regulation: Annex III, part 1, n° 82

Clinical data:

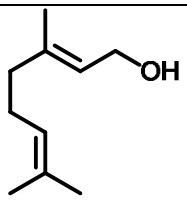
In the "background information" section of the 1999 opinion, farnesol is classified as "less frequently reported allergen"; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive PT reactions to MPR (33).

Since the last SCCNFP-opinion of 1999, farnesol is used not only for its scent, but also for its (slight) antimicrobial activity, useful, for instance, in deodorants. Thus, axillary dermatitis is a relatively typical presentation (111). In a multicentre study based on 1997/98 PT data, 0.5% positive reactions in consecutive patients were noted (Frosch 2002 a (16)). Farnesol is included in the FM II. In the original publication on single constituents of the FM II, 6 of 1701 consecutive patients reacted positively to farnesol 5%, ie., 0.35% (95% CI: 0.13 – 0.77%) (10). In a study on consecutive patients tested in 2003, 38 of 4238 patients had positive reactions to farnesol 5% pet. (0.9%, 95% CI: 0.6 – 1.2%) (4)(IVDK 2007). (A paper on farnesol previously published by the IVDK (112) presents results included in this later analysis.) In a series from Nagoya, Japan, 1.1% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at 5% pet.) (14). In the Groningen 2009 study, 0.9% (95% CI: 0.2 – 2.7%) had positive reactions (6).

Additional information:

"Farnesol is an acyclic primary sesquiterpene alcohol found in essential oils such as lemongrass, citronella, tuberose blossom, sandalwood and orange blossom" (23). A RIFM review is available (113).

GERANIOL



CAS # 106-24-1

EC # 203-377-1

(2E)-3,7-Dimethyl-2,6-octadien-1-ol

(E)-3,7-Dimethyl-2,6-octadien-1-ol; (E)-Geraniol; (E)-Nerol; 3,7-Dimethyl-trans-2,6-octadien-1-ol; Geraniol; Geranyl alcohol; Lemonol; MosquitoSafe; NSC 9279; trans-3,7-Dimethyl-2,6-octadien-1-ol; trans-Geraniol; β-Geraniol

Current regulation: Annex III, part 1, n° 78

Clinical data:

In the "background information" section of the previous opinion (33), geraniol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.4% in consecutive PT patients and accounting for 3 to 7% of reactions to the FM I. Allergic reactions had been observed in 1.2 – 30% of patients with

eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at 2%, i.e. twice the usual concentration (6). In a series from Nagoya, Japan, 0.3% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at the unusually high concentration of 5% pet.) (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=7 (0.9%) positive reactions (22). The IVDK 2010 study, 0.39% (95% CI: 0.10 – 0.69%) of 1214 consecutively tested patients reacted to the compound, while 0.87% (95% CI: 0.63 – 1.10%) of 5695 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=17 reacted positively to geraniol (48).

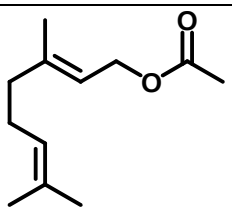
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman with urticaria at the site of application of cosmetics with generalisation (contact urticaria syndrome grade 2), which A. Yamamoto et al. diagnosed as immediate type hypersensitivity to geraniol (without CA) (115).

Additional information:

Geraniol is a component of Palmarosa oil (CYMBOPOGON MARTINI see below), geranium oil (about 40%), citronella oil (30-40%), rose oil, lavender oil, and jasmine oil. It is sensitive to heat which induces autooxidation and isomeric with linalool (53).

Geraniol forms oxidation product with increased sensitizing capacity both via spontaneous autoxidization at air exposure and via metabolic oxidation. Geranial and neral together with hydroperoxide have been identified as oxidation products when geraniol autoxidizes (84). Geranial and neral were also identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

A review is available by Hostynek and Maibach (116) and by RIFM (117). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

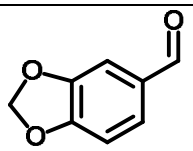
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic acid geraniol ester; Bay pine (oyster) oil; Geranyl acetate; Geranyl ethanoate; NSC 2584; trans-1-Acetoxy-3,7-dimethyl-2,6-octadiene; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-Geranyl acetate; β-Geranyl acetate	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010).

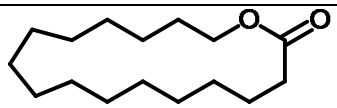
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Geliotropin; Heliotropin; Heliotropine; NSC 26826; Piperonaldehyde; Piperonylaldehyde; Protocatechuic aldehyde methylene ether	

Current regulation: /

Clinical data:

In the Frosch 2002 b study, n=2 (0.2%) positive reactions to “piperonal” (1% pet.) and n=6 (0.4%) to “piperonal” (5% pet.), respectively, in 1606 consecutive were observed (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% heliotropine in pet., tested in 106 consecutive patients in Barcelona, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

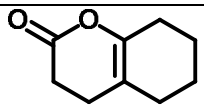
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid;1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; NSC 33546	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with previously diagnosed contact allergy to fragrance ingredients had a positive PT reaction to this compound, tested 5% pet. (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to 5% “hexadecanolide” (13).

Additional information: /

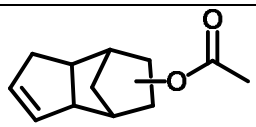
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ-Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ-1,6-2-Oxabicyclo(4.4.0)decen-3-one	

Current regulation: Annex II, n° 1135

Clinical data: /

Additional information:

A RIFM review is available (93), p. S115 ff, citing a number of positive human sensitisation experiments.

3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxydihydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.02,6]dec-3-enyl acetate; Tricyclodecenyl acetate	

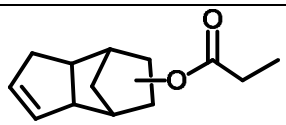
Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 1 to 5% “Cyclacet ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information:

Produced by IFF under the brand name “Cyclacet” (http://www.iff.com/Ingredients.nsf/0/1C9F2CB39EB1EF6480256993002FBC14, last accessed 2010-07-08).

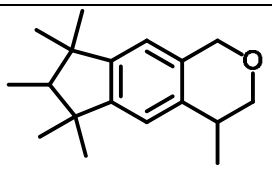
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenol propanoate	

3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.02,6]dec-3-enyl propionate; Verdyl propionate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

HEXAMETHYLINDANOPYRAN	
CAS # 1222-05-5	
EC # 214-946-9	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[γ]-2-benzopyran	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyrane; 1,3,4,6,7,8-Hexahydro-4,6,6,8,8,8-hexamethylcyclopenta-2-benzopyran; Abbalide; Galaxolide; Galaxolide 50; Galaxolide 50BB; Galaxolide 50IPM; Galaxolide White; HHCB; Pearlide	

Current regulation: /

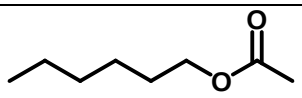
Clinical data:

In the Frosch 2002 a study, n=3 (0.2%) had positive reactions to the compound, tested 10% in isopropyl myristate (with 1 patient reacting positively to the diluent) (16). The Larsen 2001 study, testing with HHCB 7% pet., found 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to “Galaxolide 50”, tested at 5% (13) (test concentration 2% pet.). The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 25% PT preparation of HHCB (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Galaxolide 50 ®” in pet., tested in 100 consecutive patients in Stockholm, were observed (15).

Additional information:

0403/00 - Opinion concerning Hexahydro-hexamethyl-cyclopenta(γ)-2-benzopyran (HHCB)

0610/02 - Opinion on Hexahydro-hexamethyl-Cyclopenta (γ)-2-Benzopyran (HHCB) (no restrictions) It is a “top 100” substance (IFRA, pers. comm.2010).

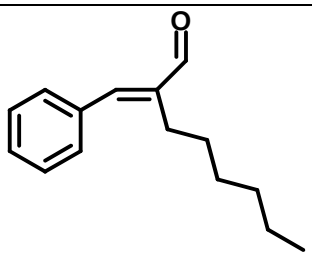
HEXYL ACETATE	
CAS # 142-92-7	
EC # 205-572-7	
Hexyl ethanoate	
Acetic acid, hexyl ester, Hexyl alcohol, acetate; 1-Hexyl acetate; Exceed 600; Hexyl acetate; Hexyl ester acetic	

acid;; NSC 7323; n-Hexyl acetate; n-Hexyl ethanoate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

HEXYL CINNAMAL	
CAS # 101-86-0	
EC # 202-983-3	
α-Hexyl-cinnamaldehyde	
2-(Phenylmethylene)octanal; 2-Hexyl-3-phenyl-2-propenal; 2-Hexylcinnamaldehyde; Hexyl cinnamic aldehyde; NSC 406799; NSC 46150; α-Hexylcinnamaldehyde; α-Hexylcinnamic aldehyde; α-Hexylcinnamyl aldehyde; α-n-Hexyl-β-phenylacrolein; α-n-Hexylcinnamaldehyde	

Current regulation: Annex III, part 1, n° 87

Clinical data:

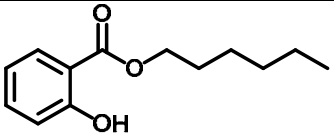
In the “background information” section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as “less frequently reported allergen”; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products were found (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the subsequent EU 2005 study, 2 of 1701 patients had positive reactions to AHCA, and n=16 doubtful or irritant to AHCA at 10% in pet. (10). The IVDK 2007 study yielded n=3, i.e, 0.2% (95% CI: 0.03 – 0.4%) positive reactions in 2019 consecutively PTed patients, using 10% pet. as test concentration (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, using a lower test concentration of 5% pet. (6).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

Hexyl cinnamal is regarded as “a recommended positive control for skin sensitization testing“, e.g., in the context of the LLNA (118).

HEXYL SALICYLATE	
CAS # 6259-76-3	
EC # 228-408-6	
Hexyl-2-hydroxybenzoate	
Salicylic acid, hexyl ester; 1-Hexyl salicylate; Hexyl salicylate; n-Hexyl salicylate	

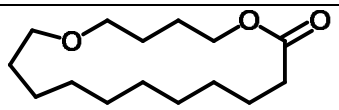
Current regulation: /

Clinical data:

None of the 218 patients with known contact allergy to fragrance ingredients reacted positively to this compound (tested at 5% in pet.) in the Larsen 2002 c study (1).

Additional information:

In a RIFM review, 2 human sensitisation experiments are mentioned which yielded no evidence of sensitising potential (HRIPT, n=103, maximisation test, n=22) (119). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

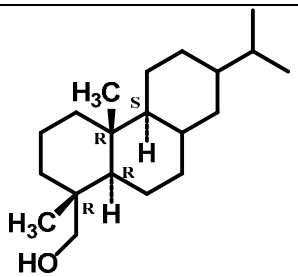
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

Current regulation: /

Clinical data:

None of the 178 patients with known contact allergy to fragrance ingredients reacted positively to "12-oxahexadecanolide" (tested at 5% in pet.) in the Larsen 2001 study (19).

Additional information: /

HYDROABIETYL ALCOHOL, when used as a fragrance ingredient	
CAS # 13393-93-6	
EC # 236-476-3	
(1R,4aR,4βS,10aR)-Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-1-Phenanthrenemethanol	
Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)- 1-henanthrenemethanol; Tetrahydroabietyl alcohol	

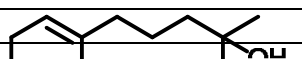
Current regulation: AnnexII, n° 440

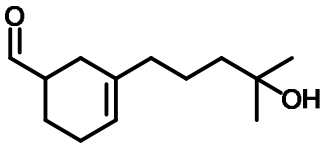
Clinical data:

In the deGroot 2000 study, 17 of 1825 consecutively tested patients had positive reactions to hydroabietyl alcohol (10% pet.) (12).

Additional information:

Commercial hydroabietyl alcohol consists of di- and tetrahydroabitetyl alcohol together with non-modified colophony (120)

HYDROXYISOHEXYL 3-CYCLOHEXENE	

CARBOXALDEHYDE *(HICC) regioisomers*	 51414-25-6
CAS # 31906-04-4 / 51414-25-6	
EC # 250-863-4 / 257-187-9	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (31906-04-4) **3-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde** (51414-25-6)	
31906-04-4: 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde; 4-(4-Methyl-4-hydroxyamyl)cyclohex-3-ene carboxaldehyde; Lyral	

Current regulation: Annex III, part 1, n° 79

Clinical data:

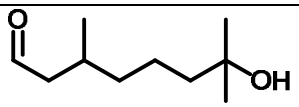
In the “background information” section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 2.7% of the 1855 consecutive patients reacted positively to HICC (5% pet.) (16). In the EU 2005 study, 28 of 1701 patients (1.7%, 95% CI: 1.1 – 2.4%) reacted positively to 5% HICC in pet. (10). In 21325 patients PTed consecutively in the IVDK 2007 study, 2.4% (95% CI: 2.2 – 2.6%) positive reactions were noted to 5% HICC in pet. (4). Similar to other studies, HICC was the most common single fragrance allergen among 320 patients tested in the Groningen 2009 study, with 3.1% (95% CI: 1.5 – 5.7%) positive reactions despite testing with a lower concentration of 2% pet. (6). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded a relatively low prevalence of 0.4% (7 of 1603; exact 95% CI (recalculated): 0.17 – 0.90%) positive reactions with 5% HICC in pet. and even less with lower test concentrations; possible reasons for the much lower prevalence were discussed. The IVDK 2010 study, 2.36% (95% CI: 2.19 - 2.53%) of 37270 consecutively tested patients reacted to HICC (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to HICC (48).

Further clinical data with a focus on quantitative dose-response (see also section 4.3), is discussed in (121).

Among the early case reports, S.A. Hendriks reported the case of a 20 year old patient developing axillary dermatitis after 5 months use of a deodorant containing HICC (122).

Additional information: /

HYDROXYCITRONELLAL	
CAS # 107-75-5	
EC # 203-518-7	
7-Hydroxy-3,7-dimethyl-octanal	
(±)-Hydroxycitronellal; 3,7-Dimethyl-7-hydroxyoctanal; 7-Hydroxy-3,7-dimethyloctanal; 7-Hydroxycitronellal; Citronellal hydrate; Citronellal, hydroxy-; Cyclalia; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguettine principle; NSC	

406740; Phixia	

Current regulation: Annex III, part 1, n° 72

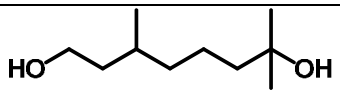
Clinical data:

In the "background information" section of the previous opinion (33), hydroxycitronellal, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.75% in consecutive PT patients and accounting for 6 to 16% of reactions to the FM I. Allergic reactions had been observed in 10 – 45% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.9 – 1.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.2% (95% CI: 0.9 – 4.5%) had positive reactions to this compound, tested at 2% pet., i.e., twice the commonly used concentration (6). The Sugiura 2000 study observed 1% positive PT reactions (test concentration 5% pet.) in 1483 patients tested for suspected cosmetic dermatitis (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 1.17% (95% CI: 0.48 – 1.85%) of 1214 consecutively tested patients reacted to the compound, while 2.95% (95% CI: 2.43 – 3.47%) of 4359 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with hydroxycitronellal, yielding 6 positive reactions (48).

Additional information:

Hydroxycitronellal is a synthetic fragrance, which only recently has been found in a few essential oils, e.g., of a *Narcissus* species and in essential oils of pepper (53)

HYDROXYCITRONELLOL	
CAS # 107-74-4	
EC # 203-517-1	
3,7-Dimethyl-7-octanediol	
2,6-Dimethyl-2,8-octanediol; 3,7-Dimethyl-1,7-octanediol; 3,7-Dimethyloctan-1,7-diol; Citronellol, hydroxy-; Hydroxyciol; Hydroxycitronellol; NSC 406140; NSC 67886	

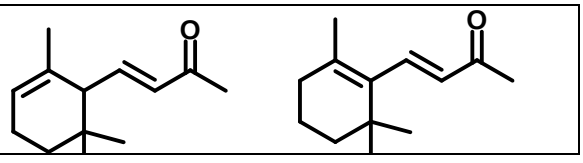
Current regulation: /

Clinical data:

This compound elicited 6.0% positive PT reactions in 218 fragrance sensitive individuals (Larsen 2002 c, (1)).

Additional information:

A RIFM review is available, reporting results of a human induction study (maximisation test) in 25 volunteers, yielding no evidence of sensitisation (123).

IONONE isomeric mixture	
CAS # 8013-90-9	
EC # 232-396-8	

Ionone	
Irisone, mixture of alpha- and beta ionone	

Current regulation: /

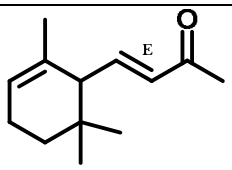
Clinical data: / (see single isomers)

Additional information:

It is a "top 100" substance, further specified with "mixed isomers" (IFRA, pers. comm.2010).

INCI: "MIXED IONONES", with CAS # 14901-07-6 / 6901-97-9 / 8013-90-9 (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=35383, last accessed 2010-07-13).

A RIFM review is available on "ionone" (124), quoting negative human and experimental results.

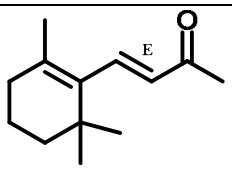
alpha-IONONE	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; 4-(2,6,6-Trimethyl-2-cyclohexenyl)-3-buten-2-one; trans-α-Ionone; α-Cyclocitrylideneacetone; α-Ionone	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% alpha-ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information: A RIFM review is available (125).

beta-IONONE	
CAS # 79-77-6	
EC # 201-224-3	
(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	
(E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; (E)-β-Ionone; Ionone beta; trans-β-Ionone; β-Ionone	

Current regulation: /

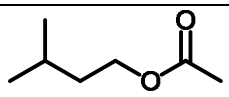
Clinical data:

In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% beta-

ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (126).

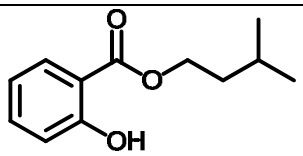
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic acid 3-methylbutyl ester; Acetic acid isopentyl ester; Banana oil; Isoamyl acetate; Isoamyl alcohol acetate; Isoamyl ethanoate; Isopentyl acetate; Isopentyl ethanoate; NSC 9260; Pear oil; i-Amyl acetate; iso-Amyl acetate; iso-Pentyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

In CosIng, it is listed as “solvent” (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=76810, last accessed 2010-07-13)

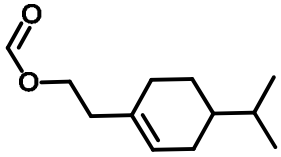
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; NSC 7952	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 50% PT preparation of this compound – this reaction may have been due to an "excited back syndrome" and is thus a limited evidence (25). In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% isoamyl salicylate in pet., tested in 95 consecutive patients, were observed (15).

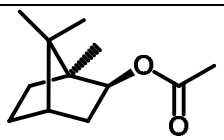
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletly defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

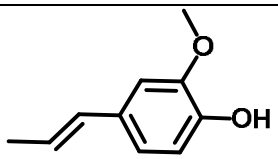
Current regulation: Annex III, part 1, n° 170

Clinical data: /

Additional information: A RIFM review is available (128).

ISOBORNYL ACETATE	
CAS # 125-12-2	
EC # 204-727-6	
(1R,2R,4R)-1,7,7-trimethyl-Bicyclo[2.2.1]hept-2-yl acetate	
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-Trimethyl-, acetate, (1R,2R,4R)-rel- ; Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-; Isoborneol, acetate; (±)-Isobornyl acetate; Isobornyl acetate; NSC 62486; Pichtosin; Pichtosine; exo-Bornyl acetate	

Current regulation: /
Clinical data: In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% isobornyl acetate in pet., tested in 107 consecutive patients in High Wycombe, were observed (15). Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ISOEUGENOL	
CAS # 97-54-1	
EC # 202-590-7	
2-Methoxy-4-(1-propen-1-yl)-phenol	
Phenol, 2-methoxy-4-(1-propenyl)- ; Phenol, 2-methoxy-4-propenyl-; 1-(3-Methoxy-4-hydroxyphenyl)-1-propene; 2-Methoxy-4-(1-propenyl)phenol; 2-Methoxy-4-propenylphenol; 3-Methoxy-4-hydroxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-β-methylstyrene; 4-Propenylguaiacol; Isoeugenol; NSC 6769	

Current regulation: Annex III, part 1, n° 73

Clinical data:

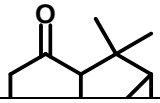
In the “background information” section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been observed in 2 – 25% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.8 – 1.8%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to isoeugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.4% positive reactions (22). At St Johns Institute of Dermatology in London 3636 subjects were patch tested with isoeugenol 2001-2005, 97 of whom were positive. Year-on-year incidence showed an increasing trend, with an overall incidence of 2.67% (129). The IVDK 2010 study, 1.62% (95% CI: 0.87 – 2.38%) of 1214 consecutively tested patients reacted to the compound, while 3.41% (95% CI: 2.90 – 3.92%) of 5747 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=11 reacted positively to isoeugenol (48).

Additional information:

Isoeugenol occurs in a cis- (CAS 5912-86-7) and a trans-isomers (CAS 5932-68-3), the latter dominating in trade products (82-88%) (53).

Isoeugenyl methyl ether caused 7.3% positive reactions in the Larsen 2002 c study (1). A number of derivatives of isoeugenol, such as isoeugenyl acetate, transisoeugenol, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and benzyl isoeugenyl have been examined in 2261 consecutive patients; a varying proportion of positive patch test reactions and a varying proportion of concomitant reactions with isoeugenol have been observed (130). In an earlier study, 5 of 7 patients positive to isoeugenol also displayed positive reactions to isoeugenol acetate (1.2% eth.) (131) (see also section 5 and 6).

ISOLONGIFOLENEKETONE	
CAS # 33407-62-4	

EC # 245-890-3	
1,3,4,6,7,8a-Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	
Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	

Current regulation: /

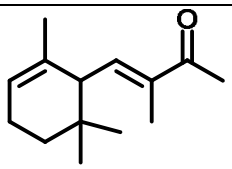
Clinical data:

The Larsen 2001 study idenfied 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

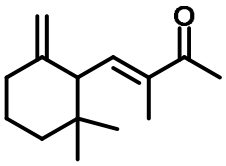
Additional information:

Not listed in CosIng under this CAS #. Other CAS # reported in RIFM review [13]:

- 29461-14-1 CosIng: INCI name "ISOLONGIFOLENE KETONE EXO";
- 23787-90-8 CosIng: INCI name "ISOLONGIFOLANONE";
- 29461-13-0: CosIng: INCI name "HEXAHYDRO-TETRAMETHYLMETHANONAPHTHALEN-8-ONE".

alpha-ISOMETHYL IONONE	
CAS 127-51-5	
EC 204-846-3	
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	
4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; Cetone Alpha; Isomethyl-α-ionone; NSC 66432; α-Cetone	

Current regulation: Annex III, part 1, n° 90

gamma-Methylionone	
CAS 7388-22-9	
EC /	

According to CosIng, "alpha-ISOMETHYL IONONE" (CAS # 127-51-5) and "gamma-Methylionone" (CAS # 7388-22-99) are synonyms, with one CAS number, and one preferred chemical name. The substance(s) are accordingly treated in the 1999 opinion (33) as one. As this treatment is also found in the literature, both substances are reviewed together.

[13] Opdyke, D. L. J.; Letizia, C. **Monographs on fragrance raw materials. Isolongifolanone.** Food and Chemical Toxicology (1983), 21(6), 859

Clinical data:

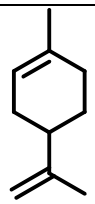
In the “background information” section of the 1999 opinion, “gamma-methylionone” is classified as “less frequently reported allergen”; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

The IVDK 2007 study yielded n=1, i.e, 0.1% (95% CI: 0.00 – 0.2%) positive reactions in 2004 consecutively PTed patients (4). In the subsequent period (2005-2008), n=986 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6).In a Korean study with 422 consecutive patients, 2.1% reacted positively to “alpha isomethyl ionone (gamma-methylionone), CAS # 127-51-5”, tested 5% pet. (13)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010) under the label of “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

A RIFM review is available, listing 4 human sensitisation experiments employing different study protocols – all yielding negative results (132). Another review is available by Hostynek and Maibach (133), both referring to “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

(DL)-LIMONENE	
CAS # 138-86-3	
EC # 231-732-0	
1-Methyl-4-(1-methylethenyl)-cyclohexene	
p-Mentha-1,8-diene; (±)-Dipentene; (±)-Limonene; (±)-α-Limonene; 1,8-p-Menthadiene; 1-Methyl-4-(1-methylethenyl)cyclohexene; 1-Methyl-4-isopropenyl-1-cyclohexene; 1-Methyl-4-isopropenylcyclohexene; 1-Methyl-p-isopropenyl-1-cyclohexene; 4-Isopropenyl-1-methyl-1-cyclohexene; 4-Isopropenyl-1-methylcyclohexene; Cajeputen; Cajeputene; Cinen; Cinene; DL-Limonene; Dipenten; Dipentene; Eulimen; Flavor orange; Goldflush II; Kautschin; Limonen; Limonene; NSC 21446; NSC 844; Nesol; Orange X; Orange flavor; PC 560; Roti-Histol; SF 001; dl-Limonene; α-Limonene	

Current regulation: Annex III, part1, n° 88, 167, 168

Clinical data:

In the “background information” section of the 1999 opinion, d-limonene (CAS 5989-27-5) is classified as “less frequently reported allergen in relation to cosmetic exposure”; with contact allergy to oxidised limonene not infrequently reported in the literature (33).

Since 1999, several studies have been performed using limonene where the oxidation state is not given, but intended to be low. In one study, 0.6% positive reactions to limonene (3% pet.) were observed in 1606 consecutive patients (17). The IVDK 2007 study yielded n=3, i.e. 0.1% (95% CI: 0.03 – 0.4%) positive reactions in 2396 patients consecutively PTed with limonene (2% pet.) (4). The IVDK 2010 study, 0.28% (95% CI: 0 – 0.57%; percentages standardised for age and sex) of 1241 patients PTed with dipentene reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed in 320 patients (6).

Regarding selected case reports, a case of a 40 year old citrus fruit picker with work related hand dermatitis and bronchial asthma has been described, who tested extreme positive to DL-limonene (2% pet.), and, less extremely, to citronellol and to the biocide dichlorophene (134). Moreover, limonene is used as a solvent in technical applications and cleaning and can lead to allergic contact dermatitis (e.g., a histopathology technicians (135, 136) or a painter and decorator (137)). In “water-free” hand cleansers it is reported to be used in concentrations around 10 – 20% (137). Wax polishes may contain dipentene and have caused one reported case of occupational ACD in a car mechanic (138). Another case of occupational ACD from dipentene in honing oil has been reported (139). In a case series from Sweden, 2 of 105 car mechanics patch tested for occupational contact dermatitis had positive reactions to oxidised *d*-limonene (5% pet.) (140).

Additional information:

Limonene is a monocyclic monoterpene existing in two enantiomers: (R)-(+)-limonene (CAS 5989-27-5) and (S)-(–)-limonene (CAS 5989-54-8). Racemic limonene is known as dipentene.

The allergenicity of limonene is closely related to oxidation (71, 72, 141, 142). It has been demonstrated that both enantiomers, R-(+)- and S-(-)-limonene sponateously autoxidize, and that the primary oxidation products formed, the hydroperoxides, are strong and clinically relevant contact allergens. Among 2411 consecutive patients in a multi-centre European study, 63 (2.6 % [95%CI: 2.0-3.3]) reacted to oxidised (R)-(+)-and/or (S)-(–)-limonene (3.0% pet.) (72). In other multi-studies also, a considerable proportion of patients showed

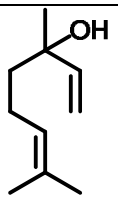
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- between 0.3% and 5.1% of subgroups of 2800 patients in Stockholm and Leuven, depending on test concentration, oxidation state and department(141),
- between 0.3% and 6.5% in 4 different departments in altogether 2273 patients (72, 143).

The primary oxidation products are the major allergens forming specific antigens (Bråred-Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. Contact Dermatitis 2006: 55: 230-237.).

Current IFRA standards emphasise "a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method" (http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56, last accessed 2009-11-11). For a more general discussion see section 5.

There is no scientific rational for the difference in peroxide value allowed for limonene (20 millimoles peroxides per litre) compared to linalool (10 millimoles peroxides per litre). Specific values for hydroperoxides, which are allergens, would be desirable.

LINALOOL	
CAS # 78-70-6 (isomeric mixture)	
EC # 201-134-4; 245-083-6 See: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=27933	
3,7-Dimethyl-1,6-octadien-3-ol	
(±)-Linalool; 2,6-Dimethyl-2,7-octadien-6-ol; 2-Methyl-1-prenyl-3-buten-2-ol; 3,7-Dimethyl-1,6-octadiene-3-ol; 3,7-Dimethyl-3-hydroxy-1,6-octadiene; L 260-2; Linalol; Linalool; Linalyl alcohol; Linanool; NSC 3789; dl-Linalool; β-Linalool	

Current regulation: Annex III, part 1, n° 84

Clinical data:

In the "background information" section of the 1999 opinion, linalool in non-oxidized form is classified as "less frequently reported allergen"; with 4 cases of contact allergy reported in 2 studies on patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, studies have been performed on contact allergy to linalool, oxidation state not given, but intended to be low. In the Larsen 2002 c study, none of the 218 patients with known contact allergy to fragrance ingredients had a positive reaction to linalool 5% pet., as prepared specially for this study (1). The IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2401 patients consecutively tested with stabilised linalool (10% pet.) (4). The IVDK 2010 study, 1 patient had a weak, and another a ++ reaction among the n=985 patients tested with 10% linalool (stabilised) in pet. (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). The deGroot 2000 study with 1825 consecutively tested patients yielded 3 positive reactions to linalool (12). The DeGroot 1985 study found no positive reactions among 179 patients using a 30 % PT preparation of linalool (25).

Additional information:

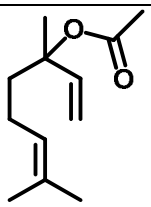
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

2003 in 6 European centres including 1511 consecutive patients, 1.3% showed a positive reaction to oxidized linalool (2.0% pet.) and 1.1% to the hydroperoxide fraction (65). A recent dose-response study in Sweden including 3400 patients in two test centres showed a positive reaction in 5.3% of the 1725 patients tested with oxidized linalool 6% pet. (145).

A review by RIFM is available both regarding linalool (146) and linalool "and related esters" (147). Another review is available by Hostynek and Maibach (148).

It is a "top 100" substance (IFRA, pers. comm.2010).

Additional CAS numbers exist for the single isomers: CAS # 126-90-9 (S-isomer), CAS # 126-91-0 (R-isomer); however, in the studies reviewed the isomeric mixture has been used throughout.

LINALYL ACETATE	
CAS # 115-95-7	
EC # 204-116-4	
3,7-Dimethyl-1,6-octadien-3-yl acetat	
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; Linalool acetate K; (±)-Linaloyl acetate; (±)-Linalyl acetate; 1,5-Dimethyl-1-vinyl-4-hexenyl acetate; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 3-Acetoxy-3,7-dimethyl-1,6-octadiene; Acetic acid linalool ester; Bergamiol; Bergamol; Bergamot mint oil; Linalyl acetate; NSC 2138; dl-Linalool acetate	

Current regulation: /

Clinical data:
In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. (15). In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted (16). Similarly, the RIFM review mentioned quotes a number of studies where no allergic reactions to this compound had been observed, with the exception of one positive reaction in a Dutch study in 1988(149).

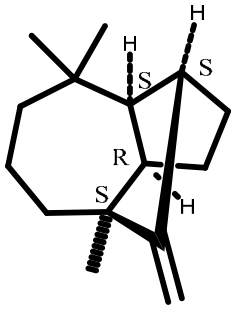
Additional information:

This is the main component of lavender oil (30%), also part of bergamot oil, neroli oil, peppermint oil, lemon oil and jasmine oil (53).

Linalyl acetate autoxidizes spontaneously at air exposure and the major allergens, the hydoperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be be metabolically hydrolysed to the corresponding alcohol cross reactions to allergens from oxidized linalool should be possible. This was indicated in a study of lavender oil and oxidised linalyl acetate which elicited positive PT reactions in some patients with known contact allergy to oxidised linalool (n=3) (151).

A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation (152).

It is a "top 100" substance (IFRA, pers. comm.2010).

Longifolene	
CAS # 475-20-7	
EC # 207-491-2	
(1S,3aR,4S,8aS)-Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-; 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1α,3aβ,4α,8aβ)]-; (+)-Longifolene; Junipen; Junipene; Kuromatsuen; Kuromatsuene; Longifolen; NSC 150808; d-Longifolene; α-Longifolene	

Current regulation: /

Clinical data: /

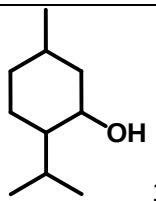
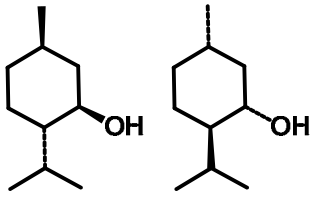
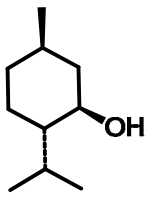
Additional information:

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=77412

This substance is listed in the Register of Flavouring Substances pursuant to Article 3(1) of Regulation EC No. 2232/96 (28 Oct 1996) that lays down a procedure for flavouring substances used or intended for use in or on foodstuffs. Adopted February 23, 1999.

A RIFM review is available citing one negative human maximisation test (n=25) with 10% pet. (153).

MENTHOL	 1490-04-6  89-78-1  2216-51-5
CAS # 1490-04-6 / 89-78-1 / 2216-51-5	
EC # 216-074-4 / 239-388-3 / 218-690-9	
5-Methyl-2-(1-methylethyl)-cyclohexanol (1490-04-6) **(1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol** (89-78-1) **(1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol** (2216-51-5)	
Other names: **1490-04-6**: Menthol; 1-Methyl-4-isopropyl-3-cyclohexanol; 2-Isopropyl-5-methylcyclohexan-1-ol; 2-Isopropyl-5-methylcyclohexanol; 3-Hydroxy-p-menthane; 5-Methyl-2-(1-methylethyl)cyclohexanol; 5-Methyl-2-isopropylcyclohexanol; Menthyl alcohol; p-Menthan-3-ol **89-78-1**: (1α,2β,5α)-5-Methyl-2-(1-methylethyl)-cyclohexanol; cis-1,3,trans-1,4-Menthol; dl-Menthol; (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)cyclohexanol; (±)-Menthol; DL-Menthol; Fisherman's Friend Lozenges; Hexahydrothymol; Menthacamphor; Menthol; Menthomenthol; NSC 2603; Peppermint camphor; Racementhol; Therapeutic Mineral Ice; Thymomenthol; rac-Menthol	

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1α,2β,5α)]-5-Methyl-2-(1-methylethyl)-cyclohexanol; (1R,3R,4S)-(-)-Menthol; (-)-Menthol; (-)-Menthyl alcohol; (-)-trans-p-Methan-cis-3-ol; (1R)-(-)-Menthol; (1R,2S,5R)-(-)-Menthol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexan-1-ol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol; (R)-(-)-Menthol; 1R-Menthol; L-Menthol; L-Mentholum; Levomenthol; NSC 62788; l-(-)-Menthol; l-Menthol	

Current regulation: /

Clinical data:

Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 10 patients had positive (+ to +++) PT reactions to menthol 5% pet. at D4, mostly reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). In 63 patients positive to the FM I, 1 had a positive PT reaction to menthol, 5% pet., in the Santucci 1987 study (28). The IVDK 2010 study, 1 of 1147 patients tested with 1% menthol in pet. had a weak positive reaction to menthol (7).

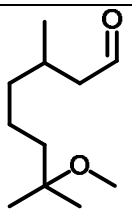
A case of contact allergy to “peppermint and menthol” in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155).Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application of 2% menthol in pet. or 5% peppermint oil in pet., has been reported (156). “A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. During the last two years, the patient had presented dyspnoea, wheezing and nasal symptoms when exposed to mentholated products such as toothpaste and candies. The aetiology was suggested by the history of exposure and diagnosis was established by skin tests and bronchial challenge with menthol. The patient achieved control of symptoms by avoiding menthol and its derivatives.“ (157).

Additional information:

Menthol is an ingredient of several essential oils, like peppermint oil, and has been identified as causative allergen in case reports listed above.

Four stereoisomeric forms are known. Natural menthol occurs as L-form (CAS 2216-51-5), trade products are DL-menthol (CAS 1490-04-6). D-form: CAS 89-78-1, racemic: CAS 15356-70-4**.** Sensitive to light, air and heat (53).

L-menthol and menthol (isomer not specified) are “top 100” substances (IFRA, pers. comm.2010). RIFM reviews are available regarding “menthol” (158), D-menthol (159), L-menthol (160), DL-menthol (161) and menthol, racemic (162). A CIR expert panel review is available (163).

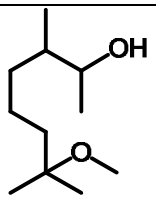
METHOXYCITRONELLAL	
CAS # 3613-30-7	
EC # 222-784-5	
7-Methoxy-3,7-dimethyl-octanal	
7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal	

Current regulation: /

Clinical data:

Nakayama et al. found 1974 (after (29)) 12 “strong positive” and 10 “weak positive” reactions to methoxycitronellal (unknown test concentration), with cross-reactions to hydroxycitronellal (proportion not given), in 183 patients.

Additional information: /

METHOXYTRIMETHYLHEPTANOL	
CAS # 41890-92-0	
EC # 255-574-7	
7-Methoxy-3,7-dimethyl-2-octanol	
3,7-Dimethyl-7-methoxy-2-octanol; Dihydromethoxyelgenol; Elesant; Osyrol	

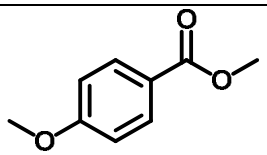
Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.9% of the patients with known contact allergy to fragrance ingredients had a positive PT reaction to this ingredient not reported as allergen previously (1).

Additional information:

A RIFM review is available (128) citing 1 negative maximisation test (n=27).

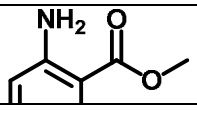
METHYL p-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

Current regulation: /

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to methyl anisate 4% pet. (24).

Additional information: /

METHYL ANTHRANILATE	
CAS # 134-20-3	

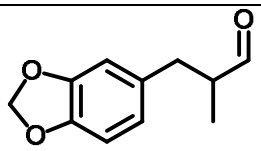
EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; Rejex-iT AP 50; Rejex-iT TP 40; Sunarome UVA; [2-(Methoxycarbonyl)phenyl]amine; o-(Methoxycarbonyl)aniline; o-Aminobenzoic acid methyl ester; o-Carbomethoxyaniline	

Current regulation: /

Clinical data:

In 91 Israeli patients with a positive or doubtful reaction to FM I or MP methyl anthranilate was tested (conc. not given), with a negative result (164).

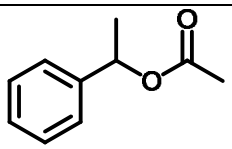
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYLENEDIOXYPHENYL METHYLPROPANAL	
CAS # 1205-17-0	
EC # 214-881-6	
3-(1,3-Benzodioxol-5-yl)-2-methylpropanal	
Hydrocinnamaldehyde, α-methyl-3,4-(methylenedioxy)-; 2-Methyl-3-(3,4-methylenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methylenedioxyphenyl)propionaldehyde; 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal; Heliobouquet; Heliofresh; Heliogan; Helional; Helipropanal; NSC 22282; Tropional; α-Methyl-1,3-benzodioxole-5-propanal; α-Methyl-3,4-(methylenedioxy)hydrocinnamaldehyde	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

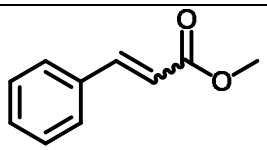
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl phenyl carbinyl acetate; Methylphenylcarbinol acetate; NSC 2397; Styrallyl acetate;	

Styrylallyl acetate; dl-1-Phenylethyl acetate; sec-Phenethyl acetate; sec-Phenylethyl acetate; α-Methylbenzenemethanol acetate; α-Methylbenzyl acetate; α-Methylbenzyl alcohol, acetate; α-Phenethyl acetate; α-Phenylethyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL CINNAMATE	
CAS # 103-26-4	
EC # 203-093-8	
Methyl 3-phenylprop-2-enoate	
3-Phenyl-2-propenoic acid methyl ester; Cinnamic acid, methyl ester; 3-Phenyl-2-propenoic acid methyl ester; 3-Phenylacrylic acid methyl ester; Methyl 3-phenyl-2-propenoate; Methyl 3-phenylacrylate; Methyl 3-phenylpropenoate; Methyl cinnamate; Methyl cinnamylate; NSC 9411; SemaSORB 9815	

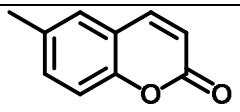
Current regulation: /

Clinical data:

Patch tests with some components of Peru balsam were carried out at 8 worldwide centers in 142 patients who had previously reacted to 25% MP. Reactions to methyl cinnamate (dose and vehicle not reported) were observed in 6 of 142 patients (no further details reported) (165).

Additional information:

A RIFM review is available (166), reviewing, e.g., a number of animal studies with conflicting results. See also under Myroxylon pereirae.

6-METHYL COUMARIN	
CAS # 92-48-8	
EC # 202-158-8	
6-Methylchromen-2-one	
Coumarin, 6-methyl-; 6-MC; 6-Methyl-2H-1-benzopyran-2-one; 6-Methyl-2H-chromen-2-one; 6-Methylbenzopyrone; 6-Methylcoumarin; 6-Methylcoumarinic anhydride; NSC 5870; Toncarine	

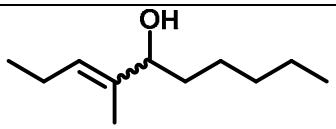
Current regulation: Annex III, part 1, n° 46

Clinical data:

Two of 24 white volunteers developed a photoallergic reaction after single epicutaneous exposure with 5% methyl coumarin in ethanol and UV-A radiation (16 J/cm²). After a photomaximisation test, 6 of 10 subjects developed photocontact allergic reactions

(167). Cardoso et al. report on 2 photoallergic patch test reactions to this substance, which were apparently clinically relevant, in 83 Portugese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

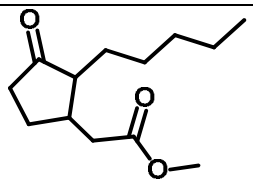
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (170), reporting 1 negative HRIPT (n=50). It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL DIHYDROJASMONATE	
CAS # 24851-98-7	
EC # 246-495-9	
Methyl 2-(3-oxo-2-pentyl cyclopentyl) acetate	
Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester; Kharismal; MDJ; Methyl (3-oxo-2-pentylcyclopentyl)acetate; Methyl 3-oxo-2-pentylcyclopentane ethanoate; Hedione	

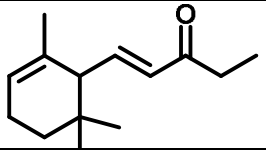
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 3 of 1606 consecutive patients (0.2%) showed positive reactions to hedione (5% pet.) (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% hedione in pet., tested in 100 consecutive patients in Belfast, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). An older RIFM review exists (128) citing 1 negative human maximisation test (n=25).

METHYL IONONE (mixture of isomers)	
CAS # 1335-46-2	
EC # 215-635-0	

1-(2,6,6-Trimethyl-1-cyclohex-2-enyl)pent-1-en-3-one	
6-Methylionone	

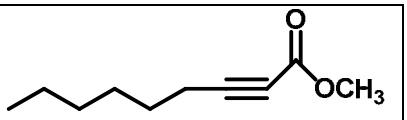
Current regulation: /

Clinical data:

See METHYLIONANTHEME for one clinical case report. Regarding methyl ionone gamma, the Frosch 1995 dose-finding pilot study found no positive reaction to 1% and 5% of this substance in pet., tested in 100 consecutive patients in Belfast (15).

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). A RIFM review is available (171).

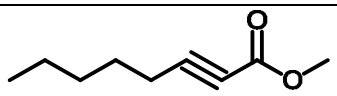
METHYL OCTINE CARBONATE	
CAS # 111-80-8	
EC #	
Methyl 2-octynoate	
Methyl 2-Nonynoate, MOC	

Current regulation: Annex III, part 1, n°173

Clinical data:

English and Rycroft reported a case of a 19-year-old laboratory technician working in the fragrance industry, who developed hand dermatitis after contact with methyl heptine and methyl octane carbonates; patch testing was strongly positive to both compounds at 1% in MEK (172).

Additional information: /

METHYL 2-OCTYNOATE	
CAS # 111-12-6	
EC # 203-836-6	
Methyl oct-2-ynoate	
M2O; Methyl heptin carbonate; Folione; Methyl hept-1-yne-1-carboxylate; Methyl pentylacetylenecarboxylate; NSC 72098; Vert de violette artificial	

Current regulation: Annex III, part 1, n° 89

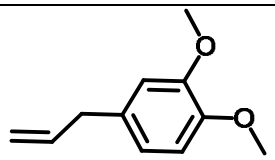
Clinical data:

In the "background information" section of the 1999 opinion, methyl 2-octynoate is classified as "less frequently reported allergen"; with only single cases of reported contact allergy, but the observation of this compound being a strong sensitizer according to IFRA (33), as also reported by Hostynek and Maibach (173)

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.49%) positive reactions in 2401 consecutively PTed patients (1% pet.) (4). The IVDK 2010 study, n=1 weak positive reaction was observed in 988 patients tested with the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%)

had positive reactions to this allergen, tested at only 2% pet. (6). In a previous case report of a fragrance laboratory assistant with work-related ACD both methyl heptin and methyl octin carbonate had been found sensitisers – probably due to their very similar chemical structure (172). In a recent bi-centric study with 350 eczema patients who were consecutively tested with 1% and 2% M2O in pet.; 0.8% positive reactions were observed. However, in 3 additional cases active sensitisation, with first reactions appearing 2 to 4 weeks after the patch test, and prompt reactions in the 2 cases repeat-patch tested, was observed (174).

Additional information: /

METHYL EUGENOL	
CAS # 93-15-2	
EC # 202-223-0	
1,2-Dimethoxy-4-(prop-2-enyl)benzene	
4-Allylveratrole; Eugenyl methyl ether extra; 1,2-Dimethoxy-4-allylbenzene; 1,3,4-Eugenol methyl ether; 1-(3,4-Dimethoxyphenyl)-2-propene; 1-Allyl-3,4-dimethoxybenzene; 3,4-Dimethoxy-1-(2-propenyl)benzene; 3,4-Dimethoxyallylbenzene; 3-(3,4-Dimethoxyphenyl)propene; 4-Allyl-1,2-dimethoxybenzene; Benzene, 4-allyl-1,2-dimethoxy-; Chavibetol methyl ether; Ent 21040; Eugenol methyl ether; Eugenyl methyl ether; Methyl eugenol ether; Methyl eugenyl ether; Methylchavibetol; NSC 209528; NSC 8900; O-Methyleugenol; Veratrole methyl ether; Veratrole, 4-allyl-	

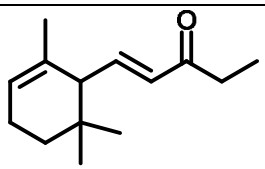
Current regulation: Annex II, 451

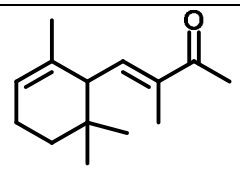
Clinical data:

In a previous study by Larsen et al (2002 c), 1.8% of patients with contact allergy to fragrance ingredients reacted positively to this compound (1).

Additional information:

Quote from the SCCS-opinion 0373/00: “Methyleugenol should not be intentionally added as a cosmetic ingredient. However, when fragrance compounds containing methyleugenol naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01 % in fine fragrance, 0.004 % in eau de toilette, 0.002 % in a fragrance cream, 0.0002 % in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.” (The reason is genotoxicity and carcinogenicity).

METHYLIONANTHEME	
CAS # 55599-63-8	
EC #	
(1E)-2-Methyl-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-penten-3-one mixt. with **(3E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one**	

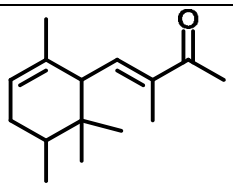
8-Methyl-α-ionone-10-methyl-α-ionone mixt.; Iralia Mixture	

Current regulation: …

Clinical data:
One case of ACD has been reported, caused by an E.d.C. (175).

Additional information:

Patented by GIVAUDAN SA 1933, is composed of isomeric n-methylionones and iso-methylionones. Methylionone has CAS # 1335-94-0 (not in CosIng) and 1335-46-2 (METHYL alpha-IONONE ISOMERS); other names: Methyl-alpha-cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41456, last accessed 2010-07-14).

5-METHYL-alpha-IONONE	
CAS # 79-69-6	
EC # 201-219-6	
4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	
Methyl-α-Ionone; 6-Methyl-α-ionone; α-Irone	

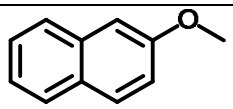
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to alpha-irone (10% pet.) (17).

Additional information:

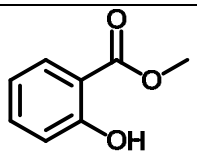
A RIFM review is available (176), citing a (negative) human maximisation test and the study results quoted.

METHYL beta-NAPHTHYL ETHER	
CAS # 93-04-9	
EC # 202-213-6	
2-Methoxynaphthalene	
beta-Naphthyl methyl ether; methyl 2-naphthyl ether; Nerolin (old); NSC 4171; Yara yara; β-Methoxynaphthalene; β-Naphthol methyl ether; β-Naphthyl methyl ether; 2-Methoxynaphthalene; Methyl β-naphthyl ether; 2-Naphthol methyl ether; 2-Naphthyl methyl ether; 6-Methoxy-2-naphthalene	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL SALICYLATE	
CAS # 119-36-8	
EC # 204-317-7	
Methyl 2-hydroxybenzoate	
Other names: Salicylic acid, methyl ester; 2-(Methoxycarbonyl)phenol; 2-Carbomethoxyphenol; 2-Hydroxybenzoic acid methyl ester; Analgit; Anthrapole ND; Ben Gay; Exagien; Flucarmit; Methyl ester of 2-hydroxy benzoic acid; Methyl o-hydroxybenzoate; Methyl salicylate; NSC 8204; Wintergreen oil; o-Hydroxybenzoic acid methyl ester; “Oil of wintergreen”	

Current regulation: /

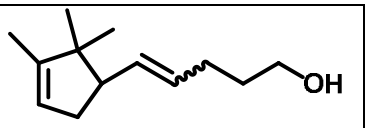
Clinical data:

The deGroot 2000 study yielded 7 positive reactions to methyl salicylate (2% pet.) in 1825 consecutive patients (12).

A case of ACD following the application of a compress bandage containing methyl salicylate has been reported, using 2% “o.o.” as PT concentration; the dose per area of methyl salicylate in the occlusive bandage was not reported (177). A similar case was reported in 1977, positive to 2% methyl salicylate in olive oil, with elicitation of pruritus and erythema after oral ingestion of acetyl salicylic acid (178).

Additional information:

A RIFM review is available (179) providing an overview on 3 human sensitisation experiments (e.g., the HRIPT) which were all negative, and clinical data. In a number of older PT studies, positive test results were seen in 6 of 4600, 3 of 183, 3 of 241, 17 of 585, 1 of 70, all employing a test concentration of 2%, usually in pet., according to above review. Methyl salicylate may occur in topical analgesic (OTC) medications, in Germany, for instance, in ”Camphopin® Salbe“ („Rote Liste 2010“).

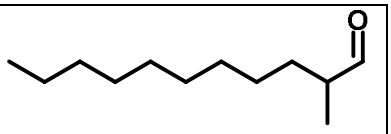
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "MTCP", tested 5% pet. (19). In the An 2005 study, 12 of 422 consecutive patients, i.e., 2.8%, had positive reactions to “ebanol”, tested at 5% (13).

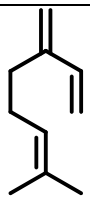
Additional information: /

METHYLUNDECANAL	
CAS # 110-41-8	
EC # 203-765-0	
2-Methylundecanal	
Aldehyde c-12 mna; undecenal, 2-methyl-; 2-Methyl-1-undecanal; Aldehyde M.N.A.; Methyl n-nonyl acetaldehyde; Methylnonylacetaldehyde; NSC 46127	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

MYRCENE	
CAS # 123-35-3	
EC # 204-622-5	
7-Methyl-3-methylideneocta-1,6-diene	
2-Methyl-6-methylene-2,7-octadiene; 7-Methyl-3-methylene-1,6-octadiene; NSC 406264; β-Geraniolene; β-Myrcene	

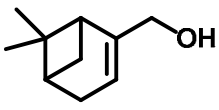
Current regulation: /

Clinical data:

In a clinical study in 6 European centres, including 1511 consecutive patients, 1 patient had a positive reaction to oxidized myrcene (65).

Additional information:

Myrcene autoxidizes spontaneously and rapidly at air exposure. In experimental studies on beta-myrcene an EC3 value of 4.3% was seen for a sample air-exposed 10 weeks (Sköld M. Contact allergy to autoxidized fragrance terpenes (180).

MYRTENOL	
CAS # 515-00-4	
EC # 208-193-5	

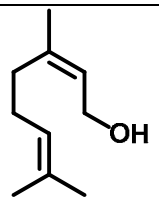
(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol

Current regulation: /

Clinical data: /

Additional information:

A RIFM review exists (181), citing 2 of 3 HRIPT studies with 1 case of sensitisation to myrtenol each.

NEROL	
CAS # 106-25-2	
EC # 203-378-7	
(2Z)-3,7-Dimethylocta-2,6-dien-1-ol	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; (Z)-3,7-Dimethyl-2,6-octadien-1-ol; (Z)-Geraniol; (Z)-Nerol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 3,7-Dimethyl-cis-2,6-octadien-1-ol; Nerol 900; Neryl alcohol; cis-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; β-Nerol; cis-geraniol – i.e., isomeric to geraniol	

Current regulation: /

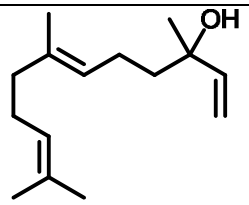
Clinical data:

In the Larsen 2002 c study, 6.0% of the fragrance sensitive patients reacted positively to 5% in pet. (1).

Additional information:

A RIFM review is available (182) citing (negative) human sensitisation experiments, an older study from Japan and the Larsen 2002 c study (see above).

Regarding autoxidation studies – see geraniol.

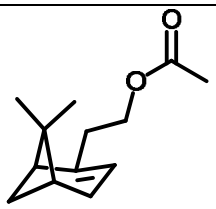
Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	

Current regulation: /

Clinical data: /

Additional information:

RIFM review is available (183) citing the occurrence of "3 positive reactions in 2273 patients". Another RIFM review is available on cis-nerolidol (184), mentioning that no data on this compound are available.

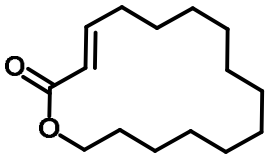
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol acetate; Citroviol; NSC 1286; NSC 404963; Nopol acetate; Nopyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 25% PT preparation of this compound – reactions may have at least partly been due to an "excited back syndrome" and thus a limited evidence (25).

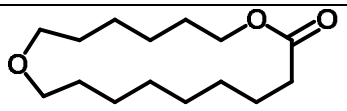
Additional information: /

OXACYCLOHEXADECENONE	
CAS # 34902-57-3	
EC # 609-040-9	
(3E)-Oxacyclohexadec-3-en-2-one	
Globalide; Oxacyclohexadecen-2-one	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

OXALIDE	
CAS # 1725-01-5	
EC # 217-033-3	
1,8-Dioxacycloheptadecan-9-one	
Nonanoic acid, 9-[(6-hydroxyhexyl)oxy]-, o-lactone; 10-Oxa-16-hexadecanolide; Oxalide; Oxalide T	

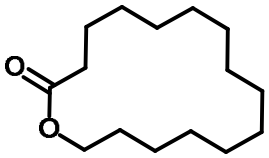
Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "10-oxahexadecanolide", tested 5% pet. (19).

Additional information:

A RIFM review is available (128), citing a negative maximisation test (n=29).

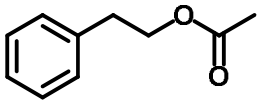
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ-lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; Muskalactone; NSC 36763; Pentadecalactone; Pentadecanolactone; Pentadecanolide; Pentalide; Thibetolide; cpd Supra; ω-Pentadecalactone; angelica lactone; hexaltolide	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). The substance has been used for clinical olfactory testing in the 60ies under the name of exaltolide.

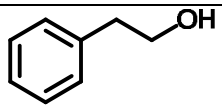
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbinyl acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl acetate; β-Phenylethanol acetate; β-Phenylethyl acetate	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). Exposure via plants (*Tanacetum parthenium*) is possible (185).

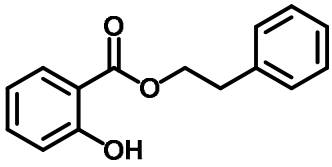
PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol; β-(Hydroxyethyl)benzene; β-PEA; β-Phenethanol; β-Phenethyl alcohol; β-Phenethylol; β-Phenylethanol; β-Phenylethyl alcohol	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 25% PT preparation of phenylethyl alcohol (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to this compound, tested 1% pet. in 100 consecutive patients in Odense, DK, was observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

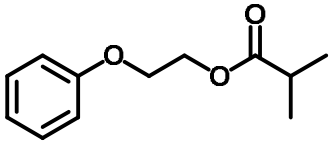
PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201-732-5	
2-Phenylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review exists (186), quoting a negative human maximisation test and a number of animal experiments, including cross-sensitisation experiments with benzyl salicylate. One LLNA study is reported yielding an EC3 value of 2.1%.

PHENOXYETHYL ISOBUTYRATE	
CAS # 103-60-6	
EC # 203-127-1	
2-Phenoxyethyl 2-methylpropanoate	
Isobutyric acid, 2-phenoxyethyl ester; Ethanol, 2-phenoxy-, isobutyrate; 2-Phenoxyethyl isobutyrate; NSC 227210; NSC	

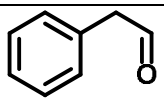
406209; Phenoxyethyl isobutyrate; β-Phenoxyethyl isobutyrate	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010).

PHENYLACETALDEHYDE	
CAS # 122-78-1	
EC # 204-574-5	
2-Phenylacetaldehyde	
Benzylcarboxaldehyde; Hyacinthin; NSC 406309; Phenacetaldehyde; Phenylacetaldehyde; Phenylacetic aldehyde; Phenylethanal; α-Phenylacetaldehyde; α-Tolualdehyde; α-Toluic aldehyde	

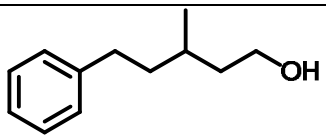
Current regulation: /

Clinical data:

In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to phenylacetaldehyde 2% pet. (24). In a case report, Sanchez-Politta et al. describe a 26-year-old worker in a perfume factory, who suffered from a spill of pure phenylacetaldehyde and became sensitised, as proven by positive patch tests with 0.5%, 1% and 2% (10 healthy controls negative) (187).

Additional information:

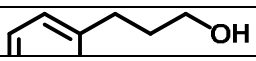
SCCS opinion: 1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assesment " (QRA: Citral, farnesol and phenylacetaldehyde)

PHENYLISOHEXANOL	
CAS # 55066-48-3	
EC # 259-461-3	
3-Methyl-5-phenylpentan-1-ol	
3-Methyl-5-phenyl-1-pentanol; 3-Methyl-5-phenylpentanol; 5-Phenyl-3-methylpentanol; Mefrosol; Phenoxanol	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

PHENYLPROPANOL	

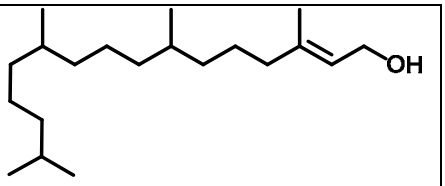
CAS # 122-97-4	
EC # 204-587-6	
3-Phenylpropan-1-ol	
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; NSC 16942; γ-Phenylpropanol; γ-Phenylpropyl alcohol; Phenethyl Carbinol	

Current regulation: /

Clinical data:

The Larsen 2002 c study yielded 0.9% positive reactions in 218 patients with contact allergy to fragrance ingredients (1).

Additional information: …

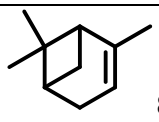
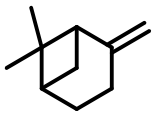
PHYTOL	
CAS # 150-86-7	
EC # 205-776-6	
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
Phytol; (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol	

Current regulation: /

Clinical data:
/

Additional information:

Phytol is a main constituent of Jasmin abs. with 7.4% reported content (17). In a human maximization study involving 25 subjects, there was one case of contact sensitization to 10% phytol (6900 µg/cm²), applied in petrolatum, as reported in a RIFM review (188).

alpha-PINENE and beta-PINENE	 80-56-8  127-91-3
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	
EC # 201-291-9 (alpha-Pinene; according to CAS service: 219-445-9); EC # 204-872-5 (beta-Pinene; according to CAS service: 245-424-9)	
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (80-56-8) **6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane** (127-91-3)	

80-56-8: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene **127-91-3**: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190; Nopinen; Nopinene; PC 600; PC 600 (pesticide); Pseudopinen; Pseudopinene; Terebenthene; β-Pinene	

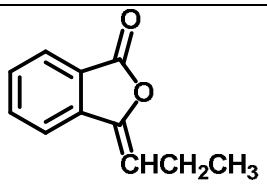
Current regulation: Annex III, part 1, n° 130 (Peroxide value less than 10 mmoles/L in substance)

Clinical data:

In 63 patients positive to the FM I, 2 had a positive PT reaction to beta-pinene (and none to alpha-pinene 5% pet.), 1% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 17 of 30 patients reacted positively to alpha-pinene, and 2 to beta-pinene (189). In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190).

A case report from Zacher and Ippen on 2 patients with allergic contact dermatitis due to bergamot oil (191) describes positive patch test reactions to alpha-pinene and beta-pinene in one, a worker in a perfume factory.

Additional information: /

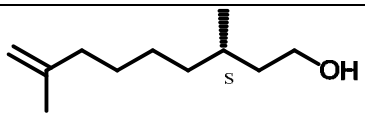
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; 3-Propylidenephthalide; Celeriax; Propylidenephthalide	

Current regulation: Annex III, part 1, n° 175

Clinical data:

In the Malten 1984 study, 2.6% of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In this paper, "3/25" positive results in human maximisation tests are listed.

Additional information: /

RHODINOL	
CAS # 6812-78-8	
EC # 229-887-4	
(3S)-3,7-Dimethyloct-7-en-1-ol	
Rhodinol; (-)-Rhodinol; α-citronellol; (-)-α-Citronellol; (S)-	

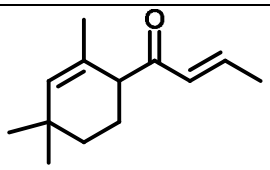
α-Citronellol	

Current regulation: /

Clinical data: / (see below)

Additional information:

A RIFM review exists citing a positive HRIPT with several cases of sensitisation, 5 of these proven upon re-challenge, and a negative human maximisation test (192).In a previous RIFM review (128), a Japanese clinical study (source not accessible) is cited: "In patch tests using cosmetics ingredients and fragrance materials on patients with eczema and dermatitis, 5% rhodinol (vehicle not specified) produced one sensitization reaction in 202 patients (Itoh et al., 1988[14])"

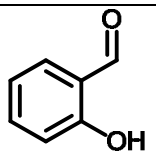
trans-ROSE KETONE-5	
CAS # 39872-57-6	
EC # 254-663-8	
(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one	
alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	

Current regulation: Annex III, part 1, n° 159 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (193) quoting 2 HRIPT studies: one with 0.2% concentration in DEP in 103 volunteers, and negative result, one with 2% concentration, sensitising 2 of 22 volunteers.

SALICYLALDEHYDE	
CAS # 90-02-8	
EC # 201-961-0	
2-Hydroxybenzaldehyde	
Salicylaldehyde; 2-Formylphenol; NSC 112278; NSC 49178; NSC 83559; NSC 83560; NSC 83561; NSC 83562; NSC 97202; Salicylal; Salicylic aldehyde; o-Formylphenol; o-Hydroxybenzaldehyde	

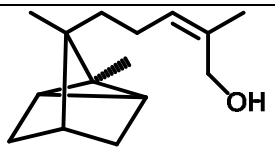
Current regulation: /

Clinical data:

[14] Itoh M., Hosono K., Kantoh H., Kinoshita M., Yamada K., Kurosaka R. and Nishimura M. (1988) Patch test results with cosmetic ingredients conducted between 1978-1986. *Nippon Koshohen Kagakkaishi* 12 (1), 27-41.

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to salicylaldehyde was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to salicylaldehyde 2% pet. (22). The IVDK 2010 study, 0.48% (95% CI: 0.18 – 0.79%; percentages standardised for age and sex) of 2729 patients PTed reacted to the compound (7). An earlier study by Bruze and Zimerson points to possible cross-reactivity between salicylaldehyde and "simple methylol phenols" occurring in synthetic resins based on phenol and formaldehyde (194). Among 24 patients sensitised to resorcinol by application of a wart remover, 2 positive reactions to salicylaldehyde were observed (195).

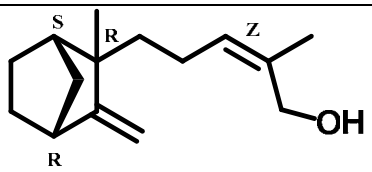
Additional information: Along with other derivates of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

alpha-SANTALOL	
CAS # 115-71-9	
EC # 204-102-8	
(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methylPent-2-en-1-ol	
2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.02,6]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.02,6]heptane, 2-penten-1-ol deriv.; (+)-(Z)-α-Santalol; (+)-α-Santalol; (Z)-α-Santalol; Sandal; Santalol a; cis-α-Santalol; d-α-Santalol	

Current regulation: /

Clinical data: / (see beta-santalol)

Additional information: Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans.

beta-SANTALOL	
CAS # 77-42-9	
EC # 201-027-2	
(2Z)-2-Methyl-5-[(1S,2R,4R)-2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl]pent-2-en-1-ol	
2-Methyl-5-(2-methyl-3-methylene-2-norbornyl)-2-penten-1-ol; [1S-[1α,2α(Z),4α]]-2-Methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)-2-penten-1-ol; β-Santalol; (-)-(Z)-β-Santalol; (-)-β-Santalol; Santalol b; cis-β-Santalol	

Current regulation: /

Clinical data:

A RIFM review is available for alpha-santalol (197) and on "santalol" (CAS # 11031-45-1 (198). The former review cites a Japanese study: "Between April 1979 and August 1990, a total of 3123 male and female patients were patch tested to 2% santalol (.alpha. or .beta. not specified) in petrolatum. Reactions were observed in 47/3123 (1.5%) of the patients. The incidence of positive reactions from 1979 to 1990 was 1.5%. The rate of reactions observed was higher during the earlier period of the patch testing than the later stage (Utsumi et al., 1992)[15]." In another Japanese study cited by the RIFM review "... patch tests were conducted with 0.05–0.5% santalol (specified as santalol 1) in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm2 lint with a 2 cm2 cellophane disc placed on the lint and then covered with a 4 cm2 plaster. Patches were applied to the back, the forearm, and the inside of the upper arm for 24–48 h. Reactions were observed in 15 patients and questionable reactions were observed in 10 patients out of the total 427 participating. A second sample of santalol (specified as santalol 2) was tested on 214 patients. Reactions were observed in three patients and questionable reactions were observed in six patients (Takenaka et al., 1986)[16]." Moreover, "The Mid-Japan Contact Dermatitis Research group (MJDCRG) conducted a 6-year (1976–1981) patch test study on facial dermatoses patients with various fragrance materials. During the year 1979, a total of 327 patients were tested with a mixture of .alpha. and .beta. santalol at concentrations of 10%, 2%, and 1% in white petrolatum. Reactions were observed in 1.5%, 0.6% and 0.6% of the 327 patients tested at concentrations 10%, 2%, and 1%, respectively (MJCDRG, 1984)[17]."

The Goossens 1997 study found 5 of 111 patients positive to "santalol 10% pet." (isoform not specified) – all sensitised to other fragrance allergens as well (23). In the Larsen 2001 study, patch testing with "2-methyl-5-(2,3-dimethyl tricyclo[2.2.1.0(2,6)]hept-3-yl-2 pentenol(.alpha.-form) and 2-methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-3-yl-2-penten-1-ol(beta-form) 5% pet." (no CAS numbers given) yielded a total of 2 positive reactions among the 178 patients with known contact allergy to fragrance ingredients (19).

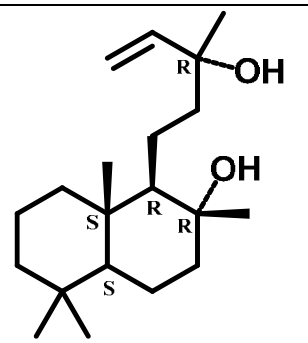
Additional information: "There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)" (A.M. Api, pers. comm., 2010).

Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans

[15] Utsumi, M., Sugai, T., Shoji, A., Watanabe, K., Asoh, S., Hashimoto, Y., 1992. Incidence of positive reactions to sandalwood oil and its related fragrance materials in patch tests and a case of contact allergy to natural and synthetic sandalwood oil in a museum worker. Skin Research 34, 209–213

[16] Takenaka, T., Hasegawa, E., Takenaka, U., Saito, F., Odaka, T., 1986. Fundamental studies of safe compound perfumes for cosmetics Part 1. The primary irritation of compound materials to the skin. Unknown Source, 313–329.

[17] Mid-Japan Contact Dermatitis Research Group, 1984. Determination of suitable concentrations for patch testing of various fragrance materials. A summary of group study conducted over a 6-year period. Journal of Dermatology, 11(1), 31–35.

SCLAREOL	
CAS # 515-03-7	
EC # 208-194-0	
(1R,2R,8aS)-1-[(3R)-3-Hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-2-ol	
(αR,1R,2R,4aS,8aS)-α-Ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1-naphthalenepropanol; [1R-[1α(R*),2β,4aβ,8aα]] - α-ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1 Naphthalenepropanol; (13R)-Labd-14-ene-8,13-diol; Sclareol; (-)-Sclareol; [1R-[1.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-.alpha.-vinyldecahydronaphthalene-1-propan-1-ol	

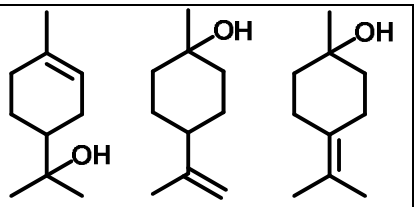
Current regulation: /

Clinical data: /

Additional information:

An older RIFM review exists (128), reporting several human maximisation tests with different samples of sclareol, yielding partly positive, partly negative results. A more recent RIFM review is available (199), citing no clincial data, but several maximisation studies, one of which was positive in a few volunteers, which was apparently due to an impurity.

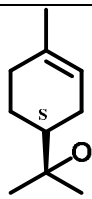
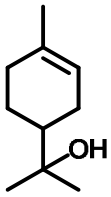
0986/06 - Opinion on Sclareol (sensitisation only) (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

TERPINEOL	
CAS # 8000-41-7	
EC # 232-268-1	
Mixtures of isomers	
Terpineol 318, mixture of terpineol isomers alfa, beta, gamma	alfa beta gamma

Current regulation: /

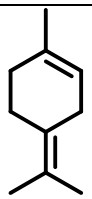
Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study “Takenaka 1986”, finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpineol in pet., tested in 100 consecutive patients in Belfast, were observed (15).

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010).

alpha-TERPINEOL	 10482-56-1  98-55-5
CAS # 10482-56-1 / 98-55-5	
EC # 233-986-8 / 202-680-6	
2-[(1S)-4-Methyl-1-cyclohex-3-enyl]propan-2-ol (10482-56-1) **2-(4-Methyl- 1-cyclohex-3-enyl)propan-2-ol** (98-55-5)	
10482-56-1: (S)-(-)-p-Menth-1-en-8-ol;(-)-α-Terpineol; (S)-(-)-Terpineol; (S)-(-)-α-Terpineol; (S)-α-Terpineol; l-α-Terpineol **98-55-5**: p-Menth-1-en-8-ol; (±)-α-Terpineol; 1,1-Dimethyl-1-(4-methylcyclohex-3-enyl)methanol; 1-p-Menthen-8-ol; 2-(4-Methyl-3-cyclohexenyl)-2-propanol; 4-(2-Hydroxy-2-propyl)-1-methylcyclohexene; 8-Hydroxy-p-menth-1-ene; NSC 21449; NSC 403665; PC 593; Pine Oil 593; Terpineol 350; dl-α-Terpineol; α,α,4-Trimethyl-3-cyclohexene-1-methanol; α-Terpineol	
Current regulation: /	

Clinical data:
A RIFM review is available (201) specifically on (-)-alpha-terpineol stating that “no data is available” regarding skin sensitisation. Another RIFM review is available on alpha-terpineol (202). In the Frosch 2002 b study, 1 of 1606 consecutive patients showed a positive reaction, but 11 patients doubtful reactions to alpha-terpineol (5% pet.) (17). The DeGroot 1985 study identified no positive reactions among 179 patients using a 15% PT preparation of terpineol (mixed isomers) (25). In 63 patients positive to the FM I, 2 had a positive PT reaction to alpha terpineol, 5% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 3 of 30 patients reacted positively to alpha-terpineol (189)

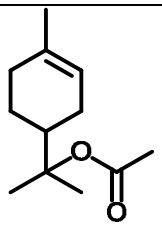
Additional information: see also terpineol (mixture of isomers). Comments on turpentine under pinene.

Terpinolene	
CAS # 586-62-9	
EC # 209-578-0	
1-Methyl-4-propan-2-ylidenecyclohexene	
p-Mentha-1,4(8)-diene; 1-Methyl-4-(1-methylethylidene)-cyclohexene; 4-Isopropylidene-1-methylcyclohexene; Isoterpinene; Nofmer TP; Terpinolen; Terpinolene; α-Terpinolene; δ-Terpinene	
Current regulation: Annex III, part 1, n° 133 (Peroxide value less than 10 mmoles/L in substance)	

Clinical data:
A 49-year-old machine cleaner developed occupational contact dermatitis due to the cleaner, which gave a positive patch test result at 1:10 000 in water. Of the ingredients identified by chromatography, only .delta.-3-carene and terpinolene, tested 5% pet.,

gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).

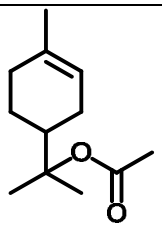
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

TERPINEOL ACETATE (Isomer mixture)	
CAS # 8007-35-0	
EC # 232-357-5	
4-Methyl-1-propan-2-yl-1-cyclohex-2-enyl acetate	
Terpinyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpinyl acetate in pet., tested in 106 consecutive patients in Barcelona, were observed (15)

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

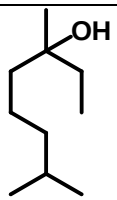
alpha-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate; α-Terpineol acetate; p-Menth-1-en-8-yl acetate; 1-Methyl-1-(4-methylcyclohex-3-enyl)ethyl ethanoate; (±)-.alpha.,.alpha.,4-trimethylcyclohex-3-ene-1-methyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified no positive reactions among 179 patients using a 10% PT preparation of “terpinyl acetate” (25).

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

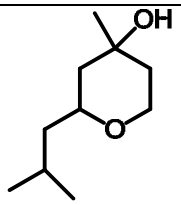
Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	

Current regulation: /

Clinical data:
/

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM

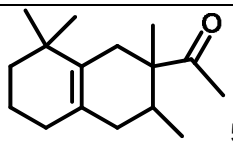
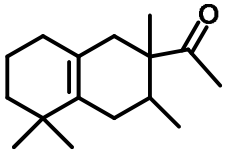
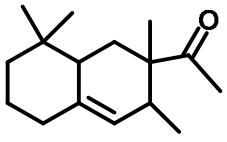
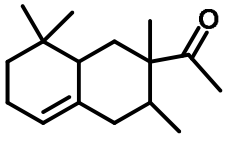
review is available (205) quoting 1 negative human maximisation test.

TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	
CAS # 63500-71-0	
EC # 405-040-6	
4- Methyl-2-(2-methylpropyl)tetrahydro-2H-4-pyranol	
2-(2-Methylpropyl)-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-methyltetrahydropyran-4-ol; 4-Hydroxy-4-methyl-2-(2-methylpropyl)tetrahydropyran; Florosa; Rozanol	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

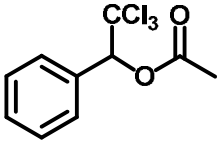
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	 54464-57-2
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9	
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2) **1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone** (54464-59-4) **1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone** (68155-66-8) **1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone** (68155-67-9)	 54464-59-4  68155-66-8  68155-67-9
54464-57-2: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone; 1',2',3',4',5',6',7',8'-Octahydro-2',3',8',8'-tetramethyl-2'-acetonaphthone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemone E; OTNE; Orbitone	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% of 1855 consecutive patients reacted to the compound (brand name mentioned: „Iso E. Super“, 5% pet.) (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction both to 1% and 5% “Iso E Super ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15). The Larsen 2001 study yielded 1.7% positive reactions (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: According to CosIng: "Mixture of isomers: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-67-9); 1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-66-8) " (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40504, last accessed 2009-11-11).

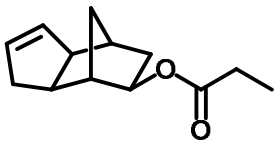
It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICHLOROMETHYL PHENYL CARBINYL ACETATE	
CAS # 90-17-5	
EC # 201-972-0	
2,2,2-Trichloro-1-phenylethyl acetate	
Benzenemethanol, α-(trichloromethyl)-, acetate; Benzyl alcohol, α-(trichloromethyl)-, acetate (Trichloromethyl)phenylcarbinyl acetate; (±)-α-(Trichloromethyl)benzyl acetate; 2-Acetoxy-1,1,1-trichloro-2-phenylethane; Crystal rose; NSC 165582; Rosacetol; Rosephenone; Rosetone; Rosone; α-(Trichloromethyl)benzyl acetate	

Current regulation: /

Clinical data: /

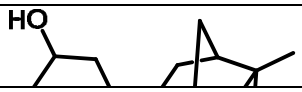
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICYCLODECENYL PROPIONATE	
CAS # 17511-60-3	
EC # 241-514-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-6-yl propionate	
4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-Hexahydro-, propionate; Cyclaprop; Florocyclene; Greenyl propionate; Tricyclo(5.2.1.02,6)dec-3-en-8-yl propionate.	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

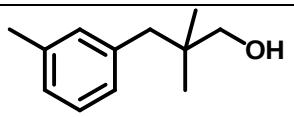
3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL	

CAS # 3407-42-9	
EC # 222-294-1	
3-(5,5,6-Trimethyl-6-bicyclo[2.2.1]heptanyl)cyclohexan-1-ol	
3-(5,5,6-Trimethyl-2-norbornyl)-cyclohexanol; 3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexan-1-ol; 3-Hydroxy-1-(5-isocamphyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of “synthetic sandalwood oil”.

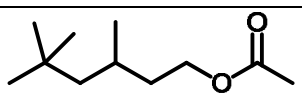
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, majantol (conc. not given, elsewhere reported as 5% pet.) caused positive PT reactions in 3.2% of patients with known contact allergy to fragrance ingredients. In a later study by the IVDK, 0.5% (95% CI: 0.3 – 0.7%) consecutive patients displayed a positive reaction to majantol 5% pet. (206). In the IVDK 2010 study, majantol was tested both in n=2189 consecutive patients, yielding 0.36 % (95% CI: 0.12—0.60%) positive reactions, and in the context in a special series, applied in an aimed fashion to n=4972 patients, yielding 0.76% (95% CI: 0.49—1.03%) (standardised) positive reactions (7). In a recent study from Copenhagen, DK, 6 of 722 patients tested with this compound were found positive, 2 of these to material used earlier provided by Symrise, 4 to material by Allmiral/Hermal/Trolab used later instead. There was no significant difference between these proportions obtained with batches of majantol from different production processes (207).

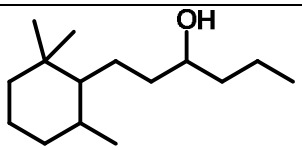
Additional information: /

TRIMETHYLHEXYL ACETATE	
CAS # 58430-94-7	
EC # 261-245-9	
3,5,5-Trimethylhexyl acetate	
1-Hexanol, 3,5,5-trimethyl-, acetate; Vanoris; neononyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

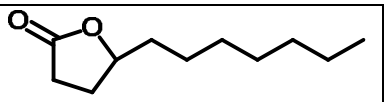
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethyl-cyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; Finotimber; Timberol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

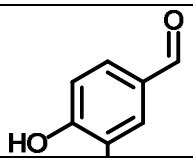
gamma-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ-lactone; (RS)-γ-Undecalactone; (±)-γ-Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 350120-1; Peach lactone; Peche Pure; Persicol; γ-(n-Heptyl)-γ-butyrolactone; γ-Heptyl-γ-butyrolactone; γ-Heptylbutyrolactone; γ-Undecalactone; γ-Undecanolactone; γ-Undecanolide; γ-n-Heptylbutyrolactone	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm. 2010)

VANILLIN	
CAS # 121-33-5	
EC # 204-465-2	

4-Hydroxy-3-methoxybenzaldehyde	
2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; 4-Formyl-2-methoxyphenol; 4-Hydroxy-5-methoxybenzaldehyde; 4-Hydroxy-m-anisaldehyde; H 0264; Lioxin; NSC 15351; NSC 403658; NSC 48383; Rhovanil; Vanillaldehyde; Vanillic aldehyde; Vanillum; m-Methoxy-p-hydroxybenzaldehyde; p-Hydroxy-m-methoxybenzaldehyde; p-Vanillin	

Current regulation: /

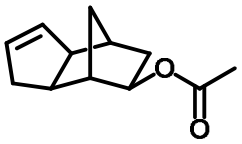
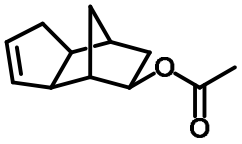
Clinical data:

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to vanillin was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to vanillin 10 % pet. (22). The IVDK 2010 study, n=10, i.e., 0.19% (95% CI: 0.07 – 0.32%; percentages standardised for age and sex) of 4377 patients PTed reacted to the compound, tested 10% pet. (7). In n=102 patients with a positive reaction to MPR, 19 compounds of this natural mixture were tested, among these, vanillin, to which none reacted positively (208). In 21 patients with contact allergy to propolis, 2 also reacted to vanillin (10% pet.) (209).

A 13-year-old girl with recurrent (peri-)cheilitis after application of a vanilla lip salve tested strongly positive to this salve (as is), “Vanilla 10% pet.” (unclear, whether natural extract or vanillin) and MPR (210). Trattner/David identified 1 / 641 consecutive patients with positive reaction to vanillin (31).

Additional information:

Naturally occurring in the fruit of *Vanilla planifolia* after a fermentation process, in styrax, clove oil, potatoes, wood, including Myroxylon pereirae resin, and other material (53). Nowadays, vanillin is synthesised from eugenol, guajakol and lignin residues from paper production, however, not fully achieving the subtle scent and taste of the natural material (53). It is a “top 100” substance and classified as R43 (IFRA, pers. comm. 2010).

VERDYL ACETATE	
CAS # 2500-83-6/ 5413-60-5	
EC # 219-700-4 / 226-501-6	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6)	2500-83-6
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat (5413-60-5)	
2500-83-6: 4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; NSC 142428; NSC 94573	5413-60-5
5413-60-5: 4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 8-Acetoxytricyclo[5.2.1.02,6]dec-3-ene; Greenyl acetate;	

Herbaflorat; Jasmacyclene; NSC 6598	

Current regulation: /

Clinical data: /

Additional information:

In CosIng, both above CAS numbers are listed under “verdyl acetate” (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41289, last accessed 2010-07-19).

In the CAS, there are 2 separate entries; moreover, there are 2 separate RIFM reviews:

- # 2500-83-6: Other names: Tricyclo[5.2.1.02,6]dec-4-en-8-yl acetate (REACH, EINECS, INCI Name according to CAS); 3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl Acetate; Tricyclodecen-4-yl 8-Acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available, stating that “no data is available” regarding the skin sensitising properties of the substance (211).
- # 5413-60-5: Other names: 3a,4,5,6,7,7a-hexahydro-4,7-methanoinden-6-yl acetate (REACH, EINECS, INCI Name according to CAS), 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 8-Acetoxytricyclo[5.2.1.02,6]dec-3-ene; Tricyclodecenyl acetate; Greenyl acetate; Herbaflorat; Jasmacyclene; NSC 6598; Verdyl acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available (212), citing 2 negative human maximisation tests and 1 negative HRIPT.

Natural extracts / essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic material. Moreover, several compounds cannot be synthesised at a competitive price, and the demand for perfumes based on natural materials is considerable (34).

The three main methods used to concentrate plant fragrance substances as essential oils comprise steam distillation, mechanical processes from the epicarp of Citrus fruits ("pressing") and dry distillation.A Essential oil is „obtained by steam distillation with addition of water in the still (hydrodistillation) or without addition of water in the still (directly by steam")(213). Essenctial oil of fruit juice is „obtained by from a fruit juice during its concntration or during UHT (flash pasteurization) treatment" (213). Cold pressed essential oil is „obtained by mechanical processes from the epicarp of the fruit of a Citrus, at ambient temperature"(213). Citrus peel oils, apart from distilled Citrus oils, are produced with varous methods (214). The oil consists of a high volume of volatile terpenes, mostly monoterpenes but also contains small amounts of non-volatile compounds such as dyes, waxes and furocoumarines.

The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concrete: an extract „obtained from a fresh plant natural raw material by extraction with a solvant"[18], containing not only volatile, but also a large proportion of non- volatile substances such as waxes; and (ii) absolute: „product, obtained by extraction with ethanol from a concrete, a floral pomade, a resinoid or a supercritical fluid extract. The ethanolic solution is generally cooled down and filtered in order to eliminate the «waxes»; the ethanol is then eliminated by distillation"[19]. Resinoids, used for their fixative properties, are „obtained from a dry plant natural raw material by extraction with a sovant"[20]. The products are usually highly viscous and thus might sometimes be diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are extracts „of spice or aromatic herb" by „treating a natural raw material with a solvent, then, after filtration if necessary, the solvent is elimitated"[21].

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case report or clinical studies regarding essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g., masseurs,

[18] ISO/DIS 9235

[19] ISO/DIS 9235

[20] ISO/DIS 9235

[21] ISO/DIS 9235

physiotherapists (215, 216), aromatherapists (217-221), beauticians doing massages (222); for further details, e.g., PT results with various essential oils, see original case reports. “Current Regulation” refers to the EU Cometics Directive only.

Catalogue of natural extracts / essential oils evaluated

ACORUS CALAMUS *ROOT OIL*	*CAS 84775-39-3; EC* 283-869-0
Calamus Oil; "Sweet Flag Oil"	*(Acorus calamus, ext. = INCI name)*

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to “calamus” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “calamus” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Acorus calamus* L. (sweet flag calamus). Acorus Calamus Root Oil is an essential oil obtained from the rhizomes of the calamus, Acorus calamus L., Araceae. It contains beta-asarone (up to 96%, depending on ploidy, and with this, origin (34)), calamene (about 4%), calamol (about 3%) alpha-asarone (about 1%), camphene (about 1%) and some beta-pinene and asaronaldehyde (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41330, last accessed 2010-01-29. Use is restricted due to potenial toxicity of beta-asarone (34).

CANANGA ODORATA and Ylang-ylang oil

Ylang-ylang and cananga oils are essential oils that are obtained from two subspecies of the cananga tree (34). In the INCI nomenclature, both are not differentiated.

CANANGA ODORATA *FLOWER EXTRACT*	*CAS* 83863-30-3; EC 281-092-1 (ylang-ylang, ext.) INCI name: CANANGA ODORATA EXTRACT
CANANGA ODORATA *FLOWER OIL*	*CAS 8006-81-3, 68606-83-7; EC / (oils, ylang-ylang) INCI name:* CANANGA ODORATA OIL

Current regulation: …

Clinical data:

Ylang-ylang oil

ISO 4720:2009 nomenclature: *Cananga odorata (Lam.)* Hook. f. et Thomson *forma*

genuina)

In the Larsen 2002 c study, "synthetic ylang-ylang oil" caused 6.4% positive reactions in 218 patients with known contact allergy to fragrance ingredients (1). In a Japanese study, M. Sugawara et al. noted a significant decline of the proportion of patients reacting positively to "ylang-ylang oil 5% pet." from 1971 to 1989, the overall number in patients with cosmetic dermatitis amounting to 176 of 1438 (12.2%, 95% CI: 10.6 – 14.0%) (223). In the Frosch 2002 b study, two fractions of Ylang-Ylang oil (I and II) were separately tested, each at 10% pet. Fraction I yielded 2.6%, fraction II 2.5% positive test reactions (no data on concomitant reactivity given) (17). The deGroot 2000 study, with 1825 consecutively tested patients, found 18 positive PT reactions to "ylang-ylang oil", tested at 4% in pet. (12). The Sugiura 2000 study with 1483 patients with suspected cosmetic dermatitis observed 0.8% positive PT reactions with ylang-ylang oil (5% pet.) (14). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with ylang-ylang oil (2% pet.) 13.4% positive reactions (9). The Belsito 2006 study (20) yielded 0.6% positive reactions to ylang-ylang oil. The subsequent NACDG 2009 study identified 1.5% positive reactions in 4434 patients PTed with 2% "ylang-ylang oil" (21). The IVDK 2010c study found 2.5% positive reactions in 3175 consecutively tested patients, and 3.9% in 2155 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to ylang-ylang oil and 3 to "cananga oil" (48).

Cananga oil

ISO 4720:2009 nomenclature: *Cananga odorata (Lam.)* Hook. f. et Thomson *forma macrophylla*. For Oil of cananga (*Cananga odorata (Lam.)* Hook. f. et Thomson, *forma macrophylla*) an ISO standard exists: ISO 3523:2002. Cananga oil is produced by steam distillation of the flowers of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *macrophylla* (*Annonaceae*). The composition resembles that of "ylang-ylang III", but with a higher content of caryophyllene (30-40%). Cananga oil originates almost exclusively in Java; annual production about 50 t. The oil is used mainly in perfuming soaps where it is more stable than ylang-ylang oils due to its lower ester content (34).

Sugiura et al. (2000) found 1.1% positive reactions to "cananga oil", tested 5% pet. (14). Cananga oil (2% pet.) mentioned in the same Portugese study already cited (9) yielded 10.4% positive reactions. In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to cananga odorata oil tested at 2% concentration (13).

Studies with both oils

The Goossens 1997 study found 3 of 111 patients positive to "ylang-ylang oil 5% pet.", and 4 to "cananga oil 15% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 1 positive reaction in 200 patients to "cananga" and 4 to "ylang-ylang" essential oil, both tested 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=10 (11.6%) positive reactions to "cananga" and n=8 (9.3%) to "ylang-ylang" essential oils, each tested at 2% pet. (27). Nakayama et al. found 1974 (after (29)) 11 "strong positive" and 15 "weak positive" reactions to "Cananga oil" and 9 and 16, resp., to "Ylang-ylang oil" (unknown test concentration) in 183 patients.

A number of case reports highlight the possibility of occupational contact and sensitisation, e.g. (222, 224).

Additional information:

Ylang-ylang oil

The composition of this essential oil is defined by a standard: ISO 3063:2004. Ylang-ylang oils are obtained by steam distillation of freshly picked blossoms of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *genuina* (*Annonaceae*). The oil is produced mainly in Madagascar and the Comoro islands. Four fractions are collected at progressively longer distillation times and are know as "extra", "I", "II" and "III". The composition of the various oil fractions depends on the duration of distillation. The first fraction has the highest content of strongly odiferous constituents such as p-cresyl methyl ether (5-16%), methyl benzoate (4-9%), (-)-linalool (7-24%), benzyl acetate (5.5-17.5%), and geranyl acetate (2.5-14%). The other fractions contain increasing amounts of sesquiterpene hydrocarbons such as caryophyllene, germacrene-D, and (E,E)-alpha-farnesene (> 70% in "ylang-ylang III"). Components such as p-cresol, eugenol and isoeugenol are important for odour, although they are present only in low concentration (34). According to (30) the maximum observed concentration in ylang-ylang I and II are (in %): germacrene-D (28); (*E*,*E*)-alpha-farnesene (21); caryophyllene (17); *linalool* (I: 19.0; II: 9.5); *benzyl benzoate* (8.0); *farnesol* (4.0); *benzyl salicylate* (4.0); (*E*,*E*)-farnesyl acetate (3.5); *geraniol* (2.5); *isoeugenol* (0.8); *benzyl alcohol* (0.5); *eugenol* (0.5); p-cresyl methyl ether (I: 5.0; II 3.5); methyl benzoate (I: 5.5; II: 3.5); benzyl acetate (I: 10.0; II: 5.0); geranyl acetate (I: 15.0; II: 12.0).

CEDRUS ATLANTICA *BARK OIL*

CAS 92201-55-3; EC 295-985-9 *(Cedrus atlantica, ext. = INCI) / 8000-27-9; EC / (Oils, cedarwood) INCI name: CEDRUS ATLANTICA OIL*

Cedarwood oil

Current regulation: /

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=5 (0.7%) positive reactions to cedarwood oil 10% pet. (22). (The exact origin of "cedarwood oil" in this study is not clear.) The IVDK 2010 c study identified 0.8% positive reactions in 6223 patients tested in the context of a special series with a cedarwood oil tagged with CAS # 8000-27-9 (30).

Additional information:

Cedrus Atlantica Bark Oil is the volatile oil obtained from the bark of *Cedrus atlantica, Pinaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55309, last accessed 2010-01-05). The main odiferous component is alpha-atlantone [32207-08-2] (39)

Nomenclature also used: Cedrus atlantica wood oil (*Cedrus atlantica* (Endl.) G.Manetti ex Carrière)[22]

See also *Juniperus virginiana*.

[22] ISO 4720:2009 nomenclature

CEDRUS DEODARA *WOOD OIL* — *CAS 91771-47-0; EC* 294-939-5 *(Cedrus deodara, ext.)*

Cedarwood oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to "cedarwood" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "Himalayan cedarwood" essential oil 2% pet. (27). (The labelling in the latter report points to *Cedrus deodara* as source of "cedarwood oil" in these 2 Polish studies.)

Additional information:

Cedrus Deodara Wood Oil, Himalayan cedarwood oil (Cedrus deodara (Roxb. ex D.Don) G. Don) [23], is the volatile oil obtained by steam distillation of the stumps of the Deodar Cedar, *Cedrus deodara, Pinaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55311, last accessed 2010-01-29).

Several other conifer species are called cedars, and the corresponding oils vary considerably in composition. These include Cedar leaf oil (Thuja oil) produced by steam distillation of fresh leaves and branch ends of *Thuja occidentalis* L. (*Cupressaceae*) from North America, containing a minimum of 60% thujone [8007-20-3] [90131-58-1] (34). Texas cedarwood oil is produced by steam distillation of chopped wood of *Juniperus mexicana* Schiede (*Cupressaceae*), containing alpha-cedrene (15-25%), thujopsene (25-35%), cedrol 20% minimum [8000-27-9] [91722-61-1] (34). Chinese cedarwood oil is similar to Texas cedarwood oil, obtained by steam distillation of *Cupressus funebris* Endl., *Cupressaceae* (*Chamaecyparis funebirs* (Endl.) France), which is a weeping cypress [8000-27-9] [85085-29-6] (34).

CINNAMOMUM CASSIA *LEAF OIL* — *94961-46-6 [invalid] / 8007-80-5; EC / (Oils, cassia) INCI name: CINNAMONUM CASSIA OIL*

Cassia Oil; Cassia leaf Oil; Cinnamon Oil Chinense

CINNAMOMUM ZEYLANICUM *BARK OIL* — *CAS 84649-98-9; EC* 284-635-0 *(Cinnamonum zeylanicum, ext. = INCI)*

Cimmamon Bark Oil Ceylon; Cinnamon Oil Ceylon

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to "cassia" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=24

[23] ISO 4720:2009 nomenclature

(27.9%) positive reactions to “cassia” essential oil 2% pet. (27).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: "cinnamon oil" (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Cinnamomum tsumu* Helms, syn. *Cinnamomum cassia* auct. and *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* J. Presl, respectively. Cassia oil (Chinese cinnamon oil) is obtained by steam distillation of the leaves, twigs, and bark of *Cinnamonum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamonum bark oil (see below), cassia oil contains a considerable amount of 2-methoxycinnamal (3-15%), in addition to its main constituent, cinnamal (70-88%). Cassia oil is predominantly used in flavouring soft drinks, with an annual production of a few hundred tons (34). For Oil of cassia, Chinese type (Cinnamomum aromaticum Nees, syn. Cinnamomum cassia Nees ex Blume) an ISO standard exists: ISO 3216:1997

Cinnamomum Zeylanicum Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, Cinnamomum zeylanicum, Lauraceae. It contains mainly cinnamaldehyde (34), e.g. 50-60%, and lesser quantities of eugenol (4-8%), phellandrene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75370, last accessed 2009-11-16). For Oil of cinnamon leaf, Sri Lanka type (Cinnamomum zeylanicum Blume) an ISO standard exists: ISO 3524:2003

Cinnamomum Cassia Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamom, Cinnamomum cassia (L.), Lauraceae. It contains 80% eugenol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75368, last accessed 2009-11-16). The cinnamon leaf oil produced by steam distillation of the leaves of Cinnamonum zeylanicum Blume (C. verum J.S. Presl) similarly has a content of 70-83% eugenol (34).

Considering the content of well-known allergenic compounds, the essential oil is considered an Established contact allergen in humans,

CITRUS AURANTIUM AMARA *FLOWER OIL*	*CAS 8016-38-4, 68916-04-1; EC / (Oils, neroli) /*
Neroli oil	
CITRUS AURANTIUM AMARA *PEEL OIL EXPRESSED*	*72968-50-4; EC* 277-143-2 *(Orange, sour, ext.)*
"Bitter Orange Oil"	
	INCI names: CITRUS AURANTIUM AMARA …

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "neroli oil" (2% pet.) 6.6% positive reactions (9). The Rudzki 1976 study found 3 positive reactions in 200 patients to “bitter orange” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%)

positive reactions to “bitter orange” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.7% positive reactions in 6220 patients tested in the context of a special series (30)

Additional information:

ISO 4720:2009 nomenclature: *Citrus aurantium* L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso. For Oil of neroli (Citrus aurantium L. spp. aurantium, syn. Citrus aurantium L. spp. amara var. pumilia) an ISO standard exists: ISO 3517:2002. Citrus Aurantium Peel Oil Expressed is an essential oil expressed from the fresh epicarps of the Sour Orange, Citrus aurantium, Rutaceae. It contains D-limonene (about 90%), citral, decanaldehyde, methyl anthranilate, linalool, terpineol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41394, last accessed 2010-01-29. The aldehyde content is lower and the ester content (e.g., linalyl and geranyl acetate) is higher than in sweet orange oil (34). It is predominantly used for flavouring alcoholic beverages. According to (30) the maximum observed concentration in neroli oil are (in %): linalool (44); limonene (18); β-pinene (17); linalyl acetate (15); *trans*-β-ocimene (8); geranyl acetate (5); *trans*-nerolidol (5); (*E*,*E*)-farnesol (4); myrcene (4); farnesol (4,0); geraniol (3,5); citral (0,3) (30).

CITRUS AURANTIUM AMARA *LEAF OIL* *72968-50-4; EC* 277-143-2 *(Orange, sour, ext.)*

Petitgrain oil Paraguay / … bigarade

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Petitgrain bigarade” and “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “Petitgrain bigarade” and n=4 (4.6%) to “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: Citrus sinensis L. Pers. X Citrus aurantium L. ssp. amara var. pumilia. Petitgrain oils in general are steam distilled from the leaves of citrus trees. Citrus Aurantium Leaf Oil is an essential oil obtained from the leaves of the Sour Orange, Citrus aurantium, Rutaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41392, last accessed 2010-02-10). Petitgrain oil Paraguay is obtained from an acclimatised variety of the bitter orange tree. Main constituents are linalool (15-30%) and linalyl acetate (40-60%). A number of trace constituents contribute essentially to the odour (34). Petitgrain oil bigarade is derived from the same species of tree grown in France, Italy, Spain and North Africa (34). For Oil of bitter orange petitgrain, cultivated (Citrus aurantium L.) an ISO standard exists: ISO 8901:2003.

Considering the content of well-known allergenic compounds, the essential oil is regarded as an established contact allergen in humans

CITRUS BERGAMIA *PEEL OIL EXPRESSED* *CAS 89957-91-5, 8007-75-8; EC*

	289-612-9 *(Bergamot, ext.)*
Bergamot Oil, Bergamot Orange Oil	*INCI: CITRUS AURANTIUM BERGAMIA EXTRACT*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “Bergamot” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found no positive reaction to “Bergamot” essential oil 2% pet. (27). In 63 patients positive to the FM I, 2 had a positive PT reaction to bergamot oil, 2% pet., in the Santucci 1987 study (28). A case report from Zacher and Ippen describes 2 patients with allergic contact dermatitis due to bergamot oil (191), one a worker in a perfume factory, the other sensitised by non-occupational use of cosmetics.

Additional information:

ISO 4720:2009 nomenclature: *Citrus bergamia* (Risso et Poit.), syn. *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler. Citrus Bergamia Peel Oil Expressed is an essential oil expressed from the epicarps of the Bergamot, Citrus bergamia risso, Rutaceae. It contains 35-45% L-linalyl acetate, about 6% linalool, D-limonene, DL-limonene and bergaptene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41398, last accessed 2009-11-27). According to Surburg/Panten: linalyl acetate 22-36%, linalool 3-15%, geranial 0.25-0.5%, citral 1%, with a relatively low terpene content of 25-50% (34, 39). Bergaptene content by HPLC is 0.18-0.38% (34). Annual production from Italy, Brazil, Spain and Ivory Coast is 100 to 150 t. For Oil of bergamot [Citrus aurantium L. subsp. bergamia (Wight et Arnott) Engler], Italian type an ISO standard exists: ISO 3520:1998.

***CITRUS LIMONUM** PEEL OIL EXPRESSED*	*CAS 84929-31-7, 8008-56-8; EC* 284-515-8 *(Lemon, ext.)*
Lemon oil	INCI names: CITRUS MEDICA LIMONUM …

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lemon oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to “lemon oil” 2% pet. (22).

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lemon” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “lemon” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus limon* (L.) Burm. f. According to (30) the maximum observed concentration in lemon oil are (in %): limonene (80); ß-pinene (16.5); γ-terpinene (12); citral (3.0); geranial (2.0); neral (1.2); β-bisabolene (0.9); geranyl

acetate (0.7); neryl acetate (0.6); linalool (0,3); geraniol (0,2) (30). An ISO standard exists for Oil of lemon [Citrus limon (L.) Burm. f.], obtained by expression: ISO 855:2003. The composition of lemon oil depends on the variety of lemon an the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

Parameter	Type		Mediterranean		Equatorial
	American Origin				
	Coast	Desert	Italy	Spain	Ivory coast, Brazil
d_{20}^{20}	0.851–0.857	0.849–0.854	0.850–0.858	0.849–0.858	0.845–0.854
n_D^{20}	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4790
α_D^{20}	+57° to +65°6′	+67° to +78°	+57° to +66°	+57° to +66°	+57° to +70°
Composition by GC [area %]					
β-Pinene	9–14	10–13	10–16.5	10–16.5	7–16
Limonene	63–70	70–80	60–68	60–70	59–75
γ-Terpinene	8.3–9.5	6.5–8	8–12	8–128–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geranial	1.0–2	0.5–0.9	0.8–2	0.6–2	0.5–2
Evaporation residue [weight %]					
	1.75–3.9		1.5–3.9	1.5–3.9	1.5–4
Carbonyl value					
	8–14	6.25–12	11–17	11–17	6–17
CD value	min. 0.2	min. 0.2	0.45–0.9	0.4–0.9	0.2–0.96

CITRUS PARADISI *PEEL OIL*

Grapefruit oil, expressed

CAS 8016-20-4 ; EC /

INCI: CITRUS GRANDIS OIL

Current regulation: II/358 R1

Clinical data: /

Additional information:

Citrus Paradisi Peel Oil is the volatile oil expressed from the peel of the Grapefruit, Citrus paradisi, Rutaceae http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=55434

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

CITRUS SINENSIS *(syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED*

CAS 97766-30-8, 8008-57-9, EC 307-891-8 *(Orange, sweet,*

	Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 *(Orange, sweet, ext.)*
(Sweet) Orange oil	INCI names: CITRUS AURANTIUM DULCIS …

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "orange oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to orange oil 2% pet. (22). The Rudzki 1976 study found 1 positive reaction in 200 patients to “sweet orange” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “sweet orange” essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, neither positive nor irritant reaction to 1% and 5% “orange oil Bras.” in pet., tested in 205 consecutive patients in Dortmund and Göttingen, were observed (15). The IVDK 2010 c study identified 0.2% positive reactions in 6246 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* (L.) Osbeck. For Oil of sweet orange (Citrus sinensis (L.) Osbeck), CAS 8008-57-9, obtained by mechanical treatment, an ISO norm exists: ISO 3140:2005. The oils have a high terpene hydrocarbon content (> 90%, mainly (+)-limonene. Important for aroma are aldehydes, mainly decanal and citral, and aliphatic and terpenoid esters. The sesquiterpene aldehydes alpha-sinensal [17909-77-2] and beta-sinensal [6066-88-8] contribute particularly to the special sweet aroma (34). According to (30) the maximum observed concentration in sweet orange oil are (in %): *limonene* (95.0); *linalool* (0.7); n-decanal (0.7); *citral* (0.3); alpha-sinensal (0.05); beta-sinensal (0.06) (30). Worldwide production is more than 30000 tons / year. Main uses comprise the flavouring of beverages and confectioneries and perfuming E.d.C, soaps and household products.

For the latter uses relevant here, both “Orange peel oil, sweet (Citrus sinensis (L.) Osbeck) (8008-57-9)“, “Orange peel, sweet, extract (Citrus sinensis L. Osbeck) (8028-48-6)“ and “Orange, sweet, Valencia, ext. (97766-30-8)” are among the top 100 used fragrance materials and classified as R43 (IFRA, pers. comm. 2010).

ORANGE OIL TERPENES (CAS # 68647-72-3) are a “top 100 mixture of substances and classified as R43 (IFRA, pers. comm.2010). Other names: ORANGE, SWEET, TERPENES (REACH); Terpenes and Terpenoids, sweet orange-oil (REACH). The CAS entry refers to a group of substances “Terpenes and Terpenoids, sweet orange-oil“ (REACH).

CITRUS TANGERINA *…*	*CAS 223748-44-5; EC /*
Oil of tangerine	[no info in CAS database]

Current regulation: /

Clinical data:

In a 17 year old girl, the perfume used for 3 months caused ACD due to the ingredient “oil of tangerine”, with a strong positive PT reaction (to 2% or 10% in pet.; 50 controls

negative) (226).

Additional information:

Citrus Tangerina Peel Oil is the volatile oil expressed from the peel of the ripe fruit the Tangerine, *Citrus Tangerina, Rutaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55441, last accessed 2010-01-29); (*Citrus tangerina* Tanaka).

***CORIANDRUM SATIVUM** HERB OIL*

Coriander oil

CAS 84775-50-8; EC 283-880-0 *(Coriander, ext.)*

INCI: CORIANDRUM SATIVUM EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to "coriander" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "coriander" essential oil 2% pet. (27).

Additional information:

Coriander Sativum Herb Oil is an essential oil obtained from the herbs of the Coriander, Coriandrum sativum L., Umbelliferae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39388, last accessed 2010-01-29). The main component of coriander oil is linalool (by GC: 65-78%) and mono- and polyunsaturated fatty aldehydes contributing to the particular aroma. In contrast to the seed oil, coriander leaf oil contains these aldehydes as main constituents, e.g. 2-deccanal and 2-dodecanal (34). For Oil of coriander fruits (Coriandrum sativum L.) an ISO standard exists: ISO 3516:1997.

***CYMBOPOGON** OILS*

Cymbopogon oils are produced from several aromatic grasses that belong to the genus *Cymbopogon* Speng. (*Poaceae*). The oils are obtained by steam distillation of the aerial parts of the plants (34).

The composition of the essential oil derived from *Cymbopogon flexuosus* (Nees ex Steudel) J.F. Watson is defined by a standard: ISO 4718:2004, as is the oil derived from *Cymbopogon citratus:* 3217:1974.

***CYMBOPOGON CITRATUS** LEAF OIL*

Cymbopogon citratus (DC.) Stapf.[24]

CAS 89998-14-1; EC 289-752-0 *(Cymbopogon citratus, ext. = INCI)*

[24] ISO 4720:2009 nomenclature

Lemon Grass Oil; Indian Verbena Oil; Indian Melissa Oil

***CYMBOPOGON SCHOENANTHUS** OIL*

Cymbopogon flexuosus (Nees ex Steudel) J.F. Watson[25]

CAS 8007-02-1; EC 289-754-1 *(oils, lemongrass) / 89998-16-3; EC* 289-752-0 *(Cymbopogon Schoenanthus, ext. = INCI)*

Lemon Grass Oil

Current regulation: /

Clinical data:

The Frosch 2002 b study on 1606 consecutive patients reported 1.6% positive reactions to "lemongrass oil (East India), CAS 8007-02-1", PTed at 2% pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 3 positive reactions to lemongrass oil were observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=6 (0.8%) positive reactions to lemongrass oil 2% pet. (22). The IVDK 2010 c study identified 0.6% positive reactions in 2435 consecutively tested patients and 2.3% positive reactions in 8445 patients tested in the context of a special series (30).

Additional information:

Cymbopogon Citratus Leaf Oil is an essential oil obtained from the leaves of the Lemon Grass, *Cymbopogon citratus* (DC., ex Nees), *Poaceae*. It contains citral (75-85%), methylheptenone, citronellal, geraniol, limonene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39457, last accessed 2009-11-12). According to Surburg/Panten, by GC: neral (31-40%), geranial (40-50%) (34).

Indian lemongrass oil is obtained by the so-called Indian variety of lemongrass, *Cymbopogon flexosus* (Nees ex Steud.) Stapf. Content by GC: 25-35% neral, 35-47% geranial (34).

Cymbopogon Schoenanthus Oil is the volatile oil obtained by the steam distillation of fresh Lemon Grass, *Cymbopogon schoenanthus* (L.), *Poaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75419, last accessed 2009-11-12). According to (30) the maximum observed concentration in lemongrass oil are (in %):*citral* (85.0); *geraniol* (7.0); *limonene* (4.0); geranyl acetate (2.2); caryophyllene (1.6); trans-isocitral (1.4); 6-methyl 5-hepten-2-one (1.3); caryophyllene oxide (1.2); 4-nonanone (1); *citronellol* (0.8); *eugenol* (0.3); *linalool* (0.2) (also according to (227))

In a LLNA study by RIFM, the lemongrass oil as used was reported to contain 68.8% citral, 6.7% limonene, 6.1% geraniol, 2.2% geranyl acetate, 1.6% caryophyllene, 1.4% trans-isocitral, 1.3% 6-methyl 5-hepten-2-one, 1.2% caryophyllene oxide and 1% 4-nonanone, according to analyses of the supplier. The EC3 value was calculated to be 6.5% (227).

***CYMBOPOGON MARTINI** HERB EXTRACT*

CAS 84649-81-0; EC 283-461-2 *(Cymbopogon Martini, ext)*

[25] ISO 4720:2009 nomenclature

INCI: CYMBOPOGON MARTINI OIL

Palmarosa oil

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon martini* (Roxb.) Will. Watson *var. motia* and var. *sofia*. Cymbopogon Martini Herb Extract is an extract obtained from the herbs of the plant, *Cymbopogon martini*, Gramineae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39460, last accessed 2009-11-24), namely, by steam distillation of wild or cultivated Cymbopogon martini (Roxb.) J.F. Wats., collected when in blossom (34).The main constituent is geraniol (72-94%) (34).

In a LLNA study by RIFM, the palmarosa oil as used was reported to contain 79.4% geraniol, 9.4% geranyl acetate and 1.9% caryophyllene, according to analyses of the supplier. The EC3 value was calculated to be 9.6% (227).

CYMBOPOGON NARDUS *HERB OIL* — *CAS 89998-15-2; EC* 289-753-6 *(Cymbopogon nardus, ext. = INCI)*

Citronella Oil (Sri Lanka)

CYMBOPOGON WINTERIANUS *HERB OIL* — *CAS 91771-61-8; EC* 294-954-7 *(Cymbopogon Winterianus, ext. = INCI)*

Citronella Oil (Java)

Current regulation: …

Clinical data:

The Rudzki 1976 study found 5 positive reactions in 200 patients to “citronella” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to “citronella” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon nardus* (L.) W. Watson *var. lenabatu* Stapf. and *Cymbopogon winterianus* Jowitt, respectively. Cymbopogon Nardus Herb Oil is an essential oil obtained from the herbs of the plant, Cymbopogon (syn: Andropogon) nardus (L.), Gramineae. The Ceylon citronella oil contains geraniol (about 60%), citronellal (about 15%), camphene, limonene, linalool, borneol. According to Surburg/Panten, the Sri Lankan oil contains citronellal (3-6%), borneol (4-7%), citronellol (3-8.5%), geraniol 15-23%) and methyl isoeugenol (7.11%) (34).

The Java citronella oil contains 25-50% citronellal, 25-45% geraniol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39469, last accessed 2009-11-24). Cymbopogon Winterianus Herb Oil as a synonym for Java citronella oil is obtained from the herbs of the plant, Cymbopogon winterianus,
Gramineae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=sea

rch.details&id=39472, last accessed 2009-11-24). This oil, produced in Taiwan and Java, contains citronellal (31-40%), geraniol (20-25%), citronellol (8.5-14%), geranyl acetate (2.5-5.5%), citronellyl acetate (2-4%) and many minor components. Annual worldwide production is currently at around 1000 t (34). For Oil of citronella, Sri Lankan type (Cymbopogon nardus (L.) W. Watson var. lenabatu Stapf.) an ISO standard exists: ISO 3849:2003, for Oil of citronella, Java type the ISO 3848:2001.

In a LLNA study by RIFM, the citronella oil as used was reported to contain 36.6% citronellal, 20.6% geraniol, 4.1% limonene, 3.7% geranyl acetate, 3.0% citronellyl acetate, 2.6% elemol, 2.2% beta-bourbonene, 1.9% delta-cadiene, 1.6% isopugenol I, 1.4% germacrene D and eugenol and linallol at < 1%, according to analyses of the supplier. The EC3 value was calculated as > 50 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

EUCALYPTUS SPP. *LEAF OIL* — *CAS 92502-70-0; EC* 296-357-7 *(Eucalyptus, ext. = INCI)*

Eucalyptus *Oil* — *CAS 8000-48-4; EC / (Oils, eucalyptus) INCI: EUCALYPTUS GLOBULUS OIL*

Current regulation: /

Clinical data:

In a study with 218 fragrance sensitive patients, 1.8% reacted positively to 10% eucalyptus oil (pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to "eucalyptus oil" was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to eucalyptus oil 2% pet. (22). The Rudzki 1976 study found 3 positive reactions in 200 patients to “eucalyptus” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to “Eucalyptus” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.2% positive reactions in 6680 patients tested in the context of a special series (30).

In a professional athlete, the use of an "analgesic and anti-inflammatory cream" over 2 years lead to ACD, which was attributed to eucalyptol (eucalyptus oil, 1% pet., 25 controls negative), the sole ingredient of the cream eliciting a positive PT reaction (228)

Additional information:

ISO 4720:2009 nomenclature: *Eucalyptus globulus* Labill. Eucalyptus oils are produced from plants belonging to the genus *Eucalyptus* (*Myrtaceae*), which includes about 500 species in Australia, the country of origin, alone. At present, few of the oils, which are used to characterise species, are commercially important (34). Some species are rich in 1,8-cineole (80-85% content). Other species contain less cineole, but 10-22% alpha-pinene. *E. citriodora* predominantly contains citronellal (min. 75% by GC), with some citronellol and isopulegol (5-10% each) (34). *E. dives* contains (-)-piperitone and 15-25% alpha-phellandrene (34). According to (30) the maximum observed concentration in eucalyptus oil are (in %): 1,8-cineole (58; 70-80 after rectification); α-pinene (22); limonene (8); para-cymene (5); trans-pinocarveol (5); aromadendrene (10); globulol (2.5) [the latter 2 components only traces after rectification] (30).

For Crude or rectified oils of Eucalyptus globulus (Eucalyptus globulus Labill.) an ISO standard exists**:** ISO 770:2002.

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

EUGENIA CARYOPHYLLUS *LEAF / FLOWER OIL* — *CAS 8000-34-8; EC / (Oils, clove)*

Clove oil — INCI: EUGENIA CARYOPHYLLUS OIL

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 19.3% of patients with known contact allergy to fragrance ingredients reacted positively to "clove bud oil" (10 % pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "clove oil" were observed (3). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with clove oil (2% pet.) 13.4% positive reactions (9). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.6% positive reactions 2% pet. (22). The Rudzki 1976 study found 2 positive reactions in 200 patients to "clove" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=12 (13.3%) positive reactions to "clove" essential oil 2% pet. (27). The IVDK 2010 c study identified 1.5% positive reactions 6893 patients tested in the context of a special series (30).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: cinnamon oil (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Syzygium aromaticum* (L.) Merr. & L. M. Perry syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison. Standards regarding the composition of clove oil are available: ISO 3141:1997, ISO 3142:1997, ISO 3143:1997. Clove oils are produced from the clove tree *Szyigium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Speng.) Bullock ex S.G. Harrison. The content of clove bud, clove leaf and clove stem oil has, with little variation, been determined by GC as 75-92% eugenol, 2-17% caryophyllene and 0.2-15% eugenyl acetate – the latter compound found in particularly high concentration in bud oil (34). According to another source, the following maximum content (%) has been observed regarding the constituents listed: eugenol (92,0);

caryophyllene (17); eugenyl acetate (15); isoeugenol (0.5) (30).

In a LLNA study by RIFM, the clove leaf oil as used was reported to contain 85.3% eugenol, 9.9% caryophyllene and 2.2% alpha humulene, according to analyses of the supplier. The EC3 value was calculated to be 7.1% (227).

EVERNIA FURFURACEA *LICHEN EXTRACT* *CAS 90028-67-4; EC* 289-860-8 *(Evernia furfuracea, ext. = INCI)*

Tree moss extract

Current regulation: /

Clinical data:

The Larsen 1977 study in 20 “perfume-sensitive patients” yielded n=6 positive reactions with “treemoss abs. in benzyl benzoate, 5% petrolatum” (18). In the IVDK 2007 study, 2.7% (95% CI: 2.0 – 3.6%) of 1658 consecutive patients had a positive reaction to “tree moss absolute” (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to the allergen, tested at 2%., i.e., twice the commonly used concentration, and not in pet., but in diethylphthalate (6). The IVDK 2010 study, 6.02% (95% CI: 4.90 – 7.14%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7).

Additional information:

Syn.: *Pseudoevernia furfuracea* (L.) Zopf (53). The lichen grows on the bark of pine and fir trees. The extraction process with carbohydrate solvents yields a “concrete” (2-5% yield) which, in a next step eliminating waxy compounds, is extracted with warm alcohol and subsequent cooling, yielding an “absolute” (40-60% yield) (53).

EVERNIA PRUNASTRI *CAS 90028-68-5; EC* 289-861-3 *(Evernia prunastri, ext. = INCI)*

Oak moss abs.

Current regulation: Annex III, part 1, n° 91

Clinical data:
In the “background information” section of the 1999 opinion, oak moss exctract is classified as “most frequently reported allergen”; in consecutive PT patients, about 2.8% positive reactions had been reported (33). ´The German MAK commission has labelled oak moss extract as ‘sensitising to the skin’ (229).

Since the last SCCNFP-opinion of 1999, a “polymer based method” was developed to reduce the natural content of these two compounds from around 1 - several percent to < 75 ppm for atranol and < 25 ppm for chloratranol. However, PTing 14 subjects with previous positive PT reactions to the “oak moss” allergen preparation with the modified Evernia prunastri material still elicited positive reactions in 8/14 subjects; thus, the reduction in allergen content was deemed unsafe for the consumer (230). In a study of 885 consecutive eczema patients tested in Gentofte, Denmark, 3.2% had a positive or follicular patch test response to oak moss absolute. Two types of oak moss absolute were tested, one contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types of oak moss absolute

(231). The IVDK 2007 study yielded 2.2% (95% CI: 1.6 – 3-0%) positive reactions in 2063 consecutively tested patients (4). In the Groningen 2009 study, 1.9% (95% CI: 0.7 – 4.0%) had positive reactions to oak moss, tested at 2% pet., i.e., twice the commonly used concentration (6). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reaction (13) (test concentration 2% pet.). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.0% positive reactions (22). The IVDK 2010 study, 1.81% (95% CI: 1.07 – 2.56%) of 1213 consecutively tested patients reacted to the compound, while 5.59% (95% CI: 4.90 – 6.27%) of 4482 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with *E. prunastri* extract, yielding 2 positive reactions (48).

L. Kanerva et al. report on a 41 year old female hairdresser in whom oak moss abs. contained in a perming solution (concentration in the product unknown) was unequivocally identified as allergen causing (i) occupational hand dermatitis and (ii) scalp dermatitis after application to the own hair (232). Another case of occupational hand dermatitis in a grinding engineer was, at least partly, attributable to contact sensitisation to "oak moss resin" contained in a soluble oil (233).

Additional information:

Source: Evernia prunastri (Oak moss) (*Evernia prunastri* var. *prunastri* L. Ach). Oak moss is extracted as described above. Chloratranol and atranol are the degradation products of chloratranorin and atranorin, resp., which are recognised as the main sensitisers in *Evernia prunastri* extracts.

ILLICIUM VERUM *FRUIT OIL*

"Anise Oil", Star anise oil

CAS 84650-59-9, 8007-70-3; EC 283-518-1

(Star anise, Illicium verum, ext. = INCI)

Current regulation: /

Clinical data:

In a study involving 100 consecutive patients, Rudzki and Grzywa found (i) a relatively high frequency of active sensitisation to star anise oil (n=5) tested with 0.5, 1 and 2% concentration (most likely in yellow petrolatum, as the other allergens in this series). Later patch testing with constituents of this essential oil (1%) in 3 patients yielded positive results to anethole in 3 cases, and to alpha-pinene and safrole in the 1 case tested to these substances. 34% of the consecutive patients reacted positively to star anise oil at 1%, which was considered as (marginally) non-irritating PT concentration (234).

Additional information:

ISO 4720:2009 nomenclature: *Illicium verum* Hook. f. Illicium Verum Fruit Oil is an essential oil distilled from the fruits of the Star Anise, Illicium verum, Illiciaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40297, last accessed 2010-01-29). The main component is trans-anethole (86-93%), which can be purified from star anise oil. Main uses are alcoholic beverages, food flavouring and oral care products (34, 39). For Oil of star anise, Chinese type (Illicium verum Hook. f.) an ISO standard exists: ISO 11016:1999.

JASMINUM GRANDIFLORUM *FLOWER EXTRACT*	*CAS 84776-64-7; EC* 283-993-5 *(Jasmine, Jasminum grandiflorum, ext. = INCI)*
Jasmine abs.	
JASMINUM OFFICINALE *FLOWER OIL*	*CAS 90045-94-6; EC* 289-960-1 *(Jasmine, Jasminum officinale, ext. = INCI)*
JASMINUM OFFICINALE *OIL*	*CAS 8022-96-6; EC* / (Oils, jasmine) INCI: JASMINUM OFFICINALE OIL

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 1.2% of 1606 consecutive patients had a positive PT to “jasmine absolute”, tested 5% in pet. (17). The deGroot 2000 study yielded 13 positive reactions to "jasmine, synthetic" in 1825 consecutively tested patients (12). In the early Larsen 1977 study, 18 of 20 “perfume sensitive patients” reacted to “Jasmin synthetic” 10% pet. (18), while 7 reacted to “Jasmin absolute” (10% pet.) – all of these also positive to the synthetic fragrance. The Sugiura 2000 study set in Nagoya, Japan, yielded 1% positive PT reactions in 1483 patients PTed for suspected cosmetic dermatitis, using 5% pet. as test concentration (14). The Larsen 2001 study in 178 patients with known contact allergy to fragrance ingredients found 16.9% positive reactions to jasmine absolute (10% pet.) (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to Jasmine officinale oil (Jasmine absolute, Egyptian), tested at 2% (13). In the NACDG 2009 study, 1.1% of 4447 patients tested with “Jasmine absolute 2% pet.” were found PT-positive (21). The Belsito 2006 study (20) yielded 0.4% positive reactions to “jasmine absolute”. The Goossens 1997 study found 5 of 111 patients positive to “jasmine absolute” (10% pet.)– all sensitised to other fragrance allergens (23). In 63 patients positive to the FM I, 13 had positive PT reactions to “jasmine absolute”, 2% pet., and 12 to “jasmine synthetic”, 2% pet. in the Santucci 1987 study – the amount of concomitant reactivity was not examined (28). Nakayama et al. found 1974 (after (29)) 19 “strong positive” and 25 “weak positive” reactions to “jasmin oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.5% positive reactions in 3668 consecutively tested patients and 1.2% positive reactions in 982 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with jasmine absolute, yielding 3 positive reactions, and with “Jasmine synthetic”, also resulting in 3 positive reactions (48).

Additional information:

Jasminum Grandiflorum Flower Extract is an extract obtained from the flowers of the Spanish Jasmine, Jasminum grandiflorum L., Oleaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39752, last accessed 2009-11-12).

Jasminum Officinale Flower Oil is an essential oil obtained by molecular distillation of the flowers from the Jasmine, Jasminum officinale L., Oleaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39754, last accessed 2009-11-25).

Jasminum Officinale Oil is the volatile oil obtained from the flowers of the Jasmine, Jasminium officinale L., Oleaceae

(http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=34776, last accessed 2010-01-05); this latter extract is used by Almirall/Hermal/Trolab for the preparation of a PT allergen.

Jasmine absolute is obtained by solvent extraction, via concrete, from the flowers of J. grandiflorum (L.) Aiton from China and India. The main volatile compound is benzyl acetate, however, minor compounds such as indole [120-72-9], cis-jasmone [488-10-8] and methyl jasmonate [1211-29-6] contribute to the typical jasmine fragrance (34). Reported compounds include the following (maximum observed concentration given in parentheses): benzyl acetate (28); benzyl benzoate (24.0); phytyl acetate (9); isophytol (8.5); phytol (7.4); linalool (7.0); eugenol (4.0); squalene (4); indole (3.5); benzyl alcohol (2.5); cis-jasmone (2.5); methyl linolenate (2.0); methyl palmitate (1.4); p-cresol (1.0); cis-3-hexenyl benzoate (1.0); benzyl salicylate (0.4); jasmin lactone (0.9); methyl jasmonate (0.7); isoeugenol (0.4) ((30), also according to (17))

JUNIPERUS VIRGINIANA *OIL*

CAS 8000-27-9; EC / (Oils, cedarwood) [this also refers to Cedrus atlantica …) / 85085-41-2; EC 285-370-3 *(Juniper, Juniperus virginiana, ext. = INCI)*

JUNIPERUS VIRGINIANA *WOOD OIL*

CAS 85085-41-2; EC 285-370-3

Cedar Wood Oil (Virginian)

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 0.6% of 1606 consecutive patients had a positive PT to “cedarwood oil (Moroccan and Chinese 1:1)”, tested 10% in pet. (17). After application of Penaten-baby™ oil as immersion oil for dermatoscopy a patient developed multiple patches of eczema at the application sites. Investigation revealed that the oil was kept in a bottle previously used for *Juniperus virginiana* oil, to which contact sensitisation was verified by patch testing (235).

Additional information:

ISO 4720:2009 nomenclature: *Juniperus virginiana* L.. Juniperus Virginiana Oil is the volatile oil obtained from the fruits and leaves of the Red Cedar, Juniperus virginiana L., Cupressaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=78070, last accessed 2010-01-05)

Juniperus Virginiana Wood Oil is an essential oil obtained from the wood and twigs of the Red Cedar, Juniperus virginiana L., Cupressaceae. It contains chiefly (alpha and beta) cedrene and cedral (cedar camphor), cuparene, thujopsene, widdrol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39767, last accessed 2009-11-12)(235). According to Surburg/Panten by GC: alpha-cedrene 22-35%, thujopsene 10-25%, cedrol 16-25% (34).

See also *Cedrus atlantica*. According to (30) the maximum observed concentration in cedar wood oil are (in %): α-cedrene (32); thujopsene (25); cedrol (25); β-cedrene (6); widdrol (5) and cuparene (traces) (30).

For Oil of cedarwood, Virginian (Juniperus virginiana L.) an ISO standard is available: ISO 4724:2004. For Oil of cedarwood, Texas (Juniperus mexicana Schiede) an ISO standard exists: ISO 4725:2004.

LAURUS NOBILIS *OIL*

CAS 8002-41-3; EC / (Oils, laurel) INCI: LAURUS NOBILIS OIL / 8007-48-5; EC / (Oils, sweet bay)/ 84603-73-6; EC 283-272-5 *(Laurus nobilis, ext.) INCI: LAURUS NOBILIS EXTRACT*

Laurel oil

Current regulation: Annex II, n° 359 (seed oil)

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to “laurel oil” 2% pet. (22).

After sensitisation by a one-time occlusive application a 36 year old Turkish patient developed widespread allergic contact dermatitis 3 days after massage with olive oil containing Laurus nobilis oil; sensitisation was proven by a strong positive reaction to the commercial test preparation and the massage oil previously used (236). Topical application of laurel oil for knee arthropathy led to an erythema exudativum multiforme-like rash on the legs of a 63 year old patient; interestingly, laurel oil yielded a “target like” strongly positive PT reaction in this case (237). In an earlier Turkish case with a similar history, the EEM-like appearance was lacking; however, a very intense, edematous reaction was noted (238). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "laurel oil" were observed (3). The IVDK 2010 c study identified 1.0% positive reactions in 6297 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Laurus nobilis* L. Laurel leaf oil is obtained by steam distillation of leaves from Laurus nobilis L. (Lauraceae), an evergreen cultivated primarily in the Mediterranean countries. The main components are 1,8-cineole (30-70%), linalool (about 10%) and eugenol (34). According to (30) the maximum observed concentration in laurel oil are (in %): 1,8-cineole (70); ß-caryophyllene (11); *linalool* (11); *limonene* (5.0); *eugenol* (2.0); *geraniol* (0.3) (30).

LAVANDULA HYBRIDA *HERB OIL*

CAS 91722-69-9; EC 294-470-6 *(Lavender, Lavandula hybrida, ext. = INCI)*

Lavandin Oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lavandin” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to “lavandin” essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% lavandin oil in pet., tested in 205 consecutive patients in Dortmund and Göttingen, and just 1 irritant reaction to

the higher concentration, were observed (15).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. Lavandula Hybrida Herb Oil is an essential oil distilled from the flowering herbs of the Lavendin, *Lavandula hybrida, Labiatae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39789, last accessed 2010-01-29. Nomenclature according to Surburg/Panten: *Lavandula* x *intermedia* Lois, which is a hybrid of lavender and spike (see below) (34). The oils from the most important variants, abrial and grosso, contain linalool (24-38%), linalyl acetate (20-38%) as well as 1,8-cineole (4-11%), and camphor (6-11%) (34). A third variant is called super because of its high concentration of linalyl acetate (35-47%), more closely resembling lavender oil (34). For Oil of lavandin Grosso (Lavandula angustifolia Mill. x Lavandula latifolia Medik.), French type an ISO standard exists: ISO 8902:2009, for Oil of lavandin Abrial (Lavandula angustifolia Miller x Lavandula latifolia Medikus), French type a different ISO standard: ISO 3054:2001.

It is a “top 100” substance (IFRA, pers. comm.2010)

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LAVANDULA OFFICINALIS *FLOWER OIL*

CAS 84776-65-8, 8000-28-0; EC 283-994-0 *(Lavender, Lavandula angustifolia angustifolia, ext. = INCI)*

Lavender oil

Current regulation: /

Clinical data:

In a large series from Nagoya, Japan, 1483 patients were tested with lavender oil 20% in pet., with overall 3.7% positive reactions from 1990 to 1998. However, within this period, a sharp increase was noted in 1997 and 1998, which as attributed to changed exposure by M. Sugiura et al. (14). On the individual level, relevance of positive reactions remained unclear in about half of the cases. The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lavender absolute" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to “Lavendula augustifolia oil” (Lavender absolute) 2% (13). The Goossens 1997 study found 4 of 111 patients positive to “lavender oil 20% pet.”)– all of them sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to “lavender” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “lavender” essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 6 “strong positive” reactions to “Lavender oil” (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with “lavender absolute”, yielding 2 positive reactions (48).

R. Goiriz et al. report on a case of photo contact allergy (10 controls negative) in a 45 year old woman developing after application of a ketoprofen-containing topical gel (“Fastum”)(239). A physiotherapist developed acute, recurrent dermatitis after use of “Difflam® gel”, scented with lavender oil. Both the gel and lavender oil (2% pet.) tested positive; avoidance resulted in clearing (240). In a study on 218 patients with known

contact allergy to fragrance ingredients, Larsen (2002 c) found positive reactions to 10% lavender oil (pet.) in 2.8% of these (1). A case of vulvovaginitis with spread and affecting the dominant hand applying various tea tree and lavender oil creams was reported by S. Varma; the PT with 10% lavender oil abs. in pet. (50 controls negative) was positive (241). In two cases, facial "pillow dermatitis" due to lavender oil, applied to the pillows, developed, confirmed by positive PT to lavender abs. (2% pet.) (242).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. *Lavandula officinalis* Flower Oil is an essential oil obtained from the fresh flowering tops of the Lavender, *Lavandula officinalis* (syn: *L. vera*), *Labiatae*. It contains 30-40% esters calculated as linalyl acetate, linalool, pinene, limonene, geraniol, some eucalyptol (cineol) (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40370, last accessed 2009-11-09). According to Surburg/Panten, lavender oil is obtained by steam distillation of freshly cut flowering tops of Lavandula angustifolia Mill. (Lamiaceae). Main constituents according to GC are linalyl acetate (25-45%), cis-ocimene (4-10%), trans-ocimene (1.5-6%), 1,8-cineole (≤ 1%) camphor (≤ 0.5%), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] (≥ 2%) (34).

In addition to distillation, both Lavandula officinalis and Lavandin are also solvent extracted, yielding concretes and, after ethanol extraction, absolutes, which are said to have a longer-lasting odour (34).

For Oil of lavender (Lavandula angustifolia Mill.) an ISO standard exists: ISO 3515:2002.

LAVANDULA SPICA *HERB OIL*

CAS 97722-12-8; EC 307-762-6 *(Lavender, Lavandula spica, ext. = INCI*

"Spike Oil"

Current regulation: …

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "spike" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=8 (9.3%) positive reactions to "spike" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: Lavandula latifolia Medik. Lavandula Spica Herb Oil is an essential oil distilled from the flowering herbs of the Spikenard, *Lavandula spica* (syn: *Lavandula latifolia*), *Labiatae*. It contains eucalyptol (35%), camphor, linalool, borneol, terpineol, D-camphene and sesquiterpenes (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40372, last accessed 2010-01-29). According to Surburg/Panten, Spanish spike lavender oil is steam distilled from the flowering tops of Lavandula latifolia Medik.. The main components are linalool (34-50%), 1,8-cineole (16-39%) and camphor (8-16%) (34). For Oil of spike lavender (Lavandula latifolia (L.f.) Medikus), Spanish type an ISO standard exists: ISO 4719:1999

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LITSEA CUBEBA *FRUIT EXTRACT*

CAS 90063-59-5, 68855-99-2; EC 290-018-7 *(Litsea cubeba, ext.) INCI: LITSEA CUBEBA OIL*

Current regulation: …

Clinical data:

The Rudzki 1976 study found 3 positive reaction in 200 patients to "Litsea cubeba" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to this essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Litsea cubeba* (Lour) Pers. Litsea Cubeba Fruit Extract is an extract obtained from the fruits of the plant, *Litsea cubeba*, *Lauraceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40036, last accessed 2009-11-24. The content by GC is: neral (25-33%), geranial (38-45%) – i.e. about ¾ citral, for which the extract had previously served as a raw material (34); direct use for perfuming is limited to household products (39). For Oil of Litsea cubeba (Litsea cubeba Pers.) an ISO standard exists: ISO 3214:2000.

In a LLNA study by RIFM, the "Litsea cubeba oil" as used was reported to contain 85.7% citral, 2.9% limonene, 1.7% linalool, 1.4% citronellal and < 1% caryophyllene and methyl heptanone, according to analyses of the supplier. The EC3 value was calculated as 8.4 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

MENTHA ARVENSIS *LEAF OIL*

Cornmint oil

CAS 68917-18-0 ; EC /

INCI: MENTHA ARVENSIS OIL

Current regulation: /

Clinical data: /

Additional information:

Mentha Arvensis Leaf Oil is the oil derived from the leaves of the Horse Mint, Mentha arvensis L., Labiatae http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=57860

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

MENTHA PIPERITA *OIL*

CAS 8006-90-4; EC / (Oils, peppermint) INCI: MENTHA

PIPERITA OIL / 84082-70-2; EC 282-015-4 *(Peppermint, ext.) INCI names: MENTHA PIPERITA …*

Peppermint oil

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% of 1606 consecutive patients reacted positively to “peppermint oil (American)”, tested 2% in pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "peppermint oil" were observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to peppermint oil 2% pet. (22). Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 6 patients had positive (+ to +++) PT reactions to “peppermint oil” 1% pet. at D4, mostly combined with positive reactions to menthol (see above) and reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). The Rudzki 1976 study found 1 positive reaction in 200 patients to “Peppermint” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=6 (6.9%) positive reactions to “peppermint” essential oil 2% pet. (27). In 63 patients positive to the FM I, 3 had positive PT reactions to peppermint oil, 2% pet., in the Santucci 1987 study (28). The IVDK 2010 c study identified 0.6% positive reactions in 6546 patients tested in the context of a special series (30).

An unusual case of “baboon-like” allergic contact dermatitis of the vulva after drinking excessive amounts of a herbal tea containing, among other ingredients, peppermint. While the PT reaction to peppermint oil was only weak to doubtful, dramatic improvement after cessation and prompt relapse after repeat ingestion proved the diagnosis (243). Recurrent foot and lower leg dermatitis after the application of a “foot spray” (containing peppermint oil) was diagnosed as allergic contact dermatitis due to this ingredient in a 59 year old golf player (244). In another case, ACD after application of a transdermal system for the treatment of lumbar pain was attributed to CA to peppermint oil (2% pet.) and its main ingredient menthol (1% pet.) (155). In a patient with toothpaste-induced cheilitis, not only *M. piperita*, but also *M. arvensis*, but not *M. spicata* or *cardica* extracts (all tested 1% pet.), as well as natural and synthetic menthol caused positive PT reactions (245).

Additional information:

ISO 4720:2009 nomenclature: Mentha x piperita L. A standard by ISO exists for Oil of peppermint (Mentha x piperita L.): ISO 856:2006. A review by the Cosmetic Ingredient Review Expert Panel, Washington, DC on the “Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water“ is available (163), stating that “Peppermint Oil is used at a concentration of < or = 3% in rinse-off formulations and < or = 0.2% in leave-on formulations. Peppermint Oil is composed primarily of menthol and menthone. Other possible constituents include pulegone, menthofuran, and limone. According to Surburg/Panten: (-)-menthol (34-46%), (-)-menthone (15-27%), (-)-menthyl acetate (2.5-7%) and menthofuran [17957-94-7] (0.5-6%) (34). According to (30) the maximum observed concentration in peppermint oil are (in %): (-)-menthol (49); (-)-menthone (28); (-)-menthyl acetate (8); mentofuran (8); isomenthone (8); neo menthol (6); pulegone (3.5); limonene (3.0); linalool (0.4) (30). Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. … Repeated

intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits ….” concluding that “with the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations”.

MENTHA SPICATA *HERB OIL*	*CAS* 84696-51-5, 8008-79-5; EC 283-656-2 (Spearmint, ext.)
Spearmint oil	*INCI: MENTHA VIRIDIS EXTRACT*

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to “spearmint oil (American)”, tested 2% in pet. (17). The CAS # quoted (8008-79-5) refers, according to CosIng, to MENTHA VIRIDIS LEAF OIL, the volatile oil obtained from the dried tops and leaves of the Garden Mint, Mentha viridis L., Labiatae. The Larsen 2001 study diagnosed 5.0% positive reactions in 178 patients with known contact allergy to fragrance ingredients, using this oil at 5% pet. test concentration (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to “Mentha viridis oil” 5% (13). PT results with toothpaste ingredients were positive in 7 patients, of whom 4 had strong positive reactions to spearmint (246).

Additional information:

ISO 4720:2009 nomenclature: Mentha spicata L. Mentha Spicata Oil is an essential oil obtained from the herbs of the Spearmint, Mentha spicata L., Labiatae (syn: Mentha viridis L., Labiatae). It contains carvone (more than 50%), limonene, pinene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40394, last accessed 2009-11-11). According to Surburg/Panten, the content is limonene (9-16.5%), (-)-carvone (60-70%), menthone (0-0.2%) and viridiflorol (0-0.5%) (34). Exposure by toothpastes, and subsequent contact allergic reaction of the lips or the oral mucosa, have been reported (e.g., (247, 248)). L-Carvone is a component of the oil from Mentha spicata (spearmint) (53) and had been tested with positive results in “toothpaste cases”, even at a concentration as low as 0.067% (68).

For Oil of spearmint -- Part 1: Native type (Mentha spicata L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearmint -- Part 2: Chinese type (80 % and 60 %) (Mentha viridis L. var. crispa Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearmint -- Part 3: Indian type (Mentha spicata L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearmint -- Part 4: Scotch variety (Mentha x gracilis Sole): ISO 3033-4:2005.

MYROXYLON PEREIRAE *RESIN*	CAS 8007-00-9; EC 232-352-8 (Balsams, Peru)
Balsam of Peru	INCI: MYROXYLON PEREIRAE / Balsams, Peru

Current regulation: Annex III, part1, n° 154

Clinical data:

This natural mixture has been employed as screening agent in Baseline series worldwide for many decades. Hence, a wealth of data is available; table 3.2 – 1 summarises results of the past 10 years.

Additional information:

ISO 4720:2009 nomenclature: *Myroxylon pereirae (Royle) Klotzsch, syn. Myroxylon balsamum var. pereirae (Royle) Harms. Myroxylon pereirae resin* (MPR, Balsamum peruvianium) is harvested from the balsam of Peru tree, *Myroxylon balsamicum* (L.) HARMS *var. pereirae* (ROYLE) HARMS, synonymous *Myroxylon pereirae* (ROYLE) KLOTZSCH (249) after thermal stress, almost exclusively in El Salvador. Main constituents of the pleasantly, vanilla-like smelling dark brown liquid are benzyl esters of cinnamic and benzoic acid (35 – 75%), up to 30% cinnamic acid, up to about 10% benzoic acid, approximately 5% alpha- and beta-nerolidol, benzyl alcohol and mostly less than 1% cinnamyl alcohol, benzyl ferulate and -isoferulate, cinnamic acid amyl ester, coniferyl alcohol, coniferyl benzoate, eugenol, isoeugenol, farnesol, vanillin, and several trace constituents (250-253). The composition of MPR varies with the origin and other factors; moreover, MPR is sometimes blended with other natural mixtures such as turpentine, styrax or colophonium (249).

MPR can be used to improve taste or smell in gargling solutions, cosmetic products such as soaps, shampoo or lipsticks, as well as sweets, tobacco and beverages (249, 254). According to EU legislation and IFRA guidelines MPR should not be used in products intended for skin contact; however, extracts and distillates of MPR may be used in a concentration of < 0,4% (IFRA-Guidelines, www.ifraorg.org (255)). E. Temesvári et al. report on the interesting case of severe ACD with subsequent hypopigmentation after a "temporary henna tattoo", which was, unexpectedly, not due to p-phenylene diamine, but to the oil used to disperse the pigment, which presumably contained allergens also included in the FM I and MPR, both of which were extreme positive on a later PT (256).

In addition to delayed type hypersensitivity reactions, MPR (and some of his constituents such as benzoic acid (257)) are capable of eliciting (non-immunological) urticarial immediate reactions (258-260). In one case, the immediate reaction to MPR (and to FM I) at the test site spread systemically in terms of a generalised urticaria, while no delayed type reactions were observed to the PT (261). Generally, there is apparently no association of immediate reactions to MPR (and cinnamal or cinnamyl alcohol) and contact sensitisation to these compounds (262). In animal experiments the sensitising potency of MPR was clearly established (250), with coniferyl benzoate identified as single compound with the most marked potency (252). However, due to the limited chemical stability of this compound is is unclear whether other, more stable compounds are, in fact, more important allergens, such as cinnamic acid and (iso-) ferulic acid esters or oxidised constituents of the resin fraction (263).

Table 3.2.2 – 1: Results with contact allergy to fragrance ingredients screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Myroxylon pereirae resin** (Balsam of Peru) 1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the reviewers.

Country	Population	Years	No. tested	Crude % positive (95% CI) [§]
Tel Aviv, Israel (264) #	Consecutive patients	1999-2000	943	6.6 % (5.1 – 8.4) [§]
South Korea (13)	Consecutive patients	04/2002 – 06/2003	422	7.3% (5.1 – 10:3%) [§]

Tel Aviv, Israel (265)	Consecutive patients	1998-2004	2156	3.6 % (2.9 – 4.5) §
Manipal, India (266)	Dermatitis patients	1989-1998	1780	n=17
Tehran, Iran (267)	Consecutive patients	2002-2004	250	2.4 % (0.9 – 5.2) §
Sevilla, Spain (268)	Consecutive patients	2002-2004	863	5.8 % (4.3 – 7.6) §
Ankara, Turkey (269)	Consecutive patients	1992-2004	1038	2.1 % (1.3 – 3.2) §
Vienna, Austria (22)	Consecutive patients of one clinic	1997-2000	2660	5.4% (4.6 – 6.3%) §
Czech Republic (270)	Consecutive patients	1997-2001	12058	7.3% (6.8 – 7.8) §
Copenhagen, Denmark (271)	Consecutive patients	1985-2007	16173	3.9 % (3.6 – 4.2) §
Sweden (272)	Consecutive patients	2000	3790	6.5%
9 European countries (273) $	Consecutive patients	2002-2003	9672	6.1 %
Germany, 3 Swiss + 1 Austrian Dept. (7)	Consecutive patients	2005-2008	36919	8.0% (7.7 – 8.3%)
10 depts. From 7 EU countries (274) *	Consecutive patients	1996-2000	26210	6.0 %
USA (Canada) (20)	Probably consecutive patients	2003	1603	6.6%
NACDG 2009 (21)	Consecutive patients	2005-2006	4449	11.9%

§ Calculated by reviewers, where possible (if actual numbers were given)

Probably included in (265)

$ > 5-fold difference between departments

* About 4-fold difference between departments

NARCISSUS SPP. *EXTRACT / OIL* *CAS: diverse*

Narcissus abs.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 1.3% positive reactions to "narcissus absolute" (2% pet.) were observed in 1606 consecutive (17). The extract used by the PT allergen provider Almirall/Hermal/Trolab has the CAS number 90064-25-8. The IVDK 2010 c study identified 0.5% positive reactions in 2445 consecutively tested patients and 0.6% positive reactions in 809 patients tested in the context of a special series (30).

Additional information:

Commonly used: *Narcissus poeticus* L. According to (30) the maximum observed concentration in Narcissus abs. are (in %): α-terpineol (23.7); trans-Isoeugenol methyl ether (20); benzyl benzoate (20); coumarin (5.7); benzyl alcohol (4.0); Δ^3-carene (3.4); cinnamyl alcohol (2.5); phenylethyl alcohol (2.2); ethyl palmitate (2.2); phenylpropyl acetate (1.7); 1,8-cineole (1.5); caryophyllene (1.0); benzyl acetate (0.7); isoeugenol (0.5); farnesol (0.3) (also according to (17)) (30).

OCIMUM BASILICUM *HERB OIL*

CAS 84775-71-3; EC 283-900-8 *(Ocimum basilicum, ext. = INCI)*

Basil Oil (sweet)

Current regulation: /

Clinical data:

/

Additional information:

ISO 4720:2009 nomenclature: *Ocimum basilicum* L. For Oil of basil, methyl chavicol type (Ocimum basilicum L.) an ISO standard exists: ISO 11043:1998. *Ocimum Basilicum* Herb Oil is an essential oil obtained from the herbs of the Sweet Basil, *Ocimum basilicum* L., *Labiatae*. (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40474, last accessed 2009-11-24). The chemical composition varies greatly with the origin (34):

- Basil oil of the methylchavicol type (Réunion type) is extracted from flowering tops or whole plants from Réunion, Comores, Madagascar, but also other countries such as Egypt. Mainly used for seasoning food. Content by GC: methylchavicol 75-87%, linalool 0.5-3%
- Basil oil, linalool type is produced mainly in the Mediterranean aera. Content by GC: Linalool 45-62%, methylchavicol trace to 30%, eugenol 2-15%
- Indian Basil oil is produced exclusively in India. Content by GC: methylchavicol trace to 70%, linalool 25%.

In a LLNA study by RIFM, the basil oil as used was reported to contain 51% linalool, 10.4% eugenol, 7.7% cineol, 3.7% bergamotene, 2.7% germacrene D, 2.7% cadinol and 1.3% cadinene, according to analyses of the supplier. The EC3 value was calculated to be < 2.5% (227).

PELARGONIUM GRAVEOLENS *FLOWER OIL*

CAS 90082-51-2; EC 290-140-0 *(Pelargonium graveolens, ext. = INCI) / 8000-46-2; EC / (Oils, geranium) INCI: GERANIUM*

Geranium Oil Bourbon

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "geranium oil Bourbon" (2% pet.) 7.4% positive reactions (9). In the Larsen 2001 study, 8.4% positive reactions were observed in 178 patients with known contact allergy to fragrance ingredients ("geranium oil Bourbon", 10% pet.) (19). The Goossens 1997 study found 3 of 111 patients positive to “geranium oil 20% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 3 positive reactions in 200 patients to “geranium” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “geranium” essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 3 “strong positive” reactions to “Geranium oil” (unknown test concentration) in 183 patients, Trattner/David 1 / 641 consecutive patients positive to “Geranium oil” (31). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to geranium oil bourbon (48).

Additional information:

ISO 4720:2009 nomenclature: *Pelargonium x ssp.* For Oil of geranium (Pelargonium X ssp.) an ISO standard exists: ISO 4731:2006 Pelargonium Graveolens Flower Oil is the volatile oil obtained from the flowers of the Bourbon Geranium, *Pelargonium graveolens* L. Hér. Ex Aiton, *P. roseum* Willdenow (and other nondefined hybrids that have developed in different regions of the world) Geraniaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details &id=57527, last accessed 2009-11-16)(34). The Bourbon type (Réunion, Madagascar) is more valuable than the North African and Chinese products, and differs in characteristic components: (-)-6,9-guaiadiene [36577-33-0] 5-9% in the Bourbon type, and 10-epi-gamma-eudesmol [15051-81-7] 3-6% in the African type, in addition to the main components (-)-citronellol, isomenthone, formates and tiglates. Chinese oil is similar to Bourbon oil, however, it contains more citronellol (32-43%) and lower amounts of linalool (2-4.5%) and geraniol (5-12%) (34).

In a LLNA study by RIFM, the geranium oil as used was reported to contain 41.1% citronellol, 9.8% 2,6-guiadine, 6.2% isomethone, 4.9% geraniol, 2.2% cis-rose oxide, 2.1% linalool, 1.5% geranyl formate, 1.3% phenyl ethyl tiglate, 1.0% trans-rose oxide, and geranyl tiglate and alpha-pinene at < 1%, according to analyses of the supplier. The EC3 value was calculated to be > 50% (227).

PELARGONIUM ROSEUM *LEAF OIL*

CAS 90082-55-6; EC 290-144-2 *(Pelargonium roseum, ext. = INCI)*

Geranium Oil; Rose Geranium Oil

Current regulation: /

Clinical data:

In the Sugiura 2000 study, among 1483 patients with suspected cosmetic dermatitis, 2.1% positive PT reactions to "geranium oil" (tested 20% in pet.) were observed (14).

Additional information:

Pelargonium Roseum Leaf Oil is an essential oil obtained from the leaves of the plant, Pelargonium roseum, Geraniaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40565, last accessed 2009-11-16.

PIMENTA RACEMOSA LEAF/FRUIT OIL CAS 85085-61-6; EC 285-385-5

Bay oil (34)

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Pimenta racemosa* (Mill.) J.W. Moore. For Oil of bay [Pimenta racemosa (Mill.) J.W. Moore] an ISO standard exists: ISO 3045:2004 Pimenta Racemosa Leaf/Fruit Oil is an essential oil obtained from the fruits of the plant, Pimenta racemosa, Myrtaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41014, last accessed 2010-02-10).

Steam distillation of the leaves of Pimenta racemosa (Mill.) J.W. Moore (Myrtaceae) yields bay oil, which consists of myrcene (20-30%), eugenol (42-56%) and chavicol (8-13%) (34).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

Pinus mugo *leaf and twig oil and extract* *CAS 90082-72-7, 8000-26-8; EC* 290-163-6

Dwarf pine needle oil (German: Latschenkiefernöl)

Current regulation: Annex III, part 1, 109

Clinical data:

In the Frosch 2002 b study, 0.7% positive reactions to dwarf pine needle oil (2% pet.) were observed in 1606 consecutive (17). The Rudzki 1976 study found 4 positive reactions in 200 patients to “Pine needle” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “pine needle” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Pinus mugo* Turra syn. *Pinus montana* Mill.) Pinus Mugo Twig Oil is an essential obtained from the twigs of the Pine, Pinus mugo, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41476&back=1, last accessed 2010-03-09). Pinus Mugo Twig Leaf Extract is an extract obtained from the twigs leaves of the Pine, Pinus mugo, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=searc

h.details&id=41473&back=1, last accessed 2010-03-09).

Dwarf pine needle oil is obtained from *Pinus mugo* Turra subsp. *mugo* and subsp. *pumilio* (Haenke) Franco (34). For Oil of dwarf pine (Pinus mugo Turra) an ISO standard exists: ISO 21093:2003. American pine oils contain almost no 3-carene or camphene (34).

PINUS PUMILA *TWIG LEAF EXTRACT / OIL*

CAS 97676-05-6; EC 307-681-6 *(Pine, Pinus pumila, ext. = INCI)*

Dwarf pine needle oil

Current regulation: Annex III, part 1, 114

Clinical data: /

Additional information:

Pinus Pumila Twig Leaf Extract obtained from the twigs leaves of the Pine, Pinus pumila, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41483&back=1, last accessed 2009-11-12), Pinus Pumila Twig Leaf Oil is the essential oil obtained from the twigs leaves of the Pine, Pinus pumila, Pinaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41484&back=1, last accessed 2009-11-12). Main constituents are alpha-pinene (60-70%) and beta-pinene (20-25%). (34) Occurrence from Siberia to Japan, classified as Endangered Species

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

POGOSTEMON CABLIN *OIL*

CAS 8014-09-3; EC / (Oils, patchouli) / 84238-39-1; EC 282-493-4 *(Patchouli, ext.)*

Patchouli oil

INCI: POGOSTEMON CABLIN / *Patchouli, ext.*

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% positive reactions to patchouli oil (10% pet.) in 1606 consecutive were observed (17). Nakayama et al. found 1974 (after (29)) 3 “strong positive” and 8 “weak positive” reactions to “Patchouli oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 0.6% positive reactions in 2446 consecutively tested patients and 1.4% positive reactions in 828 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Pogostemon cablin* (Blanco) Benth. syn. *Mentha cablin* Blanco. An ISO standard is available for Oil of patchouli (Pogostemon cablin (Blanco) Benth.): ISO 3757:2002. Pogostemon Cablin Leaf Oil is an essential oil obtained from the fermented leaves of the Patchouli, *Pogostemon cablin* (syn: *Pogostemon patchouli*),

Labiatae (Lamiaceae (34)). It contains patchouli alcohol, beta-patchoulene, azulene, eugenol, sesquiterpenes (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40927, last accessed 2009-11-12). Although the sesquiterpene alcohol (-)-patchoulol [5986-55-0] is the main component of patchouli oil (27-35%), the compound largely contributing to the characteristic odour is norpatchoulenol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-ß-patchoulene [514-51-2] (1.8-3.5%) and (-)-seychellene [20085-93-2] (1-3%) (34). According to (30) the maximum observed concentration in patchouli oil are (in %): (-)-patchoulol (35); (+)-alpha-lulnesene (21); (-)-alpha-guajene (16); β-pinene (6); (-)-ß-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulenol (1) (30).

It is a “top 100” substance (IFRA, pers. comm.2010).

ROSE FLOWER OIL (ROSA SPP.)	*CAS 8007-01-0; EC / (Oils, rose)*
ROSA ALBA FLOWER EXTRACT	*CAS 93334-48-6; EC* 297-122-1 *(Rose, Rosa alba, ext. = INCI)*
ROSA CANINA FLOWER OIL	*CAS 84696-47-9; EC* 283-652-0 *(Rose, Rosa canina, ext.) INCI: ROSA CANINA*
ROSA CENTIFOLIA FLOWER OIL	*CAS 84604-12-6, EC* 283-289-8 *(Rose, Rosa centifolia, ext.) INCI: ROSA CENTIFOLIA / Rose, Rosa centifolia, ext.*
ROSA DAMASCENA FLOWER OIL	*CAS 90106-38-0; EC* 290-260-3 *(Rose, Rosa Damascena, ext. = INCI)*
ROSA GALLICA FLOWER OIL	*CAS 84604-13-7; EC* 283-290-3 *(Rose, Rosa Gallica, ext.) INCI: ROSA GALLICA*
ROSA MOSCHATA OIL	*--*
ROSA RUGOSA FLOWER OIL	*CAS 92347-25-6; EC* 296-213-3 *(Rose, Rosa rugosa, ext.)*

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "rose oil Bulgaria" (2% pet.), yielding 0.4% positive reactions (14); Trattner/David found 2 / 641 consecutive patients positive to “Rose oil (Bulgarian)” (31). The Bulgarian rose oil usually corresponds to Rosa Damascena Flower Oil (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-16). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "rose Bulgarian oil" (2% pet.) 4.5% positive reactions (9). One case of contact allergy to “Bulgarian rose oil (2 % pet.)” – and geraniol – in a 48-year-old female with ACD after application of “Eau de Rochas” E.d.C. was diagnosed, among 326 patients with suspected contact allergy to fragrance ingredients had tested negative (275). However, other rose oils are also used (and capable of eliciting ACD) as illustrated by the case of a 27 year old woman who developed ACD after using “Rose Absolute Eau ® eau de

parfum", a "non-scented" body lotion and a number of other topicals. PTing revealed a number of (previously) relevant reaction, including "Rose centifolia" (5% alc.) and "Rose oil Bulgarian" (2% pet.) essential oil preparations (276). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Rose oil Bulgarian", tested at 2% concentration (13). Nakayama et al. found 1974 (after (29)) 4 "strong positive" reactions to "Rose oil Bulgarian" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with rose oil absolute, yielding 6 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: Rosa x damascena Mill. and Rosa sertata X Rosa rugosa. Rose Flower Oil is the volatile oil obtained from the flowers of Rosa spp. , rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details &id=59362, last accessed 2009-11-16). "Rose oil, meaning either rose otto (attar of rose, attar of roses) or rose absolute, is the essential oil extracted from the petals of various types of rose. Rose ottos are extracted through steam distillation, while rose absolutes are obtained through solvent extraction or supercritical carbon dioxide extraction, with the absolute being used more commonly in perfumery" (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-17) There are several more specifically named flower extracts used for masking or perfuming:

- Rosa Alba Flower Extract is an extract obtained from the flowers of the Rose, Rosa alba L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40969, last accessed 2009-11-16).
- Rosa Canina Flower Oil is the volatile oil obtained from the flowers of the Hip Rose, Rosa canina L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59263, last accessed 2009-11-16).
- Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of the Cabbage Rose, Rosa centifolia (L.), Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79757, last accessed 2009-11-16).
- Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of the Damask Rose, Rosa damascena, Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79760, last accessed 2009-11-16).
- Rosa Gallica Flower Oil is the volatile oil obtained from the flowers of the French Rose, Rosa gallica L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59346, last accessed 2009-11-16).
- Rosa Moschata Oil is the oil obtained from the Musk Rose, Rosa moschata, Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79761, last accessed 2009-11-16).
- Rosa Rugosa Flower Oil is the volatile oil obtained from the flowers of the Rose, Rosa rubiginosa L., Rosaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=83588, last accessed 2009-11-16).

Apparently, the Rosa Damascena and the Rosa centifolia are the species most commonly used for extraction of essential rose oils, the former mostly grown in Bulgaria, Turkey, Russia, India and China, the latter more commonly in Morocco, France and Egypt (276). Main constituents by GC are: citronellol (20-49%), geraniol (6-23%), nerol (3-12%) and phenylethyl alcohol (up to 3.5%) (34).

For Oil of rose (Rosa x damascena Miller) an ISO standard exists: ISO 9842:2003.

ROSMARINUS OFFICINALIS *FLOWER OIL*	*CAS 84604-14-8; EC* 283-291-9 *(Rosemary, ext.)*
"Rosemary Oil"	*INCI: ROSMARINUM OFFICINALIS / Rosemary, ext.*

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to “rosemary” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “rosemary” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Rosmarinus officinalis* L. Rosmarinus Officinalis Flower Oil is an essential oil obtained from the leaves and fresh flowering tops of the Rosemary, *Rosmarinus officinalis* L., Lamiaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40978, last accessed 2010-01-29). Major constituents are: 1,8-cineole (17-55%), alpha-pinene (9-26%), camphor (5-22%) and verbenone [18309-32-5] as traces in North African oils, but between 0.7 and 2.5% in Spanish oils (34). For Oil of rosemary (Rosmarinus officinalis L.) an ISO standard exists: ISO 1342:2000.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SALVIA spp. *HERB OIL*

Sage oil

SALVIA OFFICINALIS LAVANDULIFOLIA *HERB OIL*	*CAS 97952-71-1; EC* 308-365-0 *(Sage, Salvia officinalis lavandulifolia, ext. = INCI)*
SALVIA LAVANDULIFOLIA *HERB OIL*	*CAS 90106-49-3; EC* 290-272-9 *(Sage, Salvia lavandulifolia, ext. = INCI)*
SALVIA SCLAREA *FLOWER OIL*	*CAS 84775-83-7; EC* 283-911-8 *(Sage, Salvia sclarea, ext.) INCI: SALVIA SCLAREA / Sage, Salvia sclarea, ext.*
SALVIA HISPANICA *HERB OIL*	*CAS 93384-40-8; EC* 297-250-8 *(Sage, Salvia hispanica, ext. = INCI)*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Clary sage”, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to “clary sage” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Salvia officinalis* L. *Salvia Officinalis Lavandulifolia* Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia officinalis* L. *spp. lavandulifolia*, *Lamiaceae*, Syn. Dalmatian sage (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41084, last accessed 2010-01-29).

Salvia Lavandulifolia Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia lavandulifolia*, *Lamiaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40987, last accessed 2010-01-29).

Salvia Sclarea Flower Oil is an essential oil obtained from the flowers and foliage of the Clary Sage, *Salvia sclarea* L., *Lamiaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41086, last accessed 2010-01-29).

Salvia Hispanica Herb Oil is an essential oil obtained from the herbs of the Spanish Sage, *Salvia hispanica* L., *Lamiaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40985, last accessed 2010-01-29).

Clary sage oil is obtained by steam distillation of flowering tops and foliage of cultivated *Salvia sclarea* L. (Lamiaceae). Main constituents are linalyl acetate (56-78%) and linalool (6.5-24%) (34). Dalmatian sage oil is steam distilled from partially dried leaves of S. officinalis L. (Lamiaceae). The content by GC is: alpha-thujone (18-43%), beta-thujone (3-8.5%), 1,8-cineole (5.5-13%), camphor (3-8.5%) as main constituents (34). Spanish sage oil does not contain thujone, but mainly camphor (15-36%) and 1,8-cineole (11-30%), and is used mainly in pharmaceutical preparations and technical perfumery (34). For Oil of sage, Spanish (*Salvia lavandulifolia* Vahl) an ISO standard exists: ISO 3526:2005, for Oil of Dalmatian sage (Salvia officinalis L.): ISO 9909:1997.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SANTALUM ALBUM *WOOD OIL*

CAS 84787-70-2; EC 284-111-1 *(Sandalwood, ext.) INCI: SANTALUM ALBUM / Sandalwood, ext.*

Sandalwood oil ([East] India)

SANTALUM ALBUM *OIL*

CAS 8006-87-9; EC / (Oils, sandalwood)

Sandalwood oil ([East] India)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "sandalwood oil" (2% pet.), yielding 0.8% positive reactions (14). In the Frosch 2002 b study, “sandalwood oil (East India)” is mentioned with a CAS # 8015-65-4, which, however, is attributed to AMYRIS BALSAMIFERA BARK OIL, see above. Assuming that this CAS # is erroneous, study results are considered to be valid for S. album wood oil, tested at 2% and 10% concentration, yielding 0.4% and 0.9% positive reactions,

respectively (17). Out of 6 of 15 patients with a positive reaction to the higher concentration no clinical relevance was found, compared to 2 of 7 patients positive to the lower concentration (17). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "sandalwood oil" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 10 of 422 consecutive patients, i.e., 2.4%, had positive reactions to “Santalum album oil” 2% (13). The Goossens 1997 study found 4 of 111 patients positive to “sandalwood oil 10% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to “sandalwood”, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “sandalwood” essential oil 2% pet. (27). In 63 patients positive to the FM I, 1 had a positive PT reaction to sandalwood oil, 2% pet., in the Santucci 1987 study (28). Nakayama et al. found 1974 (after (29)) 6 “strong positive” and 8 “weak positive” reactions to “Sandalwood oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.3% positive reactions in 3671 consecutively tested patients and 1.8% positive reactions in 1002 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with sandalwood oil, yielding 2 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Santalum album* L. *Santalum Album* Oil is the volatile oil obtained from the heartwood of the Sandalwood, *Santalum album* L., Santalaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=80209, last accessed 2009-11-26).

Santalum Album Wood Oil is an essential oil obtained from the wood of the Sandalwood, *Santalum album* L., *Santalaceae*. It contains 75% santalol isomers (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41092, last accessed 2009-11-12), typically up to 55% .alpha.-santalol and up to 24% .beta.-santalol (30). East Indian sandalwood oil consists almost exclusively of closely related sesquiterpenoids; by far the main constituents are the alcohols alpha-santalol [115-71-9] (41-55%) and cis-beta-santalol [77-42-9] (16-24%), the latter being mainly responsible for the specific odour (34, 39).

An ISO standard regarding the composition of “*Santalum album* oil” is available: ISO 3518:2002. “Sandalwoods” are labelled as Amyris balsamifera, Eremophila mitchelli, Fusanus acuminatus (= Santalum acuminatum), Santalum album, S. austrocaledonicum, S. latifolium, S. spicatum and S. yasi. The majority of currently available trade oils, reportedly from S. album, contained approximately 50-70% santalols (Z-alpha and Z-beta), as analysed with gas chromatography-mass spectrometry (GC-MS) (277). A review on the toxicological properties of “Santalum album oil” is available (278).

AMYRIS BALSAMIFERA *BARK OIL (Sandalwood oil (Carribean)), CAS 8015-65-4; EC / (Oils, amyris) / 90320-49-3; EC* 90320-49-3 *(Amyris balsamifera, ext. = INCI name)* is used as a cheap substitute for East Indian Sandalwood in perfumes and cosmetics. Originally cultivated primarily in Haiti where it was known as 'candle wood' and used as a torch by locals due to the tree's high oil content (http://www.amphora-retail.com/sandalwood-amyris-essential-10ml-p-107.html, last accessed 2009-11-12). The major components are sesquiterpenoids such as valerianol, elemol, ß-eudesmol and epi-gamma-eudesmol (39). For Oil of amyris (Amyris balsamifera L.) an ISO standard exists: ISO 3525:2008. Amyris Balsamifera Bark Oil is the volatile oil distilled from the bark of the tree, Amyris balsamifera, Rutaceae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=74455, last accessed 2009-11-12).

SANTALUM SPICATA *WOOD OIL* *CAS 8024-35-9; EC* 296-618-5 *(Sandalwood oil, Western Australia)*

Sandalwood oil (Australia)

Current regulation: /

Clinical data:

In clinical studies, mostly S. album wood oil had been used (see above); in a number of studies this is not clear.

Additional information:

ISO 4720:2009 nomenclature: *Santalum spicatum* (R.Br.) A. DC, syn. *Eucarya spicata* (R.Br.) Sprag & Summ. For Oil of Australian sandalwood (*Santalum spicatum* (R.Br.) A.DC.) an ISO standard exists: ISO 22769:2009. Santalum Spicata Wood Oil is an essential oil obtained from the wood of the Australian Sandalwood, Santalum spicata, Santalaceae. It contains 75% santalols and 10% farnesol (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41093, last accessed 2009-11-12). This oil also contains santalols as main constituents but differs somewhat in the remaining composition. Today, it makes up a considerable part of the sandalwood oil market (34).

Considering the content of well-known allergenic compounds (santalols), this essential oil is regarded as established contact allergen in humans.

TAGETES PATULA *FLOWER OIL* *CAS 91722-29-1; EC* 294-431-3 *(Tagetes patula, ext. = INCI)*

"Marigold Oil; Tagetes Oil"

Current regulation: /

Clinical data:

In an aromatherapist, an essential oil solvent-extracted from *Tagetes patula*, patch tested at 1.5% in grapeseed oil (vehicle negative, 7 controls negative to essential oils) resulted in a +++ reaction, in accordance with a work-related bilateral hand dermatitis (217).

Additional information:

Tagetes Patula Flower Oil is an essential oil obtained by hydrodistillation of the flowers of the *Tagetes*, *Tagetes patula L., Compositae*. It contains mainly D-limonene, ocimene, 2,6-dimethyloct-7-en-4-one (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41506, last accessed 2010-01-28). According to Surburg/Panten, tagetes oil is steam distilled from the flowering plants of Tagetes minuta L. (T. glandulifera Schrank., Asteraceae). Main components comprise cis-ocimene, dihydrotagetone, tagetone, and cis- and trans-ocimenone (34, 39).

THYMUS spp. *HERB OIL*

THYMUS VULGARIS *HERB OIL*

CAS 84929-51-1, 8007-46-3; EC 284-535-7 *(Thyme, Thymus vulgaris, ext.)*

"Thyme oil"

INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to “thyme” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to “thyme” essential oil 2% pet. (27). In 63 patients positive to the FM I, none had a positive PT reaction to thymol, 1% pet., in the Santucci 1987 study (28).

Additional information:

ISO 4720:2009 nomenclature: *Thymus vulgaris* L. *Thymus vulgaris* Herb Oil is an essential oil obtained from the herbs of the Thyme, Thymus vulgaris L., Lamiaceae. It contains 20-40% thymol and carvacrol, cymene, pinene, linalool, bornyl acetate (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41133, last accessed 2010-01-29).

Other species are used for extraction, e.g., Thymus Mastichina (CAS 84837-14-9), Thymus Serpillum (CAS 84776-98-7), Thymus Zygis (CAS 85085-75-2), according to CosIng. The main constituent is thymol (37-56%) (34). For Oil of thyme containing thymol, Spanish type [Thymus zygis (Loefl.) L.] an ISO standard exists: ISO 14715:2010, for Oil of Spanish wild marjoram (Thymus mastichina L.): ISO 4728:2003.

TURPENTINE *(oil)*

CAS 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / -

Current regulation: III/124 ; III/125 ; III/126

Clinical data:

Oil of turpentine has been patch tested in a number of baseline series, i.e., in consecutive patients, although not included in the European Baseline series.

In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to “Indonesian oil of turpentine” and 8 to alpha-pinene (190)

Table 3.2.2 – 2: Overview of results with **Oil of turpentine** in patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS.

Country	Population	Years	No. tested	Crude % positive (95% CI) [§]

Lisbon, Portugal (189); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 % (1.9 – 2.8) §
Birmingham, UK (190)	Potters with occup. hand dermatitis	6 months; prior to 1996	24	14 / 24 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (279)	Consecutive patients	1992-1995	27658	0.47 % (0.39 – 0.55) §
Austria/Germany (IVDK) (280)	Consecutive patients	1996-2002	59478	Annual prevalences 1.6 to 4.4 %
Augsburg/Germany (281)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (273)	Consecutive patients	2002/03	3767	1.6 %
Austria/Germany/ Switzerland (IVDK) (7)	Consecutive patients	2005-2008	37163	1.8 %

Additional information:

ISO 4720:2009 nomenclature: *Pinus pinaster* Aiton and *Pinus massoniana* Lamb. Turpentine, oil: Any of the volatile predominately terpenic fractions or distillates resulting from the solvent extraction of, gum collection from, or pulping of softwoods. Turpentine is a mixture of terpene hydrocarbons obtained from various species of Pinus http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=41521

The composition of oil of turpentine varies with its origin, in particular, the content of .delta.-3-carene, one of its main allergenic compounds (189, 279). Similarly, the peroxide degree may vary. The main constituents are .alpha.-pinene (50-72%), .beta.-pinene (6-15%), carenes (< 0.1-17%), camphene (up to 1%), dipentene (0.5-5%), along with a number of other substances (279).

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

Verbena absolute (Lippia citriodora Kunth.) *CAS 8024-12-2, 84961-67-1; EC /)*

Current regulation: Annex III, part 1, n° 206

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Aloysia citriodora* Palau syn. *Lippia citriodora* Kunth syn. *Aloysia triphylla* (L' Hér.) Kuntze. An older RIFM review is available citing several positive human maximisation studies both with "Verbena absolute" and "Verbena oil" (128).

VETIVERIA ZIZANOIDES *ROOT OIL*

CAS 8016-96-4; EC / (Oils, vetiver) / 84238-29-9; EC 282-490-8 *(*Vetiveria zizanioides, ext. = INCI*)*

"Vetiver oil; khas khas oil"

Current regulation: …

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “vetiver” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=9 (10.4%) positive reactions to “vetiver” essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Vetiveria zizanioides* (L.) Nash. Vetiveria Zizanoides Root Oil is an essential oil distilled from the dried roots of the grass *Vetiveria zizanoides* (L.) Nash *Poaceae* (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41293, last accessed 2010-01-29). Vetiver oil has a high sesquiterpene content. The ketones alpha-vetivone [15764-04-2] (6-12%) and beta-vetivone [18444-79-6] (4-10%), which usually form more than 10% of the oil, as well as khusimol [16223-63-5] (24-36%) and isovelencenol [22387-74-2] (12-24%) are the main constituents (in Bourbon oil, i.e., from Réunion) (34). For Oil of vetiver (Vetiveria zizanioides (L.) Nash) an ISO standard exists: ISO 4716:2002.

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Annex II - Animal Data

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Substance		Vehicle (AOO=acetone:olive oil; DEP=diethyl phthalate; DMF=dimethyl formamide; DMSO=dimethyl sulphoxide; EtOH=ethanol; MEK=methyl ethyl ketone)	Conc. in vehicle (%, generally w/v)	No. animals per dose group	EC3 value *				Comment (deviation from OECD 429 etc)	Reference
INCI name *(other name)*	CAS no.				%	µg/cm²	M	lowest for the substance (%)		
Allyl phenoxyacetate	7493-74-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	3.1	775	0.16	3.1		RIFM, 2007a
Amyl cinnamal	122-40-7	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	7.6	1900	0.38	7.6		RIFM, 2006a
Amyl cinnamal	122-40-7	4:1 AOO	-	4	10.6	2650	0.52		Elahi gives ref to Basketter et al 1999, but no data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment	Elahi et al., 2004

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Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Aptula gives ref to Kimber et al 2003, but no LLNA data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment; original reference is not given.	Aptula et al., 2007
Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Original ref not given.	Roberts et al., 2007
Amylcinnamyl alcohol	101-85-9	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	> 25	>6250	>1.22	> 25	Should have been tested at higher concentrations	RIFM, 2004a
Anise alcohol	105-13-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.9	1475	0.43	5.9		RIFM, 2005a
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref	Roberts et al., 2007
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Benzyl alcohol	100-51-6	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>4.62	> 50	Should have been tested at higher concentrations	RIFM, 2005b

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Benzyl benzoate	120-51-4	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>2.36	> 50	Should have been tested at higher concentrations	RIFM, 2005c
Benzyl cinnamate	103-41-3	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	18.4	4600	0.77	18.4		RIFM, 2005d
Benzyl salicylate	118-58-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.9	725	0.13	2.9		RIFM, 2005e
p-tert-Butyl-dihydrocinnamaldehyde	18127-01-0	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	4.3	1075	0.23	4.3		RIFM, 2007b
Butylphenyl methylpropional (BMHCA)	80-54-6	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	2.9	725	0.14	2.9		RIFM, 2001a
Butylphenyl methylpropional (BMHCA)	80-54-6	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	4.1	1025	0.20			RIFM, 2001b
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	13.9	3475	0.68			RIFM, 2001c
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 DEP:EtOH	0.3, 1.0, 3.0, 10.0, 30.0	4	8.8	2200	0.43			RIFM, 2001d
Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	16.8	4200	0.82			RIFM, 2001e

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Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1, 2.5, 10, 25, 50	4	18.7	4675	0.92			Basketter et al., 2001
Camellia sinensis leaf *Tea Leaf Absolute*	84650-60-2	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	> 5.0	>1250	N/a	> 5.0	Should have been tested at higher concentrations	RIFM, 2005m
Cananga odorata leaf / flower oil *Ylang Ylang Extra*	8006-81-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	6.8	1700	N/a	6.8		RIFM, 2007f
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	10.7	2675	0.71			RIFM, 2007c
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.7	1425	0.38	5.7		RIFM, 2007d
Carvone	6485-40-1	4:1 AOO	6.0, 12, 20	4	13	3250	0.86			Nilsson et al., 2005
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015	0.2		RIFM, 2003a
Cinnamal	104-55-2	0.1% α-tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015			RIFM, 2003b
Cinnamal	104-55-2	2.0% α-tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.6	150	0.045			RIFM, 2003c
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053			RIFM, 2003d

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

		EtOH:DEP							
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003e
Cinnamal	104-55-2	2.0% α-tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.8	200	0.060		RIFM, 2003f
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.9	225	0.068		RIFM, 2003g
Cinnamal	104-55-2	0.1% α-tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.1	275	0.083		RIFM, 2003h
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.3	325	0.098		RIFM, 2003i
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.4	350	0.11		RIFM, 2003j
Cinnamal	104-55-2	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamal	104-55-2	4:1 AOO	0.5, 1, 2.5, 5, 10	4	3.1	775	0.23		Basketter et al., 2001
Cinnamal	104-55-2	4:1 AOO	-	4	1.3	325	0.10		Elahi et al., 2004
Cinnamal	104-55-2	4:1 AOO	1, 2.5	-	1.4	348	0.11	Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Cinnamal	104-55-2	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	1.7	425	0.13		Wright et al., 1995

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Cinnamal	104-55-2	MEK	1.0, 2.5, 5.0, 10.0, 25.0	4	1.1	275	0.083			Wright et al., 1996
Cinnamal	104-55-2	DMF	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.5	125	0.038			Wright et al., 1997
Cinnamal	104-55-2	propylene glycol	1.0, 2.5, 5.0, 10.0, 25.0	4	1.4	350	0.11			Wright et al., 1998
Cinnamal	104-55-2	DMSO	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.9	225	0.068			Wright et al., 1999
Cinnamal	104-55-2	90:10 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.6	400	0.12			Wright et al., 2000
Cinnamal	104-55-2	50:50 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.2	300	0.091			Wright et al., 2001
Cinnamyl alcohol	104-54-1	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamyl nitrile	1885-38-7	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 10	>2500	>0.77	> 10	Report: systemic toxicity at 25% and 50%. Should have been tested at higher concentrations	RIFM, 2005f
Citral	5392-40-5	1:3 EtOH:DEP	0.4, 2.0, 4.0, 8.0, 20.0	4	1.2	300	0.079	1.2		RIFM, 2004b
Citral	5392-40-5	0.1% α-tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	1.5	375	0.099			RIFM, 2003k

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Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	2.1	525	0.14			RIFM, 2003l
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	3.7	925	0.24			RIFM, 2003m
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30			RIFM, 2003n
Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30			RIFM, 2003o
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.3	1325	0.35			RIFM, 2003p
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.8	1400	0.38			RIFM, 2003q
Citral	5392-40-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	6.3	1575	0.41			RIFM, 2003r
Citral	5392-40-5	0.1% α-tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	6.8	1700	0.44			RIFM, 2003s
Citral	5392-40-5	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2002
Citronellol	106-22-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	43.5	10875	2.78	43.5		RIFM, 2004c

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Coumarin	91-64-5	DMF	10, 25, 50	4	>50	>12500	>3.42	>50	Should have been tested at higher concentrations	Vocanson et al., 2006
Dibenzyl ether	103-50-4	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	6.3	1575	0.32	6.3		RIFM, 2007e
Eugenol	97-53-0	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	5.3	1325	0.32	5.3		RIFM, 2001f
Eugenol	97-53-0	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	10.5	2625	0.64			RIFM, 2001g
Eugenol	97-53-0	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	10.7	2675	0.65			RIFM, 2001h
Eugenol	97-53-0	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	15.1	3775	0.92			RIFM, 2001i
Eugenol	97-53-0	4:1 AOO	2.5, 5.0, 10.0, 25.0, 50.0	-	11.9	2975	0.72			Basketter et al., 1999
Eugenol	97-53-0	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Evernia furfuracea extract *Treemoss absolute*	90028-67-4	1:3 EtOH:DEP	5.0, 10.0, 20	4	> 20	>5000	N/a	> 20	Should have been tested at higher concentrations	RIFM, 2004k
Evernia furfuracea extract *Treemoss absolute*	90028-67-4	1:3 EtOH:DEP	10.0, 25.0	4	> 25	>6250	N/a		Too few concentrations tested	RIFM, 2004d

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Evernia prunastri extract *Oakmoss*	90028-68-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.88	970	N/a	3.88		RIFM, 2004j
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	5.5	1375	0.25		Should also have been tested at lower concentrations	RIFM, 2004d
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	4.1	1025	0.18	4.1	Should also have been tested at lower concentrations	RIFM, 2004d
Geraniol	106-24-1	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	5.6	1400	0.36	5.6		RIFM, 2001j
Geraniol	106-24-1	3:1 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	11.4	2850	0.74			RIFM, 2003t
Geraniol	106-24-1	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	11.8	2950	0.76			RIFM, 2001k
Geraniol	106-24-1	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	20.4	5100	1.32			RIFM, 2001l
Geraniol	106-24-1	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	25.8	6450	1.67			RIFM, 2001m
Geraniol	106-24-1	-	-	-	26	6500	1.69			Roberts et al., 2007
trans-2-Hexenal	6728-26-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5, 10	4	2.6	650	0.26	2.6		RIFM, 2005g
trans-2-Hexenal	6728-26-3	-	-	-	5.5	1375	0.56			Roberts et al., 2007

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Hexyl cinnamal	101-86-0	generally 4:1 AOO		-O162	5.3-14.7	1325-3675	0.25-0.68	5.3		"numerous accounts in the literature"
2-Hexylidene cyclopentanone	17373-89-6	1:3 EtOH:DEP	0.1, 0.5, 1.0, 2.5, 5.0	5	2.4	600	0.14	2.4		RIFM, 2008a
Hexyl salicylate	6259-76-3	1:3 EtOH:DEP	0.05, 0.25, 0.5, 1.0, 2.5	4	0.18	45	0.008	0.18		RIFM, 2006b
Hydroxycitronellal	107-75-5	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.3	4825	1.12	19.3		RIFM, 2001n
Hydroxycitronellal	107-75-5	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.7	4925	1.14			RIFM, 2001o
Hydroxycitronellal	107-75-5	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	22.2	5550	1.29			RIFM, 2001p
Hydroxycitronellal	107-75-5	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	26.4	6600	1.53			RIFM, 2001q
Hydroxycitronellal	107-75-5	AOO	25, 50, 100	-	-	-	-		EC3 value not given	Ashby et al., 1995
Hydroxycitronellal	107-75-5	4:1 AOO	2.5, 5, 10, 25, 50	4	33.0	8250	1.92			Basketter et al., 2001
Hydroxycitronellal	107-75-5	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2002
Hydroxycitronellal	107-75-5	-	-	-	25.25	6313	1.47			Estrada et al., 2003

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Hydroxycitronellal	107-75-5	4:1 AOO	10, 25	-	23	5750	1.34		Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	4:1 AOO	1.0, 2.5, 5, 10, 25, 50	4	17.1	4275	0.81	17.1		RIFM, 2001r
p-Isobutyl-α-methyl hydrocinnamaldehdye	6658-48-6	70% EtOH	10.0, 25.0, 50.0, 100.0	4	9.5	2375	0.46	9.5	Should also have been tested at lower concentrations	RIFM, 2001w
Isocyclocitral	1335-66-6	1:3 EtOH:DEP	0.5 , 1.0 , 2.5 , 5.0, 10.0	4	7.3	1825	0.48	7.3		RIFM, 2006c
Isocyclogeraniol	68527-77-5	1:3 EtOH:DEP	5.0, 10.0, 25.0, 50.0	4	> 25	>6250	>1.62	> 25	Should have been tested at higher concentrations	RIFM, 2005h
Isoeugenol	97-54-1	4:1 AOO	0.5, 5.0	6	0.54	145	0.033	0.54	Too few concentrations tested	RIFM, 2001s
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.6	150	0.037			RIFM, 2002a
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.76	191	0.046			RIFM, 2002b
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.79	199	0.048			RIFM, 2002c

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Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.19	296	0.072		RIFM, 2001t
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.28	320	0.078		RIFM, 2004e
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	6	1.54	385	0.094		RIFM, 2001u
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.95	488	0.119		RIFM, 2001v
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0		3.3	825	0.20		Basketter et al., 1999
Isoeugenol	97-54-1	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.3	325	0.079		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.3	825	0.20		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.8	450	0.11		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.1	775	0.19		Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.6	400	0.097		Loveless et al., 1996
Isoeugenol	97-54-1	AOO	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061		Wright et al., 2001
Isoeugenol	97-54-1	MEK	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061		Wright et al., 2001
Isoeugenol	97-54-1	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	1.4	350	0.085		Wright et al., 2001

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Isoeugenol	97-54-1	propylene glycol	0.5, 1.0, 2.5, 5.0, 10.0	4	2.5	625	0.15			Wright et al., 2001
Isoeugenol	97-54-1	DMSO	0.5, 1.0, 2.5, 5.0, 10.0	4	0.9	225	0.055			Wright et al., 2001
Isoeugenol	97-54-1	90:10 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	1.8	450	0.11			Wright et al., 2001
Isoeugenol	97-54-1	50:50 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	4.9	1225	0.30			Wright et al., 2001
Jasmine absolute (Grandiflorum)	8022-96-6	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	5.9	1475	N/a	5.9		RIFM, 2006d
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	35.4	9100	N/a	35.4		RIFM, 2006e
*d-Limonene***	5989-27-5	EtOH	10.0, 20.0, 50.0, 75.0, 100.0	4	< 10	< 250	<0.73	< 10	Should also have been tested at lower concentrations	RIFM, 2004l
*d-Limonene***	5989-27-5	3:1 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	22.0	5500	1.61			RIFM, 2004m
*d-Limonene***	5989-27-5	1:3 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	38.0	9500	2.79			RIFM, 2004n
*d-Limonene***	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
*d-Limonene***	5989-27-5	4:1 AOO	25, 50, 100	4	68.5	17125	5.03			Warbrick et al., 2001

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Linalool**	78-70-6	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2002
Menthadiene-7-methyl formate	68683-20-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 10	> 2500	>0.51	> 10	Should have been tested at higher concentrations	RIFM, 2008c
4-Methoxy-α-methyl benzenpropanal	5462-06-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	23.6	5900	1.32	23.63		RIFM, 2004f
α-Methyl cinnamic aldehyde	101-39-3	-	-	-	4.5	1125	0.31	4.5		Roberts et al., 2007
Methylenedioxyphenyl methylpropanal	1205-17-0	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	16.4	4100	0.85	16.4		RIFM, 2005i
6-Methyl-3,5-heptadien-2-one	1604-28-0	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 5	> 1250	>0.40	> 5	Should have been tested at higher concentrations	RIFM, 2008d
α-iso-Methylionone	127-51-5	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	21.8	5450	1.06	21.8		RIFM, 2005j
Methyl octine carbonate	111-80-8	-	-	-	2.5	635	0.15	2.5		Roberts et al., 2007

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Methyl 2-octynoate	111-12-6	1:3 EtOH:DEP	0.5, 1.0, 2.0, 5.0, 10.0	4	< 0.5	< 125	<0.032	< 0.5	Should also have been tested at lower concentrations	RIFM, 2005k
2-Methoxy-4-methylphenol	93-51-6	-	-	-	5.8	1450	0.42	5.8		Roberts et al., 2007
1-Octen-3-yl acetate	2442-10-6	1:3 EtOH:DEP	7.5, 15.0, 30.0	5	> 30	> 7500	>1.76	> 30	Should have been tested at higher concentrations	RIFM, 2004g
Perillaldehyde *p-Mentha-1,8-dien-7-al*	2111-75-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	9.3	2325	0.62			RIFM, 2008b
Perillaldehyde *p-Mentha-1,8-dien-7-al*	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (Myroxylon pereirae Klotzsch)	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.95	987	N/a	3.95		RIFM, 2004h
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.5	625	N/a	2.5		RIFM, 2004i
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	0.5, 1.0, 2.5	4	>2.5	>625	N/a			RIFM, 2004i
Phenylacetaldehyde	122-78-1	4:1 AOO	2.5, 5, 10, 25, 50	4	3.0	750	0.25	3.0		Basketter et al., 2001

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Phenylacetaldehyde	122-78-1	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
3-Propylidenephthalide	17369-59-4	4:1 AOO	5, 10, 20	4 or 5	3.7	925	0.21	3.7	Should also have been tested at lower concentrations	Gerberick et al., 2004
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	25.14	6285	1.07	25.14		RIFM, 2005l
Trimethylbenzenepropanol *Majantol*	103694-68-4	4:1 AOO	3.0, 10.0, 30.0	4	~30	~7500	~1.68	30	Should have been tested at higher concentrations	RIFM, 2002d
Vanillin	121-33-5	4:1 AOO	2.5, 5, 10, 25, 50	4	>50.0	>1250	>3.3	>50.0		Basketter et al., 2001

* source of EC3 value value: % given in the RIFM report or references; µg/cm2 given in the RIFM report and RIFM poster; M calculated by SCCS working group

**material with low levels of oxidation according to RIFM, 2009

- = no data given; A216

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Annex III - Tabular summary of dose-elicitation studies in sensitised patients

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Chloroatranol

Chloroatranol (allergen in oak moss absolute: *Evernia prunastri*) (1)	
Design	blinded, randomised with regard to doses and controlled
Test subjects	13 patients previously identified as sensitized to chloroatranol and oak moss absolute
Controls	10 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	200 ppm to 0.0063 ppm (10 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction
ROAT	volar aspect of forearms
area	3 x 3 cm^2
applications/day	two
dose	chloroatranol in ethanol: Step 1: 5 ppm Step 2: 25 ppm
dose/application/cm^2	step 1: 0.025 µg step2: 0.125 µg
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step
Results	
PT ED10% (95% CI)	0.013 (0.002-0.03) ppm =0.0004 µg/cm^2
PT ED50% (95% CI)	0.15 (0.077-0.295) ppm =0.0045 µg/cm^2
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (5 ppm)	12/13 (92%)
Step 2 (25 ppm)	13/13 (100%)
Controls	Negative
Other information	None relevant

In a subsequent study chloroatranol and atranol, both ingredients in *Evernia prunastri*, were tested in equimolar concentrations in serial dilution in 10 eczema patients with known sensitization to chloroatranol and oak moss. A positive response was defined as any degree of reaction. Ethanol was included as the control and gave no response. No use tests were done and no control subjects included.

Results: All patients reacted to the highest concentrations of the two substances. For both substances there was a significant dose-dependence and the estimated difference in elicitation potency of chloroatranol relative to atranol was 217%. The dose-response curve is seen in figure 1 below (2).

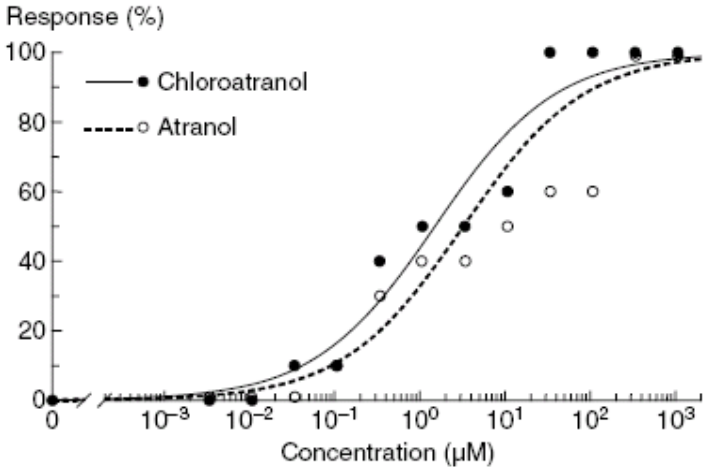


Fig. 1. Observed response rates and fitted parallel logistic dose–response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

Cinnamal

Cinnamal (3)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to cinnamal and additional 4 with a doubtful response
Controls	20 healthy controls
Substance	Purity: >98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (7 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of responses
ROAT	outer aspect of upper arm
area	5 x 5 cm^2
applications/day	two with atomizer pump
dose	Step 1: 0.02% Step 2: 0.1% Step 3: 0.8%
dose/application/cm^2	Not given
control substance	ethanol
definition of positive	The response was classified as positive no matter the degree of reaction.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.24% = 96 µg/cm^2 (calculated from the data in the paper)
PT no effect level(observed)	0.01 % in pet. = 0.4 µg/cm^2
ROAT	Cumulative responses
Step 1 (0.02%)	0/18
Step 2 (0.1%)	8/18 (44 %)
Step 3 (0.8%)	13/18 (72 %)
Controls	No eczema reactions were seen
Other information	2 patients and 2 controls developed immediate reactions to the cinnamal solution

Cinnamal *(4)*	
Design	blinded, randomised doses and controlled
Test subjects	17 patients with a positive patch test to cinnamal (8 patients in part 1 and 9 in part two)
Controls	20 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2 % to 0.00006 % (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting a + reaction
ROAT	Axilla
area	10 x 10 cm^2 (estimated)
applications/day	two with roll on deodorant (89-700 mg per application of solution) average cases: 263 mg/application controls: only range given
dose	Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32% Part two: Step 1: 0.01% Step 2: 0.032% Step 3: 0.1%
dose/application/cm^2	Part two estimated: step one: 0.26 µg; step two: 0.84 µg; 2.63 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	0.002%
ROAT	Cumulative responses
Step 1 (0.01)	2/9 (22%)
Step 2 (0.032)	6/9 (67%)
Step 3 (0.1)	8/9 (88%)
Controls	No reactions were seen
Other information	Only reactions seen to the cinnamal-containing deodorants at ROAT, difference to matrix axilla ($p<0.001$) and all control

	persons negative ($p<0.001$)

Hydroxycitronellal

Hydroxycitronellal *(5)*	
Design	blinded, randomised doses and controlled
Test subjects	7 patients with a positive patch test to hydroxycitronellal
Controls	7 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	4% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting + reaction
ROAT	Axilla
area	10 x 10 cm^2 (estimated)
applications/day	two with roll on deodorant (172-591 per application of solution) average cases: 294 mg/application controls: only range given
dose	Step 1: 0.032% Step 2: 0.1% Step: 0.32%
dose/application/cm^2	Estimated: step 1: 0.94 µg; step 2: 2.94 µg; step 3: 9.40 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	<0.00012 %
ROAT	Cumulative responses
Step 1 (0.032)	4/7 (57%)
Step 2 (0.1)	5/7 (71%)
Step 3 (0.32)	7/7 (100%)
Controls	No reactions were seen
Other information	Reactions were only seen to the hydroxycitronellal-containing deodorant at ROAT, difference to matrix treated axilla (p<0.001) and all control persons negative (p<0.001)

Hydroxycitronellal (6)	
Design	double blinded, randomised
Test subjects	13 patients with a positive patch test to hydroxycitronellal
Controls	/
Substance	purity: unknown
Patch test	confirmatory
-dilution steps	
-control/vehicle	
-definition of threshold	
ROAT	finger immersion in fragrance solution in 10% ethanol
area	/
applications/day	Once per day for 10 min
dose	Step 1: 10 ppm Step 2: 250 ppm
dose/application/cm^2	Not applicable
control substance	10% alcohol
definition of positive	clinical grading scale and laser doppler comparison between active and control
period	two weeks with each concentration: maximum four weeks
Results	
PT ED10% (95% CI)	Not relevant
PT ED50% (95% CI)	Not relevant
PT no effect level(observed)	Not relevant
ROAT	Cumulative responses
Step 1 (10 ppm)	1/13
Step 2 (250 ppm)	5/13
Vehicle control	4/13
Other information	No difference between active substance and control application was found.

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) *(7)*	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to HICC
Controls	7 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006%
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	volar aspect of lower arm
area	3 x 3 cm^2
applications/day	two with droplet bottle (theoretical:30 mg per application of solution)
dose	Step 1: 0.5% Step 2: 3%
µg/application/cm^2	Step 1: 15.3 (3.4-22.2) Step 2: 126.2 (40.5-226.2)
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step; total maximum 4 weeks
Results	
PT ED10% (95% CI)	0.9 µg/cm^2 29 (7-69) ppm
PT ED50% (95% CI)	20 µg/cm^2 662 (350-1250)ppm
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (0.5%)	11/18 (61%)
Step 2 (3%)	16/18 (89%)
Controls	No reactions were seen
Other information	Difference between test and control group statistically significant

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) *(8)*	
Design	blinded, randomised and controlled
Test subjects	15 patients with a positive patch test to HICC
Controls	10 healthy controls
Substance	Purity: > 98.8%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006% (5 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	Axilla
area	76 cm^2 (template)
applications/day	two with roll on deodorant
dose	Step 1: 200 ppm Step 2: 600 ppm Step 3: 1800 ppm
dose/application/cm^2	median 0.79 µg HICC
control substance	deodorant matrix
definition of positive	spotty erythema involving at least 25% of the exposed area and infiltration represented by at least one papule.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	0.75 µg/cm^2 25 ppm (0.69-120)
PT ED50% (95% CI)	18.3 µg/cm^2 610 ppm (120-2800)
PT no effect level (observed)	< 0.0006%
ROAT	Cumulative responses
Step 1 (200 ppm)	9/14* (64%)
Step 2 (600 ppm)	12/14* (86%)
Step 3 (1800 ppm)	14/14* (100%)
Controls	No reactions were seen
Other information	*14 patients completed the use test study Difference between HICC deodorant and matrix deodorant in cases (p=0.0001).Difference between controls and patients (p=0.004).

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) *(9)*	
Design	blinded, randomised and controlled
Test subjects	17 patients with a positive patch test to HICC
Controls	15 healthy controls
Substance	IFF lot SM/8059062
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	1500 to 0.0022 µg/cm^2 HICC (19 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of forearms
area	3 x 3 cm (5 areas)
applications/day	two with micropipette (20 µl per application)
dose	Simultaneous application to 5 areas, four doses each and vehicle
µg /application/cm^2	Dose 1:0.0357 Dose 2: 0.357 Dose 3: 3.57 Dose 4: 35.7
control substance	ethanol
definition of positive	at least 5 points on a clinical scale, corresponding to erythema in 25% of test area and at least 1 papule
period	Three weeks. All concentrations applied simultaneously (randomised)
Results	
PT ED10% (95% CI)	0.662 µg/ cm^2 (0.052-2.35)
PT ED50% (95% CI)	11.1 µg/ cm^2 (3.41- 33.1)
PT no effect level(observed)	<0.0022 µg/ cm^2
ROAT	Cumulative responses
Dose 1 (0.0357)	0/16*
Dose 2 (0.357)	3/16 (19%)
Dose 3 (3.57)	12/16 (75%)
Dose 4 (35.7)	15/16 (94%)
Controls	No reactions were seen
Other information	*16 patients completed the use test study The evaporation rate of HICC was calculated to 72% over a 24-h period. ED10% ROAT: 0.064 µg/cm^2 (more info see below)

Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application (μg HICC cm^{-2})	Number of applications after 1 week	Total accumulated dose after 1 week (μg HICC cm^{-2})	Number of applications after 2 weeks	Total accumulated dose after 2 weeks (μg HICC cm^{-2})	Number of applications after 3 weeks	Total accumulated dose after 3 weeks (μg HICC cm^{-2})
35·7	14	500	28	1000	42	1500
3·57	14	50	28	100	42	150
0·357	14	5	28	10	42	15
0·0357	14	0·5	28	1	42	1·5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

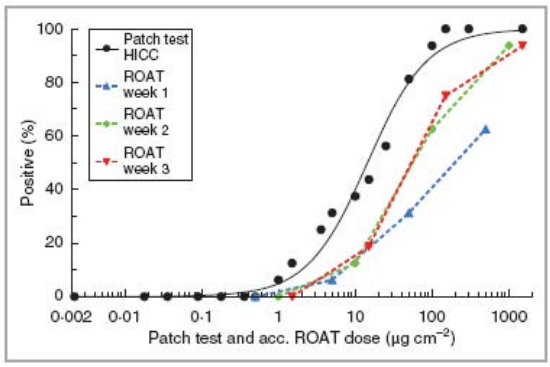


Fig 3. The fitted dose–response curve for the patch test (n = 16) and the 1-week, the 2-week 3-week accumulated ROAT doses.

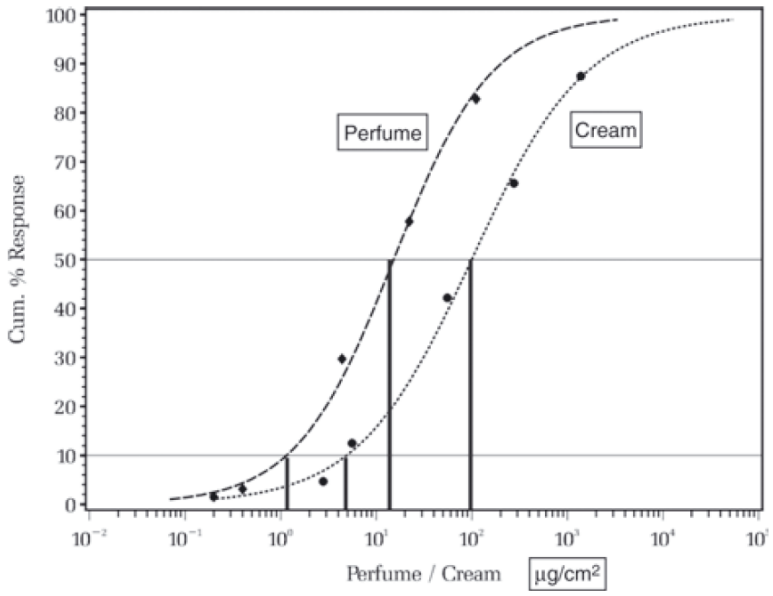
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

In a study be the German Contact Dermatitis Group, 64 persons previously diagnosed with HICC contact allergy were exposed to increasing doses of HICC in 2 different formulations, a hydrophilic cream and an ethanol solution, to mimic everyday exposures, following a standardised ROAT protocol (10). The concentration of HICC tolerated by 90% of the sensitised was estimated as 1.2 µg/cm² for perfume and 4.9 µg/cm² for cream. The dose-response curve is shown in Fig. 4.3 – 1 below.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (10)		
Design	randomised and vehicle controlled	
Test subjects	67 patients with a previous positive patch test to HICC	
Controls	None	
Substance	Provided by International Flavor & Fragrances Inc, Hilversum, NL	
Patch test		
-dilution steps	2.5% and 5%	
-control/vehicle	petrolatum	
-definition of threshold	lowest concentration giving a positive skin reaction in a continuous line to next higher concentration.	
ROAT	Volar forearms (both sides)	
area	3 x 3 cm (4 areas: one test and one control each for alcoholic solution and cream, respectively)	
applications/day	two	
dose	2.8 µg/cm² in cream 5.6 µg/cm² in cream 55.6 µg/cm² in cream 277.8 µg/cm² in cream 1388.9 µg/cm² in cream	0.2 µg/cm² in ethanol 0.4 µg/cm² in ethanol 4.4 µg/cm² in ethanol 22.2 µg/cm² in ethanol 111.1 µg/cm² in ethanol
µg /application/cm^2	See above	
control substance	Ethanol 96% and glyceryl stearate 15% in water, resp.	
definition of positive	(spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number	
period	Two weeks for each step until positive reaction or end of study, whichever occurred first	
Results		
PT ED10% (95% CI)	Not calculable; 52 of 60 Patients patch tested positive to 2.5% HICC, 57 / 60 to 5% HICC	
PT ED50% (95% CI)	Not calculable	
PT no effect level (observed)	Not calculable	
ROAT	Cumulative responses:	
	Cream preparation: 2.8 µg/cm²: 4.7%	Ethanol preparation: 0.2 µg/cm²:1.6%

	5.6 µg/cm²: 12.5% 55.6 µg/cm²: 42.2% 277.8 µg/cm²: 65.6% 1388.9 µg/cm²: 87.5%	0.4 µg/cm²: 3.1% 4.4 µg/cm²: 29.7% 22.2 µg/cm²: 57.8% 111.1 µg/cm²: 82.8%
Controls	No reactions to vehicle in the patients included into analysis	
Other information	See figure below. Three patients were excluded from the study, so results are based on 64 patients.	

Figure 4.3 – 1: Dose-response curve of 64 patients sensitised to HICC, according to a previous PT, regarding two preparations: perfume and cream, the rhomboid and dot symbol, respectively, indicating the observed response. The curve was fitted by a logistic function (10).



Isoeugenol

Isoeugenol *(11)*	
Design	blinded, randomised doses and controlled
Test subjects	20 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (8 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line
ROAT	outer aspect of upper arms
area	5 x 5 cm (2 areas: one test and one control)
applications/day	two with roll-on
dose	0.2% in ethanol
µg /application/cm^2	Doses measured to 0.14 -0.13 mg/application the first 14 days = 5.6 µg/cm^2
control substance	ethanol
definition of positive	any degree of reaction
period	Two weeks at upper arm and if negative another two weeks including application to base of neck
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.08% 32 µg/cm^2
PT no effect level (observed)	< 0.01% = 0.4 µg/cm^2
ROAT	
Dose: 0.2%	12/19 (63%)
Controls	No reactions were seen
Other information	

Isoeugenol *(12)*	
Design	blinded, randomised
Test subjects	27 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of lower arm
area	3 x 3 cm (2 areas)
applications/day	two with droplet bottle (30 mg per application)
dose	0.05% in ethanol and 0.2%
µg /application/cm^2	Doses were calculated as mean 2.2 µg/cm^2 (low conc.) and 9 µg/cm^2 (high conc.)
control substance	ethanol
definition of positive	clear visible erythema
period	28 days
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	< 0.0005% (5 ppm)
ROAT	Cumulative responses
Dose 1: 0.05%	10/24 (42%)
Dose 2: 0.2%	16/24 (67%)
Controls	No reactions were seen
Other information	Response to the low concentration in the ROAT appeared after median 15 days and to the high concentration after median 7 days.

Isoeugenol *(13)*	
Design	blinded, randomised and controlled
Test subjects	13 patients with a positive patch test to isoeugenol and 4 in part 1 (pre-test)
Controls	10 healthy controls (dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (w/v) (16 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration elicitating at least + reaction
ROAT	Axilla
area	10 x 10 cm^2 (estimated)
applications/day	two with roll-on deodorant (117-586 mg per application of solution) average cases: 266 mg/application controls: only range given
dose	Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2% Part 2: Step1:0.0063% Step 2:0.02% Step 3: 0.063%
dose/application/cm^2	Part 2: Step 1: 0.167 Step 2: 0.53 Step 3: 1.67 µg/application/ cm^2 (calculated based on data)
control substance	deodorant matrix
definition of positive	eczematous response covering 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	<0.0005% (0.15 µg/cm^2)
ROAT	
Step 1 (0.0063%)	3/13 (23%)
Step 2 (0.02%)	9/13 (69%)
Step 3 (0.063%)	10/13 (77%)
Controls	No reactions were seen
Other information	Deodorants containing cinnamal were responsible for all reactions in cinnamal sensitized individuals (p<0.001) and all control persons were negative (p<0.001)

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.0
Revision Date 27.09.2019
Print Date 02.11.2022

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

1.1 Product identifiers

Product name	:	Ethyl 2-methylbutyrate
Product Number	:	306886
Brand	:	Aldrich
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	7452-79-1

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

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Merck Life Science UK Limited New Road The Old Brickyard GILLINGHAM Dorset SP8 4XT UNITED KINGDOM
Telephone	:	+44 (0)1747 833-000
Fax	:	+44 (0)1747 833-313
E-mail address	:	TechnicalService@merckgroup.com

1.4 Emergency telephone number

Emergency Phone #	:	+44 (0)870 8200418 (CHEMTREC)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Flammable liquids (Category 3), H226

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram



Signal word Warning

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Hazard statement(s) H226	Flammable liquid and vapour.
Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	:	$C_7H_{14}O_2$
Molecular weight	:	130.18 g/mol
CAS-No.	:	7452-79-1
EC-No.	:	231-225-4

No components need to be disclosed according to the applicable regulations.

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

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SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
Use water spray to cool unopened containers.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Avoid breathing vapours, mist or gas. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid inhalation of vapour or mist.
Keep away from sources of ignition - No smoking.Take measures to prevent the build up of electrostatic charge.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

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8.2 Exposure controls

Appropriate engineering controls
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Splash contact
Material: butyl-rubber
Minimum layer thickness: 0.3 mm
Break through time: 30 min
Material tested:Butoject® (KCL 897 / Aldrich Z677647, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Impervious clothing, Flame retardant antistatic protective clothing., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: clear, liquid Colour: colourless

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b)	Odour	No data available
c)	Odour Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	133 °C - lit.
g)	Flash point	26 °C - closed cup
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	0.865 g/cm3 at 25 °C
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
Heat, flames and sparks.

10.5 Incompatible materials
Strong oxidizing agentsStrong oxidizing agents, Strong bases

10.6 Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides

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Other decomposition products - No data available
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment

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This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging

Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number

ADR/RID: 3272 IMDG: 3272 IATA: 3272

14.2 UN proper shipping name

ADR/RID: ESTERS, N.O.S. (Ethyl 2-methylbutyrate)
IMDG: ESTERS, N.O.S. (Ethyl 2-methylbutyrate)
IATA: Esters, n.o.s. (Ethyl 2-methylbutyrate)

14.3 Transport hazard class(es)

ADR/RID: 3 IMDG: 3 IATA: 3

14.4 Packaging group

ADR/RID: III IMDG: III IATA: III

14.5 Environmental hazards

ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user

No data available

SECTION 15: Regulatory information

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

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical safety assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H226 Flammable liquid and vapour.

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Further information

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